Molecular Biology Research Products Catalog and Sourcebook for Electrophoresis 2009–2010

SeaKem®, NuSieve® and MetaPhor® Agarose
PAGEr® and Reliant® Precast Gels
GelBond® Film
AccuGENE® Buffers
FlashGel® System
Dear Valued Lonza Customer,

We are pleased to introduce the 2009 - 2010 Lonza Molecular Biology Research Products Catalog. Inside, you will find all of your known and trusted Lonza research products and services, as well as many new offerings designed to advance and accelerate your research:

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- **New! The FlashGel® System** – providing separation, recovery and documentation of nucleic acid fragments in minutes, at the bench, without UV light or handling of hazardous solutions. This system will transform the workflow in your lab.

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The Lonza Molecular Biology Team
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- 6,558,521
- 6,599,711
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- 6,365,341
- 6,464,850
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Nucleic Acid Gel Electrophoresis

Chapter 1
# Nucleic Acid Gel Electrophoresis

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- GelBond® Gel Support Film
Agarose Selection Guide

Selecting the best agarose for your application can minimize opportunity for error, optimize results, and even reduce cost. Lonza offers a wide range of agarose types that are specifically engineered to optimize results by fragment size, sample type and application. The selection tools below will get you started. The following pages will guide you to the right concentration, buffer and marker to use for best performance in your experiment. If you require additional support, consult our Sourcebook for Electrophoresis beginning on page 63.

Choose the Agarose that is Right for You

Agarose and Applications

SeaKem® Gold Agarose
- In-gel Reactions, DNA & RNA Recovery, Cloning
- 200 bp - 10 Mb

SeaKem® GTG® Agarose
- DNA & RNA Recovery, Cloning
- 200 bp - 25 kb

SeaKem® GTG® Agarose
- DNA & RNA Recovery, Cloning
- 200 bp - 25 kb

SeaKem® LE Agarose
- Routine Analysis, Blotting
- 100 bp - 23 kb

NuSieve® GTG® Agarose
- DNA & RNA Recovery, Cloning
- 50 bp - 1 kb

NuSieve® 3:1 Agarose
- PCR, Blotting
- 50 bp - 1 kb

MetaPhor® Agarose
- Fine Resolution
- 200 bp - 800 bp

Agarose and Compatible Techniques

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Our agarose is GUARANTEED DNase/RNase Free
SeaKem® LE Agarose

SeaKem® LE Agarose is the ideal multipurpose, molecular biology grade agarose for any DNA or RNA application.

— Wide resolution range: 100 bp to 23 kb
— High gel strength – Ideal for Southern and Northern blotting
— Consistent lot-to-lot performance

Applications
— Broad range fragment separation
— Southern and Northern blotting
— PCR greater than 1 kb
— Immunoprecipitation techniques
— Baculovirus screening
— Colony lifts

Storage Conditions
18°C – 26°C

Recommended Lonza Ladders/Markers, see page 36
— 50 to 1,000 bp DNA Marker
— 100 bp DNA Marker

Application Support
— Preparation of Agrose Gels, Section II, page 75

Ordering Information

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<td>50005</td>
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Larger package sizes are available upon request. Please inquire for pricing and availability.

1% SeaKem® LE Agarose gel

Lane 1: Hind III digest of Lambda DNA
Lane 2: DNA Marker 1 to 10 kb (Lonza)
Lane 3: 500 bp DNA Ladder (Lonza)

See table on page 79 for analytical specifications.

Related Products

AccuGENE® Buffers 41
DNA Ladders and Markers 36
GelStar® and SYBR® Green Nucleic Acid Gel Stains 38 & 39
MetaPhor® Agarose

The highest resolution agarose available

MetaPhor® Agarose offers twice the resolution capability of standard agarose for PCR, STR and AmpFLP analysis. This intermediate melting temperature agarose rivals polyacrylamide and is capable of resolving DNA fragments differing in size by 2% between 20 bp and 800 bp.

- Rivals the resolution capability of polyacrylamide
- Eliminates the hazards associated with using polyacrylamide
- Fine separation of fragments 20 bp – 800 bp

■ Applications
  - Small PCR analysis
  - STR analysis
  - RT-PCR

■ Performance and Quality Tests
  - DNA resolution: 4 bp resolution of DNA fragments at 200 bp and 16 bp resolution at 800 bp in TBE Buffer
  - Gel background: Gel exhibits low background fluorescence after ethidium bromide staining
  - DNA binding: None detected

■ Storage Conditions
  18°C – 26°C

■ Recommended Lonza Ladders/Markers, see page 36
  - 20 bp DNA Ladder
  - 2U bp Extended Range DNA Ladder
  - 50 to 1,000 bp DNA Marker
  - 100 bp DNA Ladder
  - DNA QuantLadder
  - DNA Reverse QuantLadder

■ Application Support
  - Fast-Running protocols for High Resolution in MetaPhor® Agarose Gels, page 93

<table>
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<tr>
<td>500 bp – 1,000 bp</td>
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See table on page 79 for analytical specifications.

Ordering Information

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Larger package sizes are available upon request. Please inquire for pricing and availability.

High resolution of short DNA ladders in MetaPhor® Agarose

DNA ladders with 4 bp or 8 bp step sizes were prepared by ligation of BglII linkers. Aliquots of 0.8 μg of the ladders were separated on a 3.5% MetaPhor® Agarose gel in a horizontal format and compared to an 8% polyacrylamide gel run in a vertical format in TBE Buffer. The horizontal gel (15 cm x 20 cm and 3.0 mm thick) was run at 6.7 W/cm for 4 hours at 15°C. The vertical gel (10 cm x 20 cm and 1.0 mm thick) was run at 8 W/cm for 2 hours.

Related Products

- AccuGENE® Buffers
- DNA Ladders and Markers
- GelStar® and SYBR® Green Nucleic Acid Gel Stains
NuSieve® 3:1 Agarose was the first and still is the most reliable choice for separating and resolving PCR and RT-PCR fragments. This molecular biology grade agarose produces strong, easy-to-handle gels, making it ideal for blotting of small fragments.

- Exceptional resolution of small fragments between 50 bp and 1 kb
- Superior gel strength for blotting
- Widely cited as the choice for PCR analysis

**Applications**
- Small DNA and RNA fragment analysis
- Blotting of small fragments
- RT-PCR
- Genotyping

**Performance and Quality Tests**
- Resolution: DNA fragments 1,000 bp are finely resolved after electrophoresis
- Gel background: Gel exhibits low background fluorescence after ethidium bromide staining
- DNA binding: None detected

**Storage Conditions**
18°C – 26°C

**Recommended Lonza Ladders/Markers, see page 36**
- 20 bp DNA Ladder
- 50 to 1,000 bp DNA Marker
- 100 bp DNA Ladder
- DNA QuantLadder
- DNA Reverse QuantLadder

**Application Support**
- Preparation of Agrose Gels, Section II, page 75

---

### NuSieve® 3:1 Agarose Gel for PCR Products

A 550 bp sequence from lambda DNA was amplified (25 cycles) using primers and Taq DNA polymerase supplied in the GeneAmp® Kit (Roche Molecular Systems). PCR products and controls were electrophoresed on a 4% NuSieve® 3:1 Agarose gel in TAE Buffer at 5 V/cm for 3 hours. Lane 1, MspI digest of pBR322 DNA (1.5 μg); lane 2, Hae III digest of øX174 DNA (1.5 μg); lane 3, no DNA control; lanes 4 – 9, PCR products resulting from different reaction conditions (7 μl of 100 μl reaction mixture); and lane 10, a positive control where kit template was added.

### Related Products

- AccuGENE® Buffers...
- DNA Ladders and Markers...
- GelStar® and SYBR® Green Nucleic Acid Gel Stains...

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**Ordering Information**

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Larger package sizes are available upon request. Please inquire for pricing and availability.

---

**NuSieve® 3:1 Agarose**

<table>
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<tr>
<th>Final Agarose Concentration %</th>
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<tbody>
<tr>
<td>Colors</td>
<td>50 bp - 400 bp</td>
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See table on page 79 for analytical specifications.
NuSieve® GTG® Agarose

NuSieve® GTG® Agarose provides optimal separation and resolution of PCR and RT-PCR fragments. This low melting (65°C) temperature agarose is easy-to-handle and can be used for cloning procedures directly from remelted agarose. Genetic Technology Grade™ Agarose is quality tested to certify performance.

- Fine resolution of small fragments between 50 bp and 1 kb
- Performance certified for digestion and ligation

**Applications**
- Analysis and recovery of small DNA fragments
- In-gel PCR and In-gel ligations/transformations

**Performance and Quality Tests**
- Enzymatic activity in the presence of remelted gel: T4 DNA ligase and transformation test
- Resolution: DNA fragments fl1,000 bp are finely resolved after electrophoresis
- Gel background: Gel exhibits low background fluorescence after ethidium bromide staining
- DNase and RNase activity: None detected
- DNA binding: None detected

**Storage Conditions**
18°C – 26°C

**Recommended Lonza Ladders/Markers, see page 36**
- 20 bp DNA Ladder
- 50 to 1,000 bp DNA Marker
- DNA QuantLadder
- DNA Reverse QuantLadder

**Application Support**
- In-Gel Reactions, Section V, page 105
- Recovery of DNA from Agarose Gels, Section VI, page 115

**Ordering Information**

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Larger package sizes are available upon request. Please inquire for pricing and availability.

**β-Agarase**

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See page 11 for more information.

**Fine resolution of low molecular weight DNA fragments in NuSieve® GTG® Agarose**

DNA fragments were separated in 2%, 3%, and 4% NuSieve® GTG® Agarose gels in 1X TBE Buffer. Lane A: Hae III digest of øX174 DNA, 0.5 µg/lane. Lane B: Msp I digest of pBR322 DNA, 0.5 µg/lane. Running conditions: 1X TBE at 5 V/cm.

**Related Products**

- AccuGENE® Buffers
- DNA Ladders and Markers
- GelStar® and SYBR® Green Nucleic Acid Gel Stains
Agarose

Confidently resolve fragments from 200 bp to 25 kb prior to PCR, cloning, digesting, or sequencing in the presence of re-melted SeaPlaque® GTG® Agarose, without additional purification steps. This low melting temperature (65°C) Genetic Technology Grade™ Agarose is quality tested to certify performance.

— Optimal separation range for DNA and RNA recovery of fragments: 200 bp to 25 kb
— Performance certified

Applications
— Analysis and recovery of large DNA fragments
— In-gel PCR and In-gel ligations and transformations
— DNA and RNA digestion

Performance and Quality Tests
— Enzymatic activity in the presence of remelted gel:
  — T4 DNA ligase and transformation test
  — Hind III and EcoRI restriction digestion test
— Fine resolution of DNA fragments ≥1,000 bp with low background after ethidium bromide staining
— DNase and RNase activity: None detected
— DNA binding: None detected

Storage Conditions
18°C – 26°C

Recommended Lonza Ladders/Markers, see page 36
— 500 bp DNA Ladder
— 1 to 10 kb DNA Marker
— DNA QuantLadder

Application Support
— In-Gel Reactions, Section V, page 105

SeaPlaque® GTG® Agarose

Performance certified for large fragment recovery and in-gel reactions

Ordering Information

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Larger package sizes are available upon request. Please inquire for pricing and availability.

β-Agarase

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<td>58005</td>
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See page 11 for more information.

Resolution performance of SeaPlaque® GTG® Agarose

SeaPlaque® and SeaPlaque® GTG® Agarose

See table on page 79 for analytical specifications.

Related Products

<table>
<thead>
<tr>
<th>Product Description</th>
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<td>AccuGENE® Buffers</td>
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<td>DNA Ladders and Markers</td>
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<tr>
<td>GelStar® and SYBR® Green Nucleic Acid Gel Stains</td>
<td>38 &amp; 39</td>
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SeaKem® GTG® Agarose ensures reliable digestion and ligation from recovered DNA or RNA fragments from 100 bp to 23 kb. Our Genetic Technology Grade™ Agarose is quality tested to certify performance.

Applications
- Best choice for DNA and RNA recovery and cloning 100 bp to 23 kb

Performance and Quality Tests
- Restriction endonuclease digestion test: EcoRI and HindIII are tested for complete digestion of electroeluted, linearized pBR322 DNA
- Ligation of recovered DNA
- Fine resolution of DNA fragments ≥1,000 bp with low background after ethidium bromide staining
- DNase and RNase activity: None detected
- DNA binding: None detected

Storage Conditions
18°C – 26°C

Recommended Lonza Ladders/Markers, see page 36
- 50 to 1,000 bp DNA Marker
- 100 bp DNA Marker

Application Support
- Recovery of DNA from Agarose Gels, Section VI, page 115

Ordering Information

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Larger package sizes are available upon request. Please inquire for pricing and availability.

Resolution performance of SeaKem® GTG® Agarose

![Resolution performance of SeaKem® GTG® Agarose](image)

1 kb DNA Ladder (Lonza) 1 μg/lane, unheated

Efficient Digestions After Recovery

![Efficient Digestions After Recovery](image)

SeaKem® GTG® Agarose

![SeaKem® GTG® Agarose](image)

See table on page 79 for analytical specifications.

Related Products

- AccuGENE® Buffers 41
- DNA Ladders and Markers 36
- GelStar® and SYBR® Green Nucleic Acid Gel Stains 38 & 39
SeaPlaque® Agarose

SeaPlaque® Agarose is the original low melting temperature agarose and has been a staple in molecular biology labs for over 40 years. This molecular biology grade agarose produces gels with greater sieving capabilities from 200 bp to 25 kb, and with higher clarity than standard melting temperature agarose. Ideal for preparative DNA and RNA electrophoresis.

- Ideally suited for DNA and RNA recovery
- Also ideal for cloning of tissue culture cells and viral plaque assays

**Applications**
- Preparative DNA and RNA electrophoresis
- Viral plaque assays
- Cell culture

**Storage Conditions**
18°C – 26°C

**Recommended Lonza Ladders/Markers, see page 36**
- 100 bp DNA Marker

**Application Support**
- Recovery of DNA from Agarose Gels, Section VI, page 115

**β-Agarase**

β-Agarase is an enzyme that will completely digest the polysaccharide backbone of molten agarose into alcohol soluble oligosaccharides. DNA electrophoresed in low melting temperature agarose gels can be recovered quantitatively after the gel is melted and then digested with this enzyme. Any remaining agarose oligosaccharides will not gel or interfere with subsequent DNA manipulations such as cloning, labeling, restriction digestion, or sequencing.

**Ordering Information**

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Larger package sizes are available upon request. Please inquire for pricing and availability.

Recovery of DNA from 1% SeaPlaque® GTG® Agarose with β-Agarase

Hind III-digested lambda DNA gel stained with ethidium bromide and the DNA bands excised. Gel digested with β-Agarase, and DNA recovered by ethanol precipitation. The recovered DNA was applied to a 1% SeaKem® GTG® Agarose gel in 1X TBE Buffer and separated by electrophoresis. A photograph of the second gel stained with ethidium bromide is shown.

**1% SeaPlaque® Agarose Gel**

Lane 1: DNA Marker 1 to 10 kb [Lonza]
Lane 2: DNA Marker 50 to 2,500 bp [Lonza]
Lane 3: 500 bp DNA Ladder [Lonza]
Lane 4: 100 bp Extended Range DNA Ladder [Lonza]
SeaKem® Gold Agarose is ideal for separating very large DNA fragments or doing pulsed field gel electrophoresis (PFGE). This Genetic Technology Grade™ Agarose is ideal for rapid resolution of megabase DNA, decreasing run times by up to 50% for PFGE.

- Capable of rapid separation of large DNA from 30 kb to 50 kb by horizontal electrophoresis or 50 kb to 10 Mb for PFGE
- Good multipurpose, high gel-strength agarose for separations 1,000 bp
- Specially manufactured to create a strong gel that is easy-to-handle
- Guaranteed DNase and RNase-free

■ Applications
- Large fragment separation
- Pulsed field gel electrophoresis
- Blotting of megabase DNA

■ Performance and Quality Tests
- Relative DNA mobility: 1.3 under PFGE conditions (SeaKem® LE Agarose = 1.0)
- Restriction endonuclease digestion test: EcoR I and Hind III are tested for complete digestion of recovered DNA
- Ligation of recovered DNA
- Resolution: DNA fragments fl 1,000 bp are finely resolved after electrophoresis
- Gel background: Gel exhibits low background fluorescence after ethidium bromide staining
- DNase and RNase activity: None detected
- DNA binding: None detected

■ Storage Conditions
18°C – 26°C

■ Recommended Lonza Ladders/Markers, see page 36
- DNA Marker 1 to 10 kb
- Tandem DNA Ladder
- 500 bp DNA Ladder
- DNA QuantLadder
- DNA Reverse QuantLadder

See table on page 79 for analytical specifications.
InCert® Agarose and Megabase DNA Standards

InCert® Agarose

InCert® Agarose is a low gelling temperature agarose, certified for use in the preparation and digestion of chromosomal DNA prior to pulsed field gel electrophoresis (PFGE).
- Ideal for PFGE projects
- Certified performance for chromosomal DNA preparation and restriction endonuclease digestion

Megabase DNA Standards

Our Megabase DNA Standards are specially prepared and tested chromosomal DNA standards offered in InCert® Agarose gel plug format for easy handling during PFGE.
- Performance tested for reliable PFGE
- Saves time – Standards are ready-to-use

Applications
- Pulsed field gel electrophoresis

Performance and Quality Tests for InCert® Agarose
- Restriction endonuclease digestion in agarose gel plugs test: enzymes tested on \textit{E. coli} DNA: \textit{Eco}RI, and \textit{Hind} III
- DNase activity: None detected

Storage Conditions
- InCert® Agarose: 18°C – 26°C
- Megabase DNA Standards: 2°C – 8°C

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Larger package sizes are available upon request. Please inquire for pricing and availability.

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Performance of Megabase DNA Standards in PFGE

Lambda DNA Ladders and \textit{S. cerevisiae} DNA Standards were run on the Bio-Rad® CHEF-DR® III System.

Running conditions:
- Lambda Ladders: 1% SeaKem® GTG® Agarose, 0.5X TBE, switch angle 1° 20°, 6 V/cm, ramped switch time from 50 – 90 seconds over 22 hours.
- \textit{S. cerevisiae}: 1% SeaKem® GTG® Agarose, 0.5X TBE, switch angle 1° 20°, 6 V/cm, ramped switch time from 40 – 100 seconds over 24 hours.

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<th>Block 1</th>
<th>Block 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Switch angle</td>
<td>96°</td>
<td>100°</td>
</tr>
<tr>
<td>V/cm</td>
<td>2 V/cm</td>
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<tr>
<td>Switch time [sec.]</td>
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<td>1500</td>
</tr>
<tr>
<td>Run time [hr.]</td>
<td>24</td>
<td>24</td>
</tr>
</tbody>
</table>

Related Products

| AccuGENE® Buffers | 41 |
SeaKem® ME Agarose

SeaKem® ME Agarose is the ideal choice for serum protein electrophoresis and immunoelectrophoresis, and may be used for DNA electrophoresis.

- Enhanced resolution in serum protein electrophoresis
- High gel clarity and minimal non-specific binding

■ Applications
  - Serum protein electrophoresis
  - Immunoelectrophoresis
  - Nucleic acid electrophoresis

■ Storage Conditions
  18°C – 26°C

SeaPrep® Agarose

SeaPrep® Agarose is a unique ultra-soft agarose, ideal for high efficiency hybridoma cloning. It is also used for expanding cDNA libraries in a strictly representative fashion, decreasing the possibility that less abundant clones vanish during amplification due to differential rates of replication.

■ Specifications
  - Melting temp: ≥ 50°C @ 1%
  - Gelling temp: 8°C - 17°C @ 0.8%
  - Gel Strength: >75 g/cm² @ 2%

■ Applications
  - Cell culture
  - Hybridoma cloning
  - Encapsulation/embedding of cells

■ Storage Conditions
  18°C – 26°C

Ordering Information

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Size</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>50011</td>
<td>25 g</td>
<td>$73</td>
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<tr>
<td>50010</td>
<td>125 g</td>
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<tr>
<td>50014</td>
<td>500 g</td>
<td>$627</td>
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</table>

Larger package sizes are available upon request. Please inquire for pricing and availability.

See table on page 79 for analytical specifications.

SeaPrep® Agarose

Ideal for cell culture applications

Ordering Information

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Size</th>
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<tbody>
<tr>
<td>50302</td>
<td>25 g</td>
<td>$210</td>
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</table>

Application Support

- Amplification of cDNA libraries with SeaPrep® Agarose, Section IX, page 150
- Preparing Agarose for use in Cell Culture Applications, Section IX, page 152

Related Products

AccuGENE® Buffers 41
I.D.fifi® Agarose is specially manufactured for DNA identity testing. For reliable separation of VNTRs, HVRs, RFLPs, and DNA size standards, it is a perfect match for your DNA typing tests.

— Performance certified to assure lot-to-lot reliability for DNA identity testing
— Crisp DNA separation to accurately discriminate DNA fragments
— Strong, easy-to-handle gels allow for trouble-free high efficiency blotting

Applications
— DNA identity testing

Performance and Quality Tests
— DNase and RNase activity: None detected
— DNA binding: None detected

Storage Conditions
18°C – 26°C

Related Products

<table>
<thead>
<tr>
<th>Product Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>AccuGENE® Buffers</td>
<td>41</td>
</tr>
<tr>
<td>DNA Ladders and Markers</td>
<td>36</td>
</tr>
<tr>
<td>GelStar® and SYBR® Nucleic Acid Gel Stains</td>
<td>38 &amp; 39</td>
</tr>
<tr>
<td>Long Ranger® Singel® Packs</td>
<td>43</td>
</tr>
<tr>
<td>MetaPhor® Agarose</td>
<td>6</td>
</tr>
</tbody>
</table>

An autoradiogram of DNA size standards (LIFECODES Corp.) and Hae III-digested K562 DNA probed with D4S139 (Invitrogen). DNA was electrophoresed at 1 V/cm for 16 hours in a 1% I.D.fifi® Agarose gel, transferred, and probed. Lane 1: DNA size standards; Lane 2: alleles detected with D4S139.

Ordering Information

<table>
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<tr>
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<td>50170</td>
<td>125 g</td>
</tr>
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</table>

Larger package sizes are available upon request. Please inquire for pricing and availability.
Lonza offers a complete family of precast agarose gels for DNA and RNA electrophoresis. Our unique gel options cover the full range of separations needs, from ultra-fast PCR analysis and recovery, to fine resolution and high-throughput separations. Our custom manufacturing capabilities can support the requirements of nearly any application. All Lonza gels are precision manufactured with our high quality SeaKem® and NuSieve® Agarose and functionally tested for consistent performance.

FlashGel® System

Five minute DNA separation:
— Separate DNA 10 bp to 10 kb and RNA 0.5 kb to 9 kb
— Watch DNA migrate in real time, without UV light
— Recover samples directly, without purification

See pages 18-25 for a complete product description

Reliant® Gel Minigels

Small format gels for DNA and RNA:
— Run 8 – 24 samples
— Ideal for blotting and recovery
— Fits standard horizontal chambers

See pages 26-27 for a complete product description

Latitude® HT Gels

Large format gels for DNA:
— Run 100 – 200 samples
— Ideal for high throughput screening of DNA samples
— Fits standard horizontal chambers

See page 28 for a complete product description

PAGEr® Gold TBE Gels

Vertical polyacrylamide gels for DNA:
— Run 10 – 16 samples
— Ideal for fine resolution
— Easy to load and open

See page 31 for a complete product description
The FlashGel® System provides nearly instant results. Simply load samples, watch bands migrate and get data in as little as 2 minutes. Now the FlashGel® System has expanded to include DNA recovery and gel documentation. Say goodbye to gel preparation, band excision, purification, and UV light. Complete separation, recovery and documentation safely, at the bench, in minutes.

- **5 minute separation and recovery**
  - See bands in as little as 2 minutes
  - Recover samples directly, without UV light, band excision or purification

- **Real-time separation and documentation**
  - Watch band migration as it happens
  - Photograph gels at the bench, without DNA damaging UV light

- **Outstanding sensitivity and resolution**
  - 5–20 times more sensitive than EtBr; detect < 0.1 ng DNA or < 10 ng total RNA
  - Clean, sharp separation and straight, uniform sample lanes

The FlashGel® System consists of enclosed, disposable, precast agarose gel cassettes and a combination electrophoresis and transilluminator unit.

- **FlashGel® Cassettes** contain precast, prestained agarose gels and buffer — no need for gel preparation, buffer addition or gel staining

- **The FlashGel® Dock** is an electrophoresis apparatus with a built-in transilluminator that provides both separation and detection

- **The FlashGel® Camera** is a compact camera system designed to photograph FlashGel® Cassettes right at the bench.

- **FlashGel® Markers** are recommended for best performance

The FlashGel® System for DNA is the ideal sample screening tool. Check up to 34 PCR or restriction fragments quickly, without having to plan your day around agarose gels.

- **Fast, simple procedure**
  1. Insert cassette into dock.
  2. Pre-load wells with distilled or deionized water.
  3. Load samples.
  4. Plug in and turn on light and electrophoresis voltage.
  5. Watch until desired separation is achieved.
  6. Photograph.

FlashGel® System for DNA

5 minute DNA analysis

Separation at various run times on the FlashGel® System

Markers run on a 1.2% FlashGel® Cassette, 12+1 well format, 275V for times as shown. Sample lanes from left to right: FlashGel® DNA Marker (100 bp – 4 Kb), FlashGel® QuantLadder, Lonza 50-2500 bp Marker, Lonza 100 bp Ladder.

See page 25 for ordering information.
5 minute separation

- The FlashGel® System provides high voltage separation of fragments (275V for 2 – 7 minutes); DNA separates in a fraction of the time required by competitor precast gel systems.

Real-time visualization

- Built-in illumination, allows you to view DNA under ambient light as it migrates through the gel; stop the run when desired separation is reached (in as little as 2 minutes depending upon fragment of interest); safely view the cassette on the lighted dock without eye protection.
- DNA bands separated on FlashGel® Cassettes are also detectable by UV light and may be photographed using standard gel documentation systems. Use The FlashGel® Camera for best performance.

DNA bands as viewed during a run on the FlashGel® Dock.

Superior resolution

- Resolve fragments in 2 – 7 minutes, and see clean, sharp band separation, and straight, uniform sample lanes.

Comparison of FlashGel® System with Company I

<table>
<thead>
<tr>
<th>Lane</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-7</td>
<td>FlashGel® DNA Marker (100 bp – 4 Kb); FlashGel® QuantLadder; Lonz 100 bp Ladder; Lonz 30-2500 bp Marker; 285 bp β-actin PCR; 294 bp control PCR (Company A)</td>
</tr>
<tr>
<td>8-12</td>
<td>Samples diluted with 1X FlashGel® Loading Dye prior to loading. Dilutions and load volumes optimized for each sample in each gel system.</td>
</tr>
</tbody>
</table>

Cassette Specifications

<table>
<thead>
<tr>
<th>Specification</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimal separation range:</td>
<td>1.2% agarose: 50 bp – 4,000 bp; 2.2% agarose: 10 bp – 1,000 bp</td>
</tr>
<tr>
<td>Separation of fragments &gt;4 kb will be improved by running longer at lower voltage</td>
<td></td>
</tr>
<tr>
<td>Storage:</td>
<td>Room temperature for 5 months from date of manufacture. Shelf life may be extended with refrigerated storage.</td>
</tr>
<tr>
<td>Well volume:</td>
<td>12+1 well: 5 μl; 16+1 well: 5 μl; 8+1 well: 12 μl</td>
</tr>
<tr>
<td>Gel size:</td>
<td>70 mm (L) X 84 mm (W) X 2 mm (H)</td>
</tr>
<tr>
<td>Cassette size:</td>
<td>115 mm (L) X 107 mm (W) X 17 mm (H)</td>
</tr>
<tr>
<td>Dock size:</td>
<td>134 mm (L) X 120 mm (W) X 54 mm (H)</td>
</tr>
</tbody>
</table>

See page 25 for ordering information.

Some components and technology of the FlashGel® System are sold under licensing agreements. The nucleic acid stain in this product is manufactured and sold under license from Molecular Probes, Inc., and the FlashGel® Cassette is sold under license from Invitrogen IP Holdings, Inc, and is for use only in research applications or quality control, and is covered by pending and issued patents. The FlashGel® Dock technology contains Clare Chemical Research, Inc. Dark Reader® transilluminator technology and is covered under US Patents 6,198,107; 6,512,236; and 6,914,250. The electrophoresis technology is licensed from Temple University and is covered under US Patent 6,905,585.

Related Products

<table>
<thead>
<tr>
<th>Product</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>FlashGel® System for Recovery</td>
<td>20</td>
</tr>
<tr>
<td>FlashGel® System for RNA</td>
<td>22</td>
</tr>
<tr>
<td>FlashGel® Camera</td>
<td>24</td>
</tr>
</tbody>
</table>
Direct DNA recovery using the FlashGel® System for Recovery eliminates agarose gel preparation, band excision, and purification. The system delivers highly efficient recovery, free from inhibitors and UV-induced damage, in a simple 5-10 minute protocol.

- Go from sample loading to recovery in just 5 minutes
- Recover samples directly from the cassette, without band excision or purification
- Visualize sample recovery without UV
- Recover at 80% - 100% efficiency

**Fast, simple procedure**

1. Load samples in top tier of wells.
2. Run until samples reach the second tier of wells.
3. Stop the run and add FlashGel® Recovery Buffer.
4. Remove DNA from wells.

- The FlashGel® Recovery System eliminates the need to cut away and then purify bands. As DNA migrates to the second tier of wells, it is free from the agarose matrix and easily extracted via pipette, with the aid of the FlashGel® Recovery Buffer.
- Depending upon initial separation time in the recovery step, the same cassette may be used for analysis verification of the recovered sample.

**No DNA damaging UV or mutagenic stain exposure**
- Visible light from the compact FlashGel® Dock illuminates the recovery wells without damage to the DNA or hazard to the user
- The proprietary stain in the FlashGel® Cassettes enables separation and recovery of very small quantities of DNA, and minimizes user exposure to potential mutagens

**Efficient recovery, free from inhibitors**
- Samples are recovered at 80% - 100% efficiency, are free of inhibitors, and ready for subsequent re-amplification, cloning, or other techniques, without additional clean-up steps

**Recovery Efficiency**
on the FlashGel® System for Recovery

2-fold dilutions of 1000 bp fragments were separated and recovered using the FlashGel® Recovery System. DNA concentrations ranged from 18.25 ng – 600 ng. Images 1 and 2 show the cassette before and after recovery. Image 3 shows analysis of 5% of each recovered sample, run on a 1.2% FlashGel® DNA Cassette. Comparison to the FlashGel® QuantLadder show recovery efficiencies ranging from 80% to 90%.
DNA Size Range
on the FlashGel® System for Recovery

Samples were separated and recovered on a FlashGel® Recovery Cassette. 3 μl aliquots of recovered samples consisting of 100 ng of fragments ranging from 50 bp to 4000 bp separated on a 1.2% FlashGel® DNA Cassette and compared to the FlashGel® DNA Marker 100 bp – 4 kb and the FlashGel® QuantLadder.

Recovery Efficiency
on the FlashGel® System for Recovery

Plasmid DNA (pBR322) was subjected to restriction enzyme double digestion using PstI and BamH1. Samples of the restricted DNA were separated and 3.2 kb fragments were recovered using the FlashGel® Recovery System (FG) or spin column kits (C1 and C2). Image 1 compares 5% of each recovered sample. Aliquots of the recovered samples were ligated into PstI/BamH1 double digested pUC19 vector (V). Samples of the ligation reactions were transformed into E.coli competent cells. The number of colonies obtained with both samples were very similar. Image 2 shows examples of PstI/BamH1 cut plasmid samples from two colonies from each sample. V shows a restricted sample of vector with no insert.

FlashGel® Recovery System

Separate fragments on FlashGel® System
3 - 5 minutes

Recover DNA directly from wells
1 - 5 minutes

Total time: 4 - 10 minutes

Column Recovery Method

Separate fragments on agarose gel
30 - 60 minutes

Excise bands from agarose gel
5 - 10 minutes

Recover DNA via spin column
25 - 30 minutes

Total time: ≥ 1 hour

Cassette Specifications

<table>
<thead>
<tr>
<th>Optimal separation range:</th>
<th>1.2% agarose: 50 bp – 4,000 bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2% agarose: 10 bp – 1,000 bp</td>
<td></td>
</tr>
</tbody>
</table>

Storage: Room temperature for 5 months from date of manufacture. Shelf life may be extended with refrigerated storage.

Well volume:
- 1+1 well: 5 μl
- 16+1 well: 5 μl
- 8+1 well: 12 μl

Gel size:
- 70 mm (L) X 84 mm (W) X 2 mm (H)

Cassette size:
- 115 mm (L) X 107 mm (W) X 17 mm (H)

Dock size:
- 134 mm (L) X 120 mm (W) X 54 mm (H)

See page 25 for ordering information.

Related Products

<table>
<thead>
<tr>
<th>FlashGel® System for DNA</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>FlashGel® System for RNA</td>
<td>22</td>
</tr>
<tr>
<td>FlashGel® Camera</td>
<td>24</td>
</tr>
</tbody>
</table>
The FlashGel® System for RNA is optimized for the unique requirements of RNA, and is the ideal tool for rapid analysis of sample integrity. High quality, intact RNA is essential for consistent results in gene expression, Northern analysis, cDNA library construction and cDNA labeling for microarrays. Most protocols recommend checking RNA prior to downstream analysis.

The FlashGel® System completes RNA analysis in less than 30 minutes and requires < 10 ng total RNA for detection. The system is recommended for verification and analysis of total RNA, quick checks of native RNA and checking for RNA degradation and mRNA purity.

- Get results in 30 minutes or less
- Detect < 10 ng RNA per band
- Avoid hazardous reagents and contaminating RNases

### Rapid RNA Analysis

1. Insert cassette into FlashGel® Dock.
2. Pre-load wells with RNase-free water.
3. Load samples.
4. Plug in and turn on light and electrophoresis voltage.
5. Run for 8 minutes.
6. Turn off voltage and hold for 10 minutes, or until RNA bands are stained to the desired intensity.
7. Photograph.

### Exquisitely sensitive detection

- The FlashGel® System for RNA offers the detection sensitivity of a chip system, without the cost, and rivals the best RNA stains (SYBR® Green and GelStar® Stains), without direct handling of stain solutions. RNA quantities < 10 ng per band are clearly detected on the FlashGel® System, conserving precious RNA samples.

### Clean, enclosed system

- FlashGel® RNA Cassettes fully enclose the gel, stain and running buffer, eliminating user exposure to hazardous reagents, and protecting samples from contaminating RNases. RNA cassettes are designed for performance and purity, and are guaranteed RNase free. The FlashGel® Dock provides electrophoresis and visualization of both DNA and RNA cassettes.

### Separation of total RNA on the FlashGel® System for RNA

Samples of RNA Marker (Lonza) (lanes 1 and 2) and E. coli total RNA (lanes 3 and 4) contain 50 ng (lanes 1 and 3) or 250 ng (lanes 2 and 4) of RNA per 5 μl load. Samples prepared with Formaldehyde sample buffer (Lonza) and denatured 5 minutes at 65°C. FlashGel® RNA Cassette run for 8 minutes, followed by a 20 minute hold prior to imaging.

### Checking sample quality with the FlashGel® System for RNA

Sample degradation is visible at low levels on the FlashGel® System for RNA. FlashGel® RNA Cassette run for 8 minutes at 225 V, followed by 20 minute hold prior to imaging. Lane 1: RNA Marker (Lonza); Lane 2: 250 ng E. coli total RNA; Lanes 3 – 7: E. coli Total RNA incubated with increasing levels of RNase A. Intact, denatured RNA shows sharp, clear bands on the FlashGel® System. Partially degraded RNA shows a smeared appearance, and completely degraded RNA appears as a low molecular weight smear.
Quick check of native RNA on the FlashGel® System for RNA

FlashGel® RNA Cassette run for 4 minutes at 225 V, followed by immediate imaging. Lanes 1 – 4: native samples prepared with FlashGel® Loading Dye. Lanes 5 – 8: samples denatured with formaldehyde at 65°C for 5 minutes prior to loading. Lanes 1, 2, 5 and 6 contain a 1.25 μl load. Lanes 3, 4, 7 and 8 contain a 5 μl load.

Comparison of Agarose Gels for RNA

<table>
<thead>
<tr>
<th>Panel</th>
<th>Gel</th>
<th>Sample preparation</th>
<th>Run conditions</th>
<th>Total time</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>FlashGel® System for RNA</td>
<td></td>
<td>8 min run at 225 V. Cassette held at ambient temp for 20 min prior to imaging.</td>
<td>28 min</td>
</tr>
<tr>
<td>B</td>
<td>Reliant® 1.25% MOPS Gel (Cat. #54922)</td>
<td>Formaldehyde Sample Buffer (Cat. #50571) Samples denatured 5 Min. at 65°C.</td>
<td>60 min run at 5 V/cm. 60 min post-stain with GelStar® Stain (Cat. #50535) 1:5000 dilution</td>
<td>120 min</td>
</tr>
<tr>
<td>C</td>
<td>Reliant® 1.25% MOPS Gel</td>
<td></td>
<td>60 min run at 5 V/cm. 60 min EtBr post-stain and 60 min de-stain.</td>
<td>108 min</td>
</tr>
<tr>
<td>D</td>
<td>Hand-cast 1% SeaKem® LE Agarose Gel (Cat. #50002)</td>
<td>10 μg/ml EtBr added to Formaldehyde Sample Buffer. Samples denatured 5 Min. at 65°C.</td>
<td>Denaturing gel prepared at 2.2 M Formaldehyde in 1X MOPS Buffer (Cat. #50876). 60 min run at 6 V/cm.</td>
<td>120 min</td>
</tr>
</tbody>
</table>

Performance of RNA analysis in four agarose gel systems. Sample of RNA Marker (Cat. #50575) lanes 1 & 2, and E. coli Total RNA (Ambion) lanes 3 & 4 contain 50 ng (lanes 1 & 3) or 250 ng (lanes 2 & 4) of RNA per 5 μl load.

Specifications

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Separation range:</td>
<td>0.5 kb – 9 kb</td>
</tr>
<tr>
<td>Storage:</td>
<td>Room temperature for 3 months from date of manufacture. Shelf life may be extended with refrigerated storage.</td>
</tr>
<tr>
<td>Well volume:</td>
<td>12+1 well: 5 μl</td>
</tr>
<tr>
<td></td>
<td>16+1 well: 5 μl</td>
</tr>
<tr>
<td>Gel size:</td>
<td>70 mm (L) X 84 mm (W) X 2 mm (H)</td>
</tr>
<tr>
<td>Cassette size:</td>
<td>115 mm (L) X 107 mm (W) X 17 mm (H)</td>
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<tr>
<td>Dock size:</td>
<td>134 mm (L) X 120 mm (W) X 54 mm (H)</td>
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See page 25 for ordering information.

Related Products

<table>
<thead>
<tr>
<th>Product Description</th>
<th>Code</th>
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<tbody>
<tr>
<td>FlashGel® System for Recovery</td>
<td>20</td>
</tr>
<tr>
<td>FlashGel® System for DNA</td>
<td>18</td>
</tr>
<tr>
<td>FlashGel® Camera</td>
<td>24</td>
</tr>
</tbody>
</table>
Capture data from The FlashGel® System and say goodbye to darkrooms and UV light. Complete separation and documentation safely, at your bench in minutes. This simple digital camera in an enclosed hood connects directly to your laptop or PC via USB.

Now you can visualize band migration on The FlashGel® Dock or via image projected to your computer screen. Simply click a button to capture the desired image to a file.

- **Real time separation and documentation**
  - Complete your gel run and image capture in just 5 minutes
  - Watch DNA migrate — in the FlashGel Dock or on your computer screen
  - Photograph gels at the bench — without UV light

- **The FlashGel® Camera offers**
  - Sharp, clear high-resolution images
  - Simple user interface
  - Small, compact design
  - Optimized exposure for FlashGel® Cassettes

**FlashGel® Camera**

**Camera Specifications**

| Hood dimensions:            | 10 cm (W) x 11 cm (L) x 16 cm (H) |
| Camera type:               | Digital                           |
| Image file type:           | .jpg, .tif, .pdf                  |

See page 25 for ordering information.

**Simple user interface right from your laptop or PC**
Ordering Information

Purchase components of the FlashGel® System separately, as a complete system or as starter kits for DNA, RNA or recovery applications.

**FlashGel® System**

Ordering Information

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description/Size</th>
<th>Price</th>
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<tbody>
<tr>
<td>57025</td>
<td>Each</td>
<td>$399</td>
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<td>57040</td>
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<td>$545</td>
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<tr>
<td>57067</td>
<td>Each</td>
<td>$990</td>
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</table>

**FlashGel® Camera**

For use with all FlashGel® Cassette types.

**FlashGel® Dock**

57025 Each $399

**FlashGel® System**

57067 Each $990

Includes: FlashGel® Dock; FlashGel® Camera; 9 pk FlashGel® DNA Cassettes [1.2%, 12+1 well single-tier]; FlashGel® Loading Dye and DNA Marker.

**FlashGel® System for DNA**

Ordering Information

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description/Size</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>57023</td>
<td>1.2% agarose, 12+1 single-tier</td>
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</tr>
<tr>
<td>57029</td>
<td>1.2% agarose, 16+1 double-tier</td>
<td>$99</td>
</tr>
<tr>
<td>57031</td>
<td>2.2% agarose, 12+1 single-tier</td>
<td>$99</td>
</tr>
<tr>
<td>57032</td>
<td>2.2% agarose, 16+1 double-tier</td>
<td>$99</td>
</tr>
<tr>
<td>50462</td>
<td>5 × 1 ml</td>
<td>$126</td>
</tr>
</tbody>
</table>

**FlashGel® Loading Dye (5X)**

[contains xylene cyanol]

50462 5 × 1 ml $126

**FlashGel® DNA Marker, 50 bp – 1.5 kb**

Ready-to-Load [Recommended for 2.2% cassettes], 50 applications

57033 500 μl $114

**FlashGel® DNA Marker, 100 bp – 3 kb**

Ready-to-Load [Recommended for double-tier cassettes], 50 applications

57034 500 μl $114

**FlashGel® DNA Marker, 100 bp – 4 kb**

Ready-to-Load [Recommended for 1.2% cassettes], 50 applications

50473 500 μl $114

**FlashGel® QuantLadder, 100 bp (3 ng) – 1.5 kb (30 ng)**

Ready-to-Load, 50 applications

50475 250 μl $114

**FlashGel® DNA Starter Kit**

Includes FlashGel® Dock; FlashGel® Loading Dye; FlashGel® DNA Cassettes, 1.2%, 12 + 1 well single-tier, 9 pk; FlashGel® DNA Marker 100 bp – 4 kb.

57026 Each $497

See page 97 for detailed marker sizes.

**FlashGel® System for Recovery**

Ordering Information

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description/Size</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>57051</td>
<td>1.2% agarose, 8+1 double-tier</td>
<td>Inquire*</td>
</tr>
</tbody>
</table>

**FlashGel® Recovery Buffer**

57060 2 x 500 μl $114

**FlashGel® QuantLadder 100 bp (3 ng) – 1.5 kb (30 ng)**

Ready-to-Load, 50 applications

50475 250 μl $114

**FlashGel® Recovery Starter Kit**

Includes FlashGel® Recovery Cassettes, 1.2%, 8+1 well double-tier, 9 pk; FlashGel® Loading Dye; FlashGel® Recovery Buffer; FlashGel® QuantLadder; Visualization Glasses; Control Fragment. Dock sold separately.

57050 Each $114

*Available soon.

**FlashGel® System for RNA**

Ordering Information

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description/Size</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>57027</td>
<td>1.2% agarose, 12+1 single-tier</td>
<td>$99</td>
</tr>
<tr>
<td>57028</td>
<td>1.2% agarose, 16+1 double-tier</td>
<td>$99</td>
</tr>
<tr>
<td>50577</td>
<td>50 μg (1 μg/ml)</td>
<td>$136</td>
</tr>
</tbody>
</table>

**Formaldehyde Sample Buffer**

RNA denaturing sample buffer [contains bromophenol blue and xylene cyanol]

50571 5 × 1 ml $39

**FlashGel® Loading Dye**

For native RNA samples [contains xylene cyanol]

50462 5 × 1 ml, 5X concentrate $126

**FlashGel® RNA Marker, 0.5 kb – 9 kb**

50577 50 μg (1 μg/ml) $136

**AccuGENE® Molecular Biology Water**

RNase Free

51200 1 L $26

**FlashGel® System for RNA Starter Pack**

Includes FlashGel® RNA Cassettes 1.2%, 12 + 1 well, Single-tier, 9 pk; Sample Buffer, RNA Marker, and Molecular Biology Water. Dock sold separately.

57024 Each $264

NOTE: Due to varying storage requirements, kit components may arrive in separate shipping containers.
Reliant® Minigels

Versatile minigels for routine DNA separation and recovery

Reliant® Gels are versatile and convenient minigels for nearly any application. Each gel is precision manufactured for rapid and reproducible resolution of DNA sizes from 8 bp to 10 kb. Reliant® Gels are available in a variety of well formats and agarose concentrations, in TAE and TBE Buffer and most are pre-stained with ethidium bromide.

— Manufactured with high quality SeaKem® and NuSieve® Agarose for reliability
— Compatible with most minigel chambers
— Versatile format options

Ordering Information

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Stain</th>
<th>Buffer</th>
<th>Separation Range (bp)</th>
<th>Agarose</th>
<th>Price</th>
</tr>
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<tbody>
<tr>
<td>54801</td>
<td>No Stain</td>
<td>TAE</td>
<td>400 fi 10,000</td>
<td>1% SeaKem® Gold Agarose</td>
<td>$121</td>
</tr>
<tr>
<td>54803</td>
<td>Ethidium bromide (0.5 μg/ml)</td>
<td>TAE</td>
<td>400 fi 10,000</td>
<td>1% SeaKem® Gold Agarose</td>
<td>$121</td>
</tr>
<tr>
<td>54925</td>
<td>Ethidium bromide (0.5 μg/ml)</td>
<td>TAE</td>
<td>20 fi 1,000</td>
<td>4% NuSieve® 3:1 Plus Agarose</td>
<td>$121</td>
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<tr>
<td>54903</td>
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<td>TBE</td>
<td>300 fi 8,000</td>
<td>1% SeaKem® Gold Agarose</td>
<td>$121</td>
</tr>
<tr>
<td>54927</td>
<td>Ethidium bromide (0.5 μg/ml)</td>
<td>TBE</td>
<td>8 fi 1,000</td>
<td>4% NuSieve® 3:1 Plus Agarose</td>
<td>$121</td>
</tr>
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</table>

Reliant® Gel – 12 well

20 gels/box

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Stain</th>
<th>Buffer</th>
<th>Separation Range (bp)</th>
<th>Agarose</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>54921</td>
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<td>TAE</td>
<td>400 fi 10,000</td>
<td>1% SeaKem® Gold Agarose</td>
<td>$121</td>
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<tr>
<td>54920</td>
<td>Ethidium bromide (0.5 μg/ml)</td>
<td>TBE</td>
<td>300 fi 8,000</td>
<td>1% SeaKem® Gold Agarose</td>
<td>$121</td>
</tr>
<tr>
<td>54925</td>
<td>Ethidium bromide (0.5 μg/ml)</td>
<td>TBE</td>
<td>100 fi 3,000</td>
<td>2% SeaKem® Gold Agarose</td>
<td>$121</td>
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<tr>
<td>54923</td>
<td>Ethidium bromide (0.5 μg/ml)</td>
<td>TBE</td>
<td>8 fi 1,000</td>
<td>4% NuSieve® 3:1 Plus Agarose</td>
<td>$121</td>
</tr>
</tbody>
</table>

Reliant® Gel – 24 well

20 gels/box

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Stain</th>
<th>Buffer</th>
<th>Separation Range (bp)</th>
<th>Agarose</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>54905</td>
<td>Ethidium bromide (0.5 μg/ml)</td>
<td>TBE</td>
<td>300 fi 8,000</td>
<td>1% SeaKem® Gold Agarose</td>
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<tr>
<td>54813</td>
<td>Ethidium bromide (0.5 μg/ml)</td>
<td>TBE</td>
<td>100 fi 3,000</td>
<td>2% SeaKem® Gold Agarose</td>
<td>$121</td>
</tr>
<tr>
<td>54929</td>
<td>Ethidium bromide (0.5 μg/ml)</td>
<td>TBE</td>
<td>8 fi 1,000</td>
<td>4% NuSieve® 3:1 Plus Agarose</td>
<td>$121</td>
</tr>
</tbody>
</table>

Reliant® Gel – 20 well

20 gels/box

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Stain</th>
<th>Buffer</th>
<th>Separation Range (bp)</th>
<th>Agarose</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>54938</td>
<td>No Stain</td>
<td>TBE</td>
<td>100 fi 3,000</td>
<td>2% SeaKem® Gold Agarose</td>
<td>$121</td>
</tr>
<tr>
<td>54944</td>
<td>No Stain</td>
<td>TBE</td>
<td>8 fi 1,000</td>
<td>4% NuSieve® 3:1 Plus Agarose</td>
<td>$121</td>
</tr>
<tr>
<td>54907</td>
<td>Ethidium bromide (0.5 μg/ml)</td>
<td>TBE</td>
<td>300 fi 8,000</td>
<td>1% SeaKem® Gold Agarose</td>
<td>$121</td>
</tr>
<tr>
<td>54939</td>
<td>Ethidium bromide (0.5 μg/ml)</td>
<td>TBE</td>
<td>100 fi 3,000</td>
<td>2% SeaKem® Gold Agarose</td>
<td>$121</td>
</tr>
<tr>
<td>54928</td>
<td>Ethidium bromide (0.5 μg/ml)</td>
<td>TBE</td>
<td>8 fi 1,000</td>
<td>4% NuSieve® 3:1 Plus Agarose</td>
<td>$121</td>
</tr>
</tbody>
</table>

Visit www.lonza.com/research or contact Scientific Support to inquire about custom precast gels.

Specifications

- Gels per box: 20
- Gel dimensions: 6.0 cm × 9.5 cm
- Gel thickness: 5.5 mm
- Tray dimensions: 6.8 cm × 10.2 cm
- Well volume: <15 μl
Reliant® Minigels

Continued

Performance of Reliant® Minigels

Panel A 20 bp Ladder (1 μl), 100 bp Ladder (1 μl) and 50-1000 bp Marker (2.5 μl) (all Lonza), loaded and run in a 4% NuSieve® 3:1 Plus Reliant® Gel containing ethidium bromide. Gel was run at 7 V/cm for 50 minutes using 1X TBE Buffer containing 0.5 μg/ml ethidium bromide.

Panel B. A repeating pattern of 500 bp DNA Ladder (1 μl/lane) and 1-10 kb DNA Marker (2.5 μl/lane) (Lonza) run in a 1% SeaKem® Gold Reliant® Gel containing ethidium bromide. Gel was run at 5 V/cm for 60 minutes using 1X TAE Buffer containing 0.5 μg/ml ethidium bromide.

Applications
- DNA analysis
- Restriction digests
- Recovery
- PCR
- RT-PCR
- Cloning
- Blotting

Performance and Quality Tests
- DNase: No activity detected
- Gel performance: Sharp bands and low background fluorescence

Storage Conditions
18°C – 26°C for 6 – 12 months depending upon agarose concentration

Application Support
- Loading and Running DNA in Agarose Gels, Section III, page 89
- Detection and Sizing of DNA in Agarose Gels, Section IV, page 95

Related Products

DNA Ladders and Markers 36
GelStar® and SYBR® Green Nucleic Acid Gel Stains 38 & 39
Latitude® HT Precast Agarose Gels are large format agarose gels designed for high throughput screening applications. These gels are precision manufactured for rapid, reproducible resolution of DNA sizes from 8 bp to 10 kb. Latitude® HT Gels are available in multiple well formats (from 100 – 200 wells) and agarose concentrations, in TAE and TBE Buffer, all prestained with ethidium bromide.

— Manufactured with high quality SeaKem® or NuSieve® Agarose for reliability
— Versatile design allows you to run gels in most large submerged electrophoresis systems
— Multichannel pipette compatible

### Resolution of DNA markers in a Latitude® HT Precast Agarose Gel

Alternate loads of 50 - 1000 bp Marker and 100 bp Ladder (Lonza) run in a 2% SeaKem® LE Plus Agarose Gel in 1X TBE buffer containing 0.5 μg/ml ethidium bromide. Gels run at 6 V/cm, 1 hour run using the TruBand® Anchor.

### Ordering Information

For optimal performance, use the TruBand® Anchor with Latitude® HT Gels, see page 29.

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Stain</th>
<th>Buffer</th>
<th>Separation Range (bp)</th>
<th>Agarose</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>57204</td>
<td>Ethidium bromide (0.5 μg/ml)</td>
<td>TAE</td>
<td>400 to 10,000</td>
<td>1% SeaKem® LE Plus Agarose</td>
<td>$1.32</td>
</tr>
<tr>
<td>57206</td>
<td>Ethidium bromide (0.5 μg/ml)</td>
<td>TAE</td>
<td>100 to 3,000</td>
<td>2% SeaKem® LE Plus Agarose</td>
<td>$1.32</td>
</tr>
<tr>
<td>57224</td>
<td>Ethidium bromide (0.5 μg/ml)</td>
<td>TBE</td>
<td>300 to 8,000</td>
<td>1% SeaKem® LE Plus Agarose</td>
<td>$1.32</td>
</tr>
<tr>
<td>57225</td>
<td>Ethidium bromide (0.5 μg/ml)</td>
<td>TBE</td>
<td>8 to 1,000</td>
<td>4% NuSieve® 3:1 Plus Agarose</td>
<td>$1.32</td>
</tr>
<tr>
<td>57226</td>
<td>Ethidium bromide (0.5 μg/ml)</td>
<td>TBE</td>
<td>100 to 2,000</td>
<td>2% SeaKem® LE Plus Agarose</td>
<td>$1.32</td>
</tr>
</tbody>
</table>

Visit www.lonza.com/research or contact Scientific Support to inquire about custom precast gels.
Latitude® HT Gels

Continued

Applications
- High-throughput DNA analysis
- Restriction digests
- PCR
- Multiplex PCR
- RT-PCR
- Genotyping
- Fingerprinting
- Quality control for sequencing or microarray construction
- Library construction

Performance and Quality Tests
- DNase: No activity detected
- Gel performance: Sharp bands and low background fluorescence

Specifications
| Gels per box: | 5 |
| Gel dimensions: | 24 cm × 14 cm |
| Gel thickness: | 6.5 mm |
| Ethidium Bromide: | 0.5 μg/ml |
| Tray dimensions: | 25 cm × 15 cm |
| Well volume: | 10 μl – 12 μl for 50 well gels
25 μl – 30 μl for 25 well gels |

Chamber Compatibility Information
- Latitude® HT Gels fit most large submerged electrophoresis systems. We recommend the following systems:
  - Owl Centipede™ Horizontal System
  - Owl Millipede™ Horizontal System
  - FisherBiotech® Wide Format System FB-SB-2318
  - Bio-Rad® Sub-Cell® Models 96 and 192
  - Shelton JSB-96

Storage Conditions
18°C – 26°C for 6 – 12 months depending upon agarose concentration

Application Support
- Loading and Running DNA in Agarose Gels, Section III, page 89
- Detection and Sizing of DNA in Agarose Gels, Section IV, page 95

Ordering Information
Latitude® HT Precast Agarose Gels — Supporting Products

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>56991</td>
<td>Owl Millipede™, Shelton JSB-96, Fisher SB-2318</td>
<td>$39</td>
</tr>
<tr>
<td>56993</td>
<td>Other standard chambers</td>
<td>$39</td>
</tr>
</tbody>
</table>

DNA Loading Buffer (6X) (contains bromophenol blue and xylene cyanol)
| 50655   | 5 × 1 ml | $30   |

AccuGENE® 50X TAE Buffer
| 51216   | 1 L | $67   |

AccuGENE® 10X TAE Buffer
| 50844   | 1 L | $29   |
| 50841   | 4 L | $87   |

AccuGENE® 10X TBE Buffer
| 50843   | 1 L | $20   |
| 50840   | 4 L | $56   |
| 50837   | 10 L | $127  |
| 50838   | 20 L | $234  |

AccuGENE® 5X TBE Buffer
| 50839   | 4 L | $38   |
| 50835   | 10 L | $79   |
| 50836   | 20 L | $152  |

Related Products
DNA Ladders and Markers 36

Ordering Information
Latitude® HT Pre-cast Agarose Gels — Supporting Products

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>56991</td>
<td>Owl Millipede™, Shelton JSB-96, Fisher SB-2318</td>
<td>$39</td>
</tr>
<tr>
<td>56993</td>
<td>Other standard chambers</td>
<td>$39</td>
</tr>
</tbody>
</table>

DNA Loading Buffer (6X) (contains bromophenol blue and xylene cyanol)
| 50655   | 5 × 1 ml | $30   |

AccuGENE® 50X TAE Buffer
| 51216   | 1 L | $67   |

AccuGENE® 10X TAE Buffer
| 50844   | 1 L | $29   |
| 50841   | 4 L | $87   |

AccuGENE® 10X TBE Buffer
| 50843   | 1 L | $20   |
| 50840   | 4 L | $56   |
| 50837   | 10 L | $127  |
| 50838   | 20 L | $234  |

AccuGENE® 5X TBE Buffer
| 50839   | 4 L | $38   |
| 50835   | 10 L | $79   |
| 50836   | 20 L | $152  |

Related Products
DNA Ladders and Markers 36

1-800-638-8174 www.lonza.com/research

Precast Gels
Latitude® Precast Agarose Midigels are designed for high sample throughput DNA analysis applications requiring increased resolution distance. These gels are precision manufactured for rapid and reproducible resolution of DNA sizes from 8 bp to 10 kb. Latitude® Gels are available in a variety of well formats and agarose concentrations, in TAE and TBE Buffer.

- Manufactured with high quality SeaKem® or NuSieve® Agarose for reliability
- Latitude® Gels fit most midigel chambers and provide optimal performance in the Latitude® Chamber

Performance and Quality Tests
- DNase: No activity detected
- Gel performance: Sharp bands and low background fluorescence

Storage Conditions
18°C – 26°C for 6 – 12 months depending upon agarose concentration

Specifications
- Gels per box: 8
- Gel dimensions: 10 cm x 15 cm
- Gel thickness: 6.0 mm
- Ethidium Bromide: 0.5 μg/ml
- Tray dimensions: 10.4 cm x 15.6 cm
- Well volume: 10 μl – 12 μl

Ordering Information
For optimal performance, use Latitude® Gels with the TruBand® Anchor, see below.

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description</th>
<th>Stain Buffer</th>
<th>Separation Range (bp)</th>
<th>Agarose</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>57200</td>
<td>Latitude® Midigel – 20 Well</td>
<td>Ethidium bromide (0.5 μg/ml)</td>
<td>TAE</td>
<td>400 &gt; 10,000</td>
<td>1% SeaKem® LE Plus Agarose</td>
</tr>
<tr>
<td>57220</td>
<td>Latitude® Midigel – 20 Well</td>
<td>Ethidium bromide (0.5 μg/ml)</td>
<td>TBE</td>
<td>300 – 8,000</td>
<td>1% SeaKem® LE Plus Agarose</td>
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<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description</th>
<th>Stain Buffer</th>
<th>Separation Range (bp)</th>
<th>Agarose</th>
<th>Price</th>
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<tbody>
<tr>
<td>57210</td>
<td>Latitude® Midigel – 20 Well</td>
<td>Ethidium bromide (0.5 μg/ml)</td>
<td>TAE</td>
<td>400 &gt; 10,000</td>
<td>1% SeaKem® LE Plus Agarose</td>
</tr>
<tr>
<td>57211</td>
<td>Latitude® Midigel – 20 Well</td>
<td>Ethidium bromide (0.5 μg/ml)</td>
<td>TAE</td>
<td>100 – 3,000</td>
<td>2% SeaKem® LE Plus Agarose</td>
</tr>
<tr>
<td>57220</td>
<td>Latitude® Midigel – 20 Well</td>
<td>Ethidium bromide (0.5 μg/ml)</td>
<td>TBE</td>
<td>300 – 8,000</td>
<td>1% SeaKem® LE Plus Agarose</td>
</tr>
<tr>
<td>57230</td>
<td>Latitude® Midigel – 20 Well</td>
<td>Ethidium bromide (0.5 μg/ml)</td>
<td>TBE</td>
<td>100 – 2,000</td>
<td>2% SeaKem® LE Plus Agarose</td>
</tr>
<tr>
<td>57231</td>
<td>Latitude® Midigel – 20 Well</td>
<td>Ethidium bromide (0.5 μg/ml)</td>
<td>TBE</td>
<td>8 – 1,000</td>
<td>4% NuSieve® 3:1 Plus Agarose</td>
</tr>
</tbody>
</table>

Visit www.lonza.com/research or contact Scientific Support to inquire about custom precast gels.

Performance of the 40 well Latitude® Precast Agarose Midigels

Alternate loads of 100 bp DNA Ladder and Lonza 20 bp DNA Ladder (Lonza) [1 μl marker/lane] run in a 4% NuSieve® 3:1 Plus Agarose Gel in 1X TBE Buffer containing 0.5 μg/ml Ethidium Bromide.
6 V/cm, 70 minute run in a 10 cm x 15 cm Latitude® Gel Chamber using the TruBand® Gel Anchor.
PAGEr® Gold TBE Gels

Polyacrylamide minigels for DNA separation

PAGEr® Gold TBE Precast Gels provide fine resolution of DNA fragments < 2,000 bp, and are optimal for resolving 1% differences in DNA fragment size. These ready-to-use gels are specially designed for maximum user convenience. Opening the cassette requires a simple snap of the comb.

— Easy-to-load: unique gold colored cassette and marked lanes are easy-to-see
— Easy-to-open: simple snap-open cassette does not require a special opening device

Separation ranges for nucleic acids in PAGEr® Gold TBE Gels

<table>
<thead>
<tr>
<th>Polyacrylamide Concentration</th>
<th>Size Separation Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>6%</td>
<td>75 bp – 2,000 bp</td>
</tr>
<tr>
<td>10%</td>
<td>30 bp – 1,000 bp</td>
</tr>
<tr>
<td>4-20%</td>
<td>10 bp – 2,000 bp</td>
</tr>
</tbody>
</table>

Applications

— Fine resolution of PCR products
— Oligo analysis

Performance and Quality Tests

— Each lot of PAGEr® Gold TBE Gels is functionally tested
— Certificate of Analysis available upon request

Specifications

<table>
<thead>
<tr>
<th>Gels per box:</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gel dimensions:</td>
<td>8.3 cm × 7.1 cm × 0.1 cm</td>
</tr>
<tr>
<td></td>
<td>8.3 cm × 8.3 cm × 0.1 cm</td>
</tr>
<tr>
<td>Cassette thickness:</td>
<td>0.49 cm (9 cm × 10 cm tray)</td>
</tr>
<tr>
<td></td>
<td>0.55 cm (10 cm × 10 cm tray)</td>
</tr>
<tr>
<td>Cassette dimensions:</td>
<td>9 cm × 10 cm (L × W)</td>
</tr>
<tr>
<td></td>
<td>10 cm × 10 cm (L × W)</td>
</tr>
<tr>
<td>Well volume:</td>
<td>10 well – 32 μl</td>
</tr>
<tr>
<td></td>
<td>12 well – 20 μl</td>
</tr>
<tr>
<td></td>
<td>16 well – 14 μl</td>
</tr>
</tbody>
</table>

Storage Conditions

2°C – 8°C for 3.5 months from date of manufacture

Application Support

— Separation of DNA in Polyacrylamide Gels, Section VII, page 125

Ordering Information

<table>
<thead>
<tr>
<th>Description</th>
<th>Cassette size</th>
<th>10 well</th>
<th>12 well</th>
<th>16 well</th>
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</thead>
<tbody>
<tr>
<td>PAGEr® Gold TBE Gels</td>
<td>6%</td>
<td>9 × 10 cm</td>
<td>58525</td>
<td>58528</td>
</tr>
<tr>
<td></td>
<td>10%</td>
<td>9 × 10 cm</td>
<td>58526</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>4-20% gradient</td>
<td>9 × 10 cm</td>
<td>58527</td>
<td>58530</td>
</tr>
</tbody>
</table>

Performance of PAGEr® Gold TBE Gels

Alternating lanes of the 50 bp-1,000 bp DNA Marker (2 μl/lane) and 100 bp DNA Ladder (1 μl/lane) (Lonza) separated on a 4–20% PAGEr® Gold TBE Gel. Gel run at 200 V for 60 minutes, stained for 15 minutes in 0.5 μg/ml EtBr, and destained for 5 minutes.

See page 72 for chamber compatibility.

Related Products

| AccuGENE® Buffers | 41 |
| DNA Ladders and Markers | 36 |
| DNA Loading Buffer     | 41 |
| GelStar® Nucleic Acid Gel Stain | 38 |
| PAGEr® Minigel Chamber | 50 |
Reliant® and Latitude® Precast RNA Gels

Reliant® and Latitude® Precast RNA Agarose Gels are precision cast in 1.25% SeaKem® Gold Agarose with MOPS buffer and are guaranteed RNase-free. These gels are ideal for Northern blotting and analysis of RNA transcripts. Our RNA markers, stains, and buffers are designed to optimize RNA separations.

Ordering Information

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Buffer Description</th>
<th>No. of Wells</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reliant® RNA Gel System 20 minigels/box</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>54922</td>
<td>MOPS 1.25% SeaKem® Gold for RNA</td>
<td>8 well</td>
<td>$144</td>
</tr>
<tr>
<td>54948</td>
<td>MOPS 1.25% SeaKem® Gold for RNA</td>
<td>20 well (landscape)</td>
<td>$144</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Gel Description</th>
<th>Sample Buffer Description</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reliant® RNA Analysis Kits</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>54975</td>
<td>8 well Reliant® RNA Gel</td>
<td>Formaldehyde Sample Buffer</td>
<td>$428</td>
</tr>
<tr>
<td>54976</td>
<td>20 well Reliant® RNA Gel</td>
<td>Formaldehyde Sample Buffer</td>
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<tr>
<td>54977</td>
<td>8 well Reliant® RNA Gel</td>
<td>Glyoxal Sample Buffer</td>
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</tr>
<tr>
<td>54978</td>
<td>20 well Reliant® RNA Gel</td>
<td>Glyoxal Sample Buffer</td>
<td>$428</td>
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</table>

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Buffer Description</th>
<th>No. of Wells</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latitude® RNA Midigels 8 midigels/box</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>57237</td>
<td>MOPS 1.25% SeaKem® Gold</td>
<td>2 × 20 well</td>
<td>$156</td>
</tr>
<tr>
<td>57238</td>
<td>MOPS 1.25% SeaKem® Gold</td>
<td>1 × 20 well</td>
<td>$156</td>
</tr>
</tbody>
</table>

NOTE: Due to varying storage requirements, kit components may arrive in separate shipping containers.

Applications
- Northern blotting
- RNA electrophoresis
- RNA integrity checks

Performance and Quality Tests
- Agarose: No RNase activity detected
- Gel performance: Sharp RNA bands and low background fluorescence are visible with ethidium bromide, SYBR® Green II and GelStar® Nucleic Acid Gel Stains

Application Support
- Separation of RNA in Agarose Gels, Section VIII, page 131

Resolution of RNA Markers run in a Reliant® RNA Gel

Gel loaded with samples of RNA Marker 0.5 kb - 9 kb. Marker loaded at 200 ng (lanes 3 and 6) and 1 μg (lanes 1, 4, 5 and 8). Gel run at 5 V/cm for 2 hours using AccuGENE® MOPS Buffer (1X). RNA stained for 30 minutes using GelStar® Nucleic Acid Gel Stain (1:10,000 dilution).
Sample Buffers

These ready-to-use buffers are used for denaturation of RNA samples for electrophoresis on Reliant® and Latitude® Precast RNA Gels, and are ideal for Northern blotting.

- **Storage Conditions**
  - Glyoxal: 18°C – 26°C for 12 months; storage at 4°C will extend the stability to 2 years
  - Formaldehyde: -20°C for 12 months

AccuGENE® 10X MOPS Buffer

AccuGENE® 10X MOPS Buffer is specially formulated for use with Latitude® and Reliant® Precast Gels for RNA electrophoresis. It is manufactured with the same reagents used in our precast gels. The buffer contains 0.2 M MOPS (free acid), 0.05 M sodium acetate, 0.01 M EDTA (disodium salt), and 0.01 M EGTA (free acid), pH 7.0.

- **Storage Conditions**
  - 18°C – 24°C

RNA Marker 0.5-9 kb

RNA Markers 0.5-9 kb, are suitable for sizing single stranded RNA in glyoxal or formaldehyde denaturing systems. The RNA Marker consists of ten RNA transcripts: 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, and 9 kb in length. Markers can be denatured with standard procedures, and visualized on Northern blots with labeled lambda sequence. Detect: 4 μg with ethidium bromide, or smaller quantities with GelStar® or SYBR® Green II Gel Stains.

- **Storage Conditions**
  - -80°C for 24 months or -20°C for 6 months

---

Ordering Information

<table>
<thead>
<tr>
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<th>Description</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glyoxal Sample Buffer</strong></td>
<td>Ideal for Northern blotting</td>
<td></td>
</tr>
<tr>
<td>50560</td>
<td>1.7 ml</td>
<td>$43</td>
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<tr>
<td><strong>Formaldehyde Sample Buffer</strong></td>
<td>Ideal for visualization/analysis contains bromophenol blue and xylene cyanol</td>
<td></td>
</tr>
<tr>
<td>50571</td>
<td>5 × 1 ml</td>
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Ordering Information

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<th>Description</th>
<th>Price</th>
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<tr>
<td><strong>AccuGENE® 10X MOPS Buffer</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50876</td>
<td>1 L</td>
<td>$60</td>
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Ordering Information

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<tr>
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<th>Description</th>
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</thead>
<tbody>
<tr>
<td><strong>RNA Marker 0.5 - 9 kb</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50575</td>
<td>50 μg (1 μg/μl)</td>
<td>$133</td>
</tr>
</tbody>
</table>

---

**Resolution of RNA Markers run in a Reliant® RNA Gel**

- 9 kb
- 6 kb
- 4 kb
- 3 kb
- 2.5 kb
- 2 kb
- 1.5 kb
- 1 kb
- 0.5 kb

---

Related Products

| FlashGel® System for RNA | 22 |
GelStar® Nucleic Acid Gel Stain

GelStar® Nucleic Acid Gel Stain is a fast-acting, fluorescent stain that is up to fifteen times more sensitive than Ethidium Bromide for RNA detection.

- Detects 3 ng of RNA or 20 pg of dsDNA

RNA Detection with GelStar® Stain

Samples of *E. coli* total RNA were denatured using the following denaturants: Lane A: Formaldehyde/Formamide; Lane B: Formamide; Lane C: Glyoxal. Samples were loaded at 2 μg/lane for the formaldehyde/formamide and formamide only denatured samples, and 4 μg/lane for the glyoxal denatured samples. Reliant® RNA Precast Agarose Gels were run at 7 V/cm for 40 minutes in 1X MOPS Buffer and post stained with GelStar® Gel Stain and photographed on the Clare Chemical Research, Inc., Dark Reader® Transilluminator.

SYBR® Green II Nucleic Acid Gel Stain

SYBR® Green II Nucleic Acid Gel Stain is a highly sensitive fluorescent stain that is ideal for detection of RNA.

- Detects 2 ng of RNA or 100 pg of dsDNA

Storage Conditions

-20°C for stain
18°C – 26°C for photographic filter

RNA Detection with SYBR® Green II Stain

Samples of *E. coli* total RNA were denatured using the following denaturants: Lane A: Formaldehyde/Formamide; Lane B: Formamide; Lane C: Glyoxal. Samples were loaded at 2 μg/lane for the formaldehyde/formamide and formamide only denatured samples, and 4 μg/lane for the glyoxal denatured samples. Reliant® RNA Precast Agarose Gels were run at 7 V/cm for 40 minutes in 1X MOPS Buffer and post stained with SYBR® Green II Gel Stain and photographed on the Clare Chemical Research, Inc., Dark Reader® Transilluminator.

Ordering Information

### GelStar® Nucleic Acid Gel Stain

<table>
<thead>
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<th>Cat. No.</th>
<th>Description</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>50535</td>
<td>2 x 250 μl</td>
<td>$146</td>
</tr>
<tr>
<td></td>
<td>Product licensed from Molecular Probes, Inc.</td>
<td></td>
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</tbody>
</table>

### GelStar® Gel Stain Photographic Filter

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description</th>
<th>Price</th>
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<tbody>
<tr>
<td>50536</td>
<td>3 inch square</td>
<td>$58</td>
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<td></td>
<td>(Wratten® #9)</td>
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### SYBR® Green II Nucleic Acid Gel Stain

<table>
<thead>
<tr>
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<th>Description</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>50523</td>
<td>10 x 50 μl</td>
<td>$212</td>
</tr>
<tr>
<td>50522</td>
<td>2 x 500 μl</td>
<td>$379</td>
</tr>
<tr>
<td></td>
<td>Product licensed from Molecular Probes, Inc.</td>
<td></td>
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</table>

### SYBR® Green Gel Stain Photographic Filter

<table>
<thead>
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<th>Cat. No.</th>
<th>Description</th>
<th>Price</th>
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</thead>
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<td>50530</td>
<td>3 inch square</td>
<td>$58</td>
</tr>
<tr>
<td></td>
<td>(Wratten® #15)</td>
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</table>
Great performance starts with high quality agarose and gels, but for complete assurance, you need to use high quality markers, ladders, stains, and buffers. Lonza supports a broad offering of products that complement and match the performance of our agarose and precast gels.

Rapidly estimating fragment size requires clear sharp banding patterns on each and every gel. We offer two types of ladders and markers: Standard and SimplyLoad®. Standard Markers and Ladders are ready to dilute prior to loading your gel, while our convenient SimplyLoad® Ladders are premixed, ready for direct loading. Our DNA quantitation ladders are ideal for the accurate estimation of molecular mass of fragments from 10 ng to 100 ng.

Seeing all of your data is critical to the overall success of your experiment. GelStar® Nucleic Acid Gel Stain clearly detects fragments down to 20 pg of DNA. Maximize your performance by adding the stain directly to your gel prior to casting or post-stain your gel. We also offer SYBR® Green Nucleic Acid Gel Stains.

Finally, Lonza offers a complete line of AccuGENE® Electrophoresis and Molecular Biology Buffers to support your research. Our AccuGENE® Buffers are formulated to optimize performance of our agarose and precast gel products.
DNA Ladders and Markers

Sizing made easy

SimplyLoad® Ladders are supplied in ready-to-load concentrations.

See page 96 for detailed size information

1-800-638-8174  www.lonza.com/research
DNA Ladders and Markers

**Standard Ladders and Markers** are ready-to-dilute prior to loading on your gel. Plasmid-free to ensure minimal background.

**Ordering Information**

**Standard DNA Ladders**

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Volume</th>
<th>Description</th>
<th>Applications</th>
<th>Price</th>
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<tbody>
<tr>
<td>20 bp DNA Ladder</td>
<td>50330</td>
<td>150 μl</td>
<td>20 bp – 500 bp</td>
<td>150</td>
</tr>
<tr>
<td>20 bp Extended Range DNA Ladder</td>
<td>50320</td>
<td>150 μl</td>
<td>20 bp – 1,000 bp</td>
<td>100</td>
</tr>
<tr>
<td>100 bp DNA Ladder</td>
<td>50321</td>
<td>160 μl</td>
<td>100 bp – 1,000 bp</td>
<td>100</td>
</tr>
<tr>
<td>100 bp Extended Range DNA Ladder</td>
<td>50322</td>
<td>150 μl</td>
<td>100 bp – 3,000 bp</td>
<td>100</td>
</tr>
<tr>
<td>500 bp DNA Ladder</td>
<td>50323</td>
<td>300 μl</td>
<td>500 bp – 8,000 bp</td>
<td>200</td>
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**Standard Quantitation Ladders**

<table>
<thead>
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<th>Volume</th>
<th>Description</th>
<th>Applications</th>
<th>Price</th>
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</thead>
<tbody>
<tr>
<td>DNA QuantLadder</td>
<td>50334</td>
<td>125 μl</td>
<td>100 bp – 1,000 bp</td>
<td>50</td>
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<tr>
<td>DNA Reverse QuantLadder</td>
<td>50335</td>
<td>125 μl</td>
<td>100 bp – 1,000 bp</td>
<td>50</td>
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**Standard DNA Markers**

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Volume</th>
<th>Description</th>
<th>Applications</th>
<th>Price</th>
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</thead>
<tbody>
<tr>
<td>DNA Marker 50 – 1,000 bp</td>
<td>50461</td>
<td>250 μl</td>
<td>50 bp – 1,000 bp</td>
<td>50</td>
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<tr>
<td>DNA Marker 1 – 10 kb</td>
<td>50471</td>
<td>2 × 250 μl</td>
<td>1 kb – 10 kb</td>
<td>100</td>
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<tr>
<td>DNA Marker 50 – 2,500 bp</td>
<td>50631</td>
<td>250 μl</td>
<td>50 bp – 2,500 bp</td>
<td>50</td>
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</tbody>
</table>

**SimplyLoad® Ladders** are supplied ready-to-load on your gel. No need for mixing, heating or diluting prior to loading. Plasmid-free to ensure minimal background.

**Ordering Information**

**SimplyLoad® DNA Ladders**

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Volume</th>
<th>Description</th>
<th>Applications</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>SimplyLoad® 20 bp DNA Ladder</td>
<td>50331</td>
<td>500 μl</td>
<td>20 bp – 500 bp</td>
<td>100</td>
</tr>
<tr>
<td>SimplyLoad® 20 bp Extended Range DNA Ladder</td>
<td>50326</td>
<td>500 μl</td>
<td>20 bp – 1,000 bp</td>
<td>100</td>
</tr>
<tr>
<td>SimplyLoad® 100 bp DNA Ladder</td>
<td>50327</td>
<td>500 μl</td>
<td>100 bp – 1,000 bp</td>
<td>100</td>
</tr>
<tr>
<td>SimplyLoad® 100 bp Extended Range DNA Ladder</td>
<td>50328</td>
<td>500 μl</td>
<td>100 bp – 3,000 bp</td>
<td>100</td>
</tr>
<tr>
<td>SimplyLoad® 500 bp DNA Ladder</td>
<td>50329</td>
<td>500 μl</td>
<td>500 bp – 8,000 bp</td>
<td>100</td>
</tr>
<tr>
<td>SimplyLoad® Tandem DNA Ladder</td>
<td>50333</td>
<td>500 μl</td>
<td>100 bp – 12,000 bp</td>
<td>100</td>
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**SimplyLoad® Quantitation Ladders**

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Volume</th>
<th>Description</th>
<th>Applications</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>SimplyLoad® DNA QuantLadder</td>
<td>50336</td>
<td>250 μl</td>
<td>100 bp – 1,000 bp</td>
<td>100</td>
</tr>
<tr>
<td>SimplyLoad® DNA Reverse QuantLadder</td>
<td>50337</td>
<td>250 μl</td>
<td>100 bp – 1,000 bp</td>
<td>100</td>
</tr>
</tbody>
</table>

**Storage Conditions**

- Standard Ladders and Markers: 4°C or -20°C
- SimplyLoad® Ladders: 4°C

**Related Products**

| Agarose | 4 |
| Precast Gels | 17 |
GelStar® Nucleic Acid Gel Stain is a highly sensitive fluorescent stain for detecting both DNA and RNA.

Add GelStar® Stain to your agarose solution prior to casting, or post-stain your gels. GelStar® Stain exhibits exceptional signal-to-noise ratio with minimal background.

— Maximum sensitivity, detect as little as 20 pg of dsDNA or 3 ng of RNA
— Versatile, use for agarose or polyacrylamide gel electrophoresis, ideal alternative to silver staining
— Ultimate user flexibility, add GelStar® Stain prior to gel casting or post-stain, no destaining required
— Complete staining solution for all types of nucleic acids
— Detect fragments with either a standard 300 nm UV transilluminator or the Clare Chemical Research, Inc., Dark Reader® Transilluminator
— Document your results with either Polaroid® or CCD-based Camera Systems

GelStar® Stain versus Ethidium Bromide

Serial dilution of SimplyLoad® DNA QuantLadder on 2% Reliant® Precast Gels post-stained with 1X GelStar® Stain (left) or 0.5 μg/ml Ethidium Bromide (right) for 45 minutes.

Ordering Information

Cat. No. | Description | Price
---|---|---
50535 | GelStar® Nucleic Acid Gel Stain | $146
| Supplied as a 10,000X concentrated solution in DMSO. Product licensed from Molecular Probes, Inc.
---|---|---
50536 | GelStar® Gel Stain Photographic Filter | $58
| (Wratten® #9)

GelStar® Gel Stain Photographic Filter

— Use for optimal sensitivity with black and white film
— Suitable for use with most Polaroid® Documentation or Camera Systems

Applications

— DNA and RNA detection
— SSCP and heteroduplex analysis

Storage Conditions

-20°C for stain
18°C – 26°C for photographic filter

Application Support

— Detecting DNA with Gelstar® and SYBR® Green Nucleic Acid Stains. Section IV, pages 98,
— Detecting DNA in Polyacrylamide Gels with Gelstar® and SYBR® Green Nucleic Acid Stains, Section VII, page 127
— Detection of RNA in Agarose Gels, Section VIII, page 138

Related Products

<table>
<thead>
<tr>
<th>Product Description</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>AccuGENE® Buffers</td>
<td>41</td>
</tr>
<tr>
<td>Agarose</td>
<td>4</td>
</tr>
<tr>
<td>DNA Ladders</td>
<td>36</td>
</tr>
<tr>
<td>DNA Ladders</td>
<td>44</td>
</tr>
<tr>
<td>Precast Gels</td>
<td>17</td>
</tr>
</tbody>
</table>
SYBR® Green Nucleic Acid Gel Stains

SYBR® Green Nucleic Acid Gel Stains are fluorescent stains for detecting DNA and RNA, exhibiting excellent signal-to-noise ratio with minimal background. SYBR® Green Stains are more sensitive than standard stains, making them convenient alternatives to silver staining and radioisotopes. For maximum detection, gels should be post-stained and photographed with the SYBR® Green Photographic Filter.

SYBR® Green I Stain
— Detects as little as 60 pg of dsDNA and 1 ng oligonucleotides
— Optimal for PCR, apoptosis studies, and heteroduplex analysis

SYBR® Green II Stain
— Detects 100 pg of ssDNA and 2 ng of RNA
— Optimal for RNA gel electrophoresis and SSCP analysis

SYBR® Green Gel Stain Photographic Filter
— Required for optimal sensitivity with black and white film
— Suitable for use with most Polaroid® Systems

RNA Detection with SYBR® Green II Stain

DNA samples (pBR322 Msp I digest) ranging from 1 to 200 ng per lane were separated on a 10 cm × 16 cm × 0.1 cm, 45 vertical MetaPhor® Agarose gel prepared in 1X TBE Buffer. The gel was run for 1 hour at 488 V/cm. Following electrophoresis the gel was divided into two, and one half was stained with 1 μg/ml ethidium bromide while the other was stained with SYBR® Green I Stain (1:10,000 dilution of stock). Detection was achieved with standard 300 nm UV transillumination.

Applications
— DNA and RNA detection
— SSCP and heteroduplex analysis

Storage Conditions
-20°C for stain
18°C – 26°C for photographic filter

Ordering Information

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<thead>
<tr>
<th>Cat. No.</th>
<th>Size</th>
<th>Price</th>
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</thead>
<tbody>
<tr>
<td>SYBR® Green I Nucleic Acid Gel Stain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50513</td>
<td>10 × 50 μl</td>
<td>$212</td>
</tr>
<tr>
<td>50512</td>
<td>2 × 500 μl</td>
<td>$379</td>
</tr>
<tr>
<td>Supplied as a 10,000X concentrated solution in DMSO. Product licensed from Molecular Probes, Inc.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| SYBR® Green II Nucleic Acid Gel Stain |
| 50523    | 10 × 50 μl | $212   |
| 50522    | 2 × 500 μl | $379   |
| Supplied as a 10,000X concentrated solution in DMSO. Product licensed from Molecular Probes, Inc. |

| SYBR® Green Gel Stain Photographic Filter |
| 50530  | 3 inch square | $58   |
| (Wratten® #15) |

Related Products

AccuGENE® Buffers 41
Agarose 4
MDE® Gel Solution 44
Precast Gels 17

Sample of E. coli total RNA were denatured using the following denaturants: Lane A: Formaldehyde/Formamide; Lane B: Formamide; Lane C: Glyoxal. Samples were loaded at 2 μg/lane for the formaldehyde/formamide and formamide only denatured samples, and 4 μg/lane for the glyoxal denatured samples. Reliant® RNA Precast Agarose Gels were run at 7 V/cm for 40 minutes in 1X MOPS Buffer and post stained with SYBR® Green II Gel Stain and photographed on the Clare Chemical Research, Inc., Dark Reader® Transilluminator.
Markers, Stains, and Buffers

AccuGENE® Molecular Biology Buffers are ready-to-use solutions ideal for a wide range of molecular biology applications.

- **Reliable** – Manufactured according to strict quality control standards to ensure lot-to-lot consistency
- **High quality** – Guaranteed DNase, RNase, and protease-free
- **Efficient** – Ready-made solutions eliminate experiment preparation time
- **Flexible** – Customized solutions are available to meet individual needs

### Storage Conditions

18°C – 24°C

### Ordering Information

<table>
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<tr>
<th>Cat. No.</th>
<th>Size</th>
<th>Price</th>
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<tbody>
<tr>
<td><strong>AccuGENE® Molecular Biology Water</strong></td>
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<td></td>
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<tr>
<td>51200</td>
<td>1 L</td>
<td>$26</td>
</tr>
<tr>
<td>51244</td>
<td>4 L</td>
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<tr>
<td>51223</td>
<td>10L</td>
<td>$119</td>
</tr>
<tr>
<td>51224</td>
<td>20 L</td>
<td>$204</td>
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</tbody>
</table>

| **AccuGENE® 0.5 M EDTA Solution** | Disodium salt, pH 8.0 |       |
| 51201   | 100 ml | $24   |
| 51234   | 1 L    | $62   |

| **AccuGENE® 5 M Sodium Chloride** |       |       |
| 51202   | 1 L    | $28   |

| **AccuGENE® 10% SDS** | Monosodium salt |       |
| 51206   | 100 ml | $24   |
| 51213   | 500 ml | $38   |

| **AccuGENE® 3 M Sodium Acetate, pH 5.2** |       |       |
| 51203   | 500 ml | $45   |

| **AccuGENE® 20X SSC Buffer** | 3.0 M NaCl, 0.3 M sodium citrate, pH 7.0 |       |
| 51205   | 1 L    | $28   |
| 51233   | 10 L   | $148  |
| 51232   | 20 L   | $287  |

| **AccuGENE® 20X SSPE Buffer** | 3.0 M NaCl, 0.2 M Na2HPO4, 0.02 M EDTA, pH 7.4 |       |
| 51214   | 1 L    | $40   |
| 51246   | 10 L   | $246  |

| **AccuGENE® 1 M Tris HCl Buffer** |       |       |
| 51236   | 7.2 L  | $33   |
| 51237   | 7.4 L  | $34   |
| 51238   | 8.0 L  | $34   |

| **AccuGENE® LB Broth (Luria Bertani Medium)** | 10 g/L Bacto-Tryptone, 5 g/L Bacto-Yeast Extract, and 10 g/L NaCl |       |
| 51217   | 500 ml | $23   |

| **AccuGENE® Super Broth (Terrific Broth)** | 12 g/L Bacto-Tryptone, 24 g/L Bacto-Yeast Extract, 6.3 g/L glycerol, 2.5 g/L K2HPO4, and 3.8 g/L KH2PO4, pH 7.2 |       |
| 51219   | 500 ml | $34   |
| 51220   | 10 L   | $206  |

| **AccuGENE® Neutralization Solution** | 1.5 M NaCl, 1.0 M Tris, pH 7.5 |       |
| 51229   | 1 L    | $49   |
| 51230   | 10 L   | $206  |

| **AccuGENE® 1X PBS** | 1.7 mM KH2PO4, 5 mM Na2HPO4, 150 mM NaCl, pH 7.4 |       |
| 51225   | 1 L    | $30   |

| **AccuGENE® 10X PBS** | 0.017 M KH2PO4, 0.05 M Na2HPO4, 1.5 M NaCl, pH 7.4 |       |
| 51226   | 1 L    | $34   |

---

AccuGENE® Molecular Biology Buffers are convenient and ready-to-use solutions for a wide range of molecular biology applications. Reliability is ensured through strict quality control standards, guaranteeing consistency lot-to-lot. High quality is maintained by ensuring the buffers are DNase, RNase, and protease-free. Efficiency is achieved through ready-made solutions that eliminate the need for experiment preparation time. Flexibility is provided with customized solutions tailored to individual needs.

### Storage Conditions

Store at 18°C – 24°C

### Ordering Information

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Size</th>
<th>Price</th>
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<tbody>
<tr>
<td><strong>AccuGENE® Molecular Biology Water</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>51200</td>
<td>1 L</td>
<td>$26</td>
</tr>
<tr>
<td>51244</td>
<td>4 L</td>
<td>$51</td>
</tr>
<tr>
<td>51223</td>
<td>10 L</td>
<td>$119</td>
</tr>
<tr>
<td>51224</td>
<td>20 L</td>
<td>$204</td>
</tr>
</tbody>
</table>

| **AccuGENE® 0.5 M EDTA Solution** | Disodium salt, pH 8.0 |       |
| 51201   | 100 ml | $24   |
| 51234   | 1 L    | $62   |

| **AccuGENE® 5 M Sodium Chloride** |       |       |
| 51202   | 1 L    | $28   |

| **AccuGENE® 10% SDS** | Monosodium salt |       |
| 51206   | 100 ml | $24   |
| 51213   | 500 ml | $38   |

| **AccuGENE® 3 M Sodium Acetate, pH 5.2** |       |       |
| 51203   | 500 ml | $45   |

| **AccuGENE® 20X SSC Buffer** | 3.0 M NaCl, 0.3 M sodium citrate, pH 7.0 |       |
| 51205   | 1 L    | $28   |
| 51233   | 10 L   | $148  |
| 51232   | 20 L   | $287  |

| **AccuGENE® 20X SSPE Buffer** | 3.0 M NaCl, 0.2 M Na2HPO4, 0.02 M EDTA, pH 7.4 |       |
| 51214   | 1 L    | $40   |
| 51246   | 10 L   | $246  |

1-800-638-8174  www.lonza.com/research
AccuGENE® Electrophoresis Buffers

Optimal performance

AccuGENE® Electrophoresis Buffers are formulated for maximum performance and convenience, and are optimized for use with our agarose and precast gels. AccuGENE® Buffers for DNA, RNA, and protein electrophoresis are prepared with high quality reagents and use 18 megOhm water. Products are filtered using a 0.2-micron filter, and are guaranteed DNase/RNase free.

— Reliable — Manufactured according to strict quality control standards to ensure lot-to-lot consistency
— Efficient — Ready-to-use solutions eliminate experiment preparation time
— Flexible — Customized solutions are available to meet individual needs

Storage Conditions
18°C – 24°C
4°C for CE Buffer

Application Support
— Buffers for Electrophoresis, Section II, page 81

Ordering Information

Buffers for DNA Electrophoresis

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Size</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>AccuGENE® 10X TAE Buffer</td>
<td>0.4 M Tris-acetate, 0.01 M EDTA (disodium salt), pH 8.0</td>
<td></td>
</tr>
<tr>
<td>50844</td>
<td>1 L</td>
<td>$29</td>
</tr>
<tr>
<td>50841</td>
<td>4 L</td>
<td>$87</td>
</tr>
<tr>
<td>AccuGENE® 50X TAE Buffer</td>
<td>2.0 M Tris-acetate, 0.05 M EDTA, pH 8.3</td>
<td></td>
</tr>
<tr>
<td>51216</td>
<td>1 L</td>
<td>$67</td>
</tr>
<tr>
<td>AccuGENE® 5X TBE Buffer</td>
<td>0.45 M Tris-borate, 0.01 M EDTA (disodium salt), pH 8.3</td>
<td></td>
</tr>
<tr>
<td>50839</td>
<td>4 L</td>
<td>$38</td>
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<tr>
<td>50835</td>
<td>10 L</td>
<td>$79</td>
</tr>
<tr>
<td>50836</td>
<td>20 L</td>
<td>$152</td>
</tr>
<tr>
<td>AccuGENE® 10X TBE Buffer</td>
<td>0.89 M Tris-borate, 0.02 M EDTA (disodium salt), pH 8.3</td>
<td></td>
</tr>
<tr>
<td>50843</td>
<td>1 L</td>
<td>$20</td>
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<tr>
<td>50840</td>
<td>4 L</td>
<td>$56</td>
</tr>
<tr>
<td>BE08-23824</td>
<td>4 L</td>
<td>Europe only</td>
</tr>
<tr>
<td>BE08-238210</td>
<td>10 L</td>
<td>Europe only</td>
</tr>
<tr>
<td>50837</td>
<td>10 L</td>
<td>$127</td>
</tr>
<tr>
<td>50838</td>
<td>20 L</td>
<td>$234</td>
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</table>

Buffers for RNA Electrophoresis

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Size</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>AccuGENE® 10X MOPS Buffer</td>
<td>0.2 M MOPS (free acid), 0.05 M sodium acetate, 0.01 M EDTA (disodium salt), 0.01 M EGTA (free acid), pH 7.0. No detectable RNase activity</td>
<td></td>
</tr>
<tr>
<td>50876</td>
<td>1 L</td>
<td>$60</td>
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</table>

Electrophoresis Loading Buffers

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Size</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA Loading Buffer (6X)</td>
<td>Ficoll® based with bromophenol blue and xylene cyanol</td>
<td></td>
</tr>
<tr>
<td>50655</td>
<td>5 x 1 ml</td>
<td>$30</td>
</tr>
<tr>
<td>Triple-Dye Loading Buffer (6X)</td>
<td>Contains bromophenol blue, xylene cyanol, and orange G</td>
<td></td>
</tr>
<tr>
<td>50632</td>
<td>1.1 ml</td>
<td>$35</td>
</tr>
</tbody>
</table>

Related Products

- Agarose
- Long Ranger® Gel Solutions 43
- MDE® Gel Solution 44
- Precast Gels 17
- Protein Electrophoresis Products 45
GelBond® Film is a transparent, flexible polyester film designed to support agarose gels. Gels cast on GelBond® Film remain permanently attached to the film through electrophoresis or immunodiffusion and all subsequent fixing, staining, destaining, and drying procedures (gels remain flexible after drying). GelBond® Film is available either as precut sheets or rolls.

- Reliable – Agarose gels cast on GelBond® Film retain their original dimensions during staining and after drying
- Durable – Gels, particularly thin ones, are easier to handle during staining, destaining, and drying when supported
- Convenient – Gel orientation can be recorded directly on the GelBond® Film prior to casting

NOTE: Polyester films will not transmit light of less than 310 nm, and will fluoresce at higher wavelengths.

**Applications**
- Drying and support of agarose gels

**Storage Conditions**
- 18°C – 26°C

### GelBond® PAG Film

Polyacrylamide support film

GelBond® PAG Film is a transparent, flexible polyester film designed to support polyacrylamide or MDE® Gels. The acrylamide monomers covalently attach to the coating on the film during the polymerization reaction. Gels remain permanently attached to the film through electrophoresis and all subsequent fixing, staining, destaining, and drying procedures.

- Reliable – Polyacrylamide gels retain their original dimensions during staining and after drying
- Durable – Gels, particularly thin ones, are easier to handle during staining, destaining, and drying when supported
- Convenient – Gel orientation can be recorded directly on the GelBond® PAG Film prior to casting

NOTE: Polyester films will not transmit light of less than 310 nm, and will fluoresce at higher wavelengths.

**Applications**
- Drying and support of polyacrylamide gels

**Storage Conditions**
- 18°C – 26°C, protect from light

### Ordering Information

#### GelBond® Film Sheets

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Sheet Size (mm)</th>
<th>Chamber Compatibility</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>53734</td>
<td>85 x 100</td>
<td>—</td>
<td>$161</td>
</tr>
<tr>
<td>53745</td>
<td>110 x 125</td>
<td>—</td>
<td>$257</td>
</tr>
<tr>
<td>53746</td>
<td>100 x 150</td>
<td>Bio-Rad® Wide Mini-Sub® Cell, Sub-Cell® [H]</td>
<td>$289</td>
</tr>
<tr>
<td>53748</td>
<td>110 x 205</td>
<td>—</td>
<td>$368</td>
</tr>
<tr>
<td>53749</td>
<td>160 x 180</td>
<td>Hoefer® SE400, SE600, Bio-Rad PROTEAN® II xi [V]</td>
<td>$514</td>
</tr>
<tr>
<td>53759</td>
<td>125 x 245</td>
<td>—</td>
<td>$547</td>
</tr>
<tr>
<td>53761</td>
<td>124 x 258</td>
<td>GE Multiphor® [H]</td>
<td>$553</td>
</tr>
</tbody>
</table>

Custom-cut GelBond® Film is available upon special request. Please inquire for pricing and availability.

#### GelBond® Film Rolls

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Roll Width</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>53740</td>
<td>102 mm</td>
<td>$289</td>
</tr>
<tr>
<td>53750</td>
<td>127 mm</td>
<td>$386</td>
</tr>
<tr>
<td>53760</td>
<td>152 mm</td>
<td>$451</td>
</tr>
<tr>
<td>53780</td>
<td>203 mm</td>
<td>$579</td>
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</table>

#### Related Products

- Agarose

### Ordering Information

#### GelBond® PAG Film Sheets

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Sheet Size (mm)</th>
<th>Chamber Compatibility</th>
<th>Quantity (sheets)</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>54721</td>
<td>138 x 158</td>
<td>—</td>
<td>50</td>
<td>$238</td>
</tr>
<tr>
<td>54723</td>
<td>160 x 180</td>
<td>Hoefer® SE400, SE600, Bio-Rad PROTEAN® II</td>
<td>50</td>
<td>$289</td>
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<td>54727</td>
<td>124 x 258</td>
<td>GE Multiphor®</td>
<td>50</td>
<td>$321</td>
</tr>
<tr>
<td>54729</td>
<td>220 x 165</td>
<td>—</td>
<td>50</td>
<td>$353</td>
</tr>
<tr>
<td>54731</td>
<td>199 x 264</td>
<td>—</td>
<td>50</td>
<td>$514</td>
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<td>54733</td>
<td>203 x 260</td>
<td>GE Multiphor® II</td>
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<td>$514</td>
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<tr>
<td>54735</td>
<td>195 x 370</td>
<td>Biometra® SA-32</td>
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<td>$643</td>
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<tr>
<td>54746</td>
<td>350 x 430</td>
<td>X-ray size</td>
<td>10</td>
<td>$289</td>
</tr>
</tbody>
</table>

Custom-cut GelBond® PAG Film is available upon special request. Please inquire for pricing and availability.

#### Related Products

- MDE® Gel Solution
- ProSieve® 50 Acrylamide Gel Solution

1-800-638-8174

www.lonza.com/research
Long Ranger® Sequencing Products

Reliable and convenient slab gel sequencing

Long Ranger® products are designed for high performance sequencing on ABI Prism® 377 and manual sequencers.

— Long Ranger® Gel Solution offers exceptional results for sequencing and STR analysis
— Singel® Packs contain all necessary reagents for casting a single gel in a pre-measured, ready-to-use pouch
— Gel Slick® Solution is a safe alternative to hazardous silane-based coating solutions and is suitable for sequencing gels and coating microcentrifuge tubes

Applications

— DNA and RNA sequencing
— STR analysis
— Identity testing
— Genotyping
— Differential display

Storage Conditions
18°C – 26°C

Comparison of Long Ranger® Gel Solution and polyacrylamide on the ABI Prism® 377 Automated DNA Sequencer

This comparison used 5% Long Ranger® Gel Solution, 4% polyacrylamide, 36 cm well-to-read, Amplitaq® DNA Polymerase, FS, dye primer on M13 mp18 template.

Typical Results on the ABI Prism® DNA 377 Automated DNA Sequencer

<table>
<thead>
<tr>
<th>% Gel</th>
<th>WTR</th>
<th>Run Condition</th>
<th>Read Length @ 98.5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long Ranger® Gel Solution</td>
<td>5%</td>
<td>36 cm</td>
<td>3 kV – 3.5 hrs</td>
</tr>
<tr>
<td></td>
<td>5%</td>
<td>36 cm</td>
<td>1.68 kV – 9 hrs</td>
</tr>
<tr>
<td></td>
<td>5%</td>
<td>48 cm</td>
<td>2.88 kV – 7.5 hrs</td>
</tr>
</tbody>
</table>

Ordering Information

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Instrument</th>
<th>Plate Length</th>
<th>Gel Matrix</th>
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<tbody>
<tr>
<td>50691</td>
<td>ABI Prism® 377</td>
<td>36</td>
<td>5% Long Ranger® Singel® Pack, 6 M urea</td>
</tr>
<tr>
<td>50686</td>
<td></td>
<td>48</td>
<td>4.75% Long Ranger® Singel® Pack, 6 M urea</td>
</tr>
<tr>
<td>50689</td>
<td>Li-COR®</td>
<td>33 or 41</td>
<td>6% Long Ranger® Singel® Pack, 7 M urea</td>
</tr>
<tr>
<td>50694</td>
<td>Manual Sequencing</td>
<td>—</td>
<td>5.75% Long Ranger® Singel® Pack, 7 M urea</td>
</tr>
</tbody>
</table>

Long Ranger® Singel® Pack for ABI Sequencers 377-36 cm

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Quantity/box</th>
<th>Price</th>
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<tbody>
<tr>
<td>50691</td>
<td>5</td>
<td>$132</td>
</tr>
</tbody>
</table>

Long Ranger® Singel® Pack for ABI Sequencers 377-48 cm

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<th>Quantity/box</th>
<th>Price</th>
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<tbody>
<tr>
<td>50686</td>
<td>5</td>
<td>$132</td>
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</tbody>
</table>

Long Ranger® Singel® Pack for Li-COR® Sequencers 33 or 41 cm

<table>
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<tr>
<th>Cat. No.</th>
<th>Quantity/box</th>
<th>Price</th>
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<tbody>
<tr>
<td>50689</td>
<td>5</td>
<td>$132</td>
</tr>
</tbody>
</table>

Long Ranger® Singel® Pack for manual sequencing

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Quantity/box</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>50694</td>
<td>5</td>
<td>$132</td>
</tr>
</tbody>
</table>

Singel® instrument compatibility

See page 41 for sequencing buffers.

Related Products

AccuGENE® Buffers 41
Mutation Detection Enhancement (MDE®) Gel Solution is a highly sensitive, high resolution, unique gel formulation designed to separate based on conformational differences. Increased sensitivity means you will find more mutations than standard polyacrylamide gels.

- Detects more mutations. Unique formulation
- Easy-to-cast and handle, especially with GelBond® PAG Film
- Eliminate messy silver staining by using GelStar® Nucleic Acid Gel Stain
- Greater flexibility; can be used on standard manual or automated equipment
- Try our complete MDE® Heteroduplex Kit containing MDE® Gel Solution, Heteroduplex Control DNA, Triple Dye Loading Buffer, GelStar® Nucleic Acid Gel Stain, and AccuGENE® 10X TBE Buffer

NOTE: Protocols are available for both manual and automated heteroduplex and SSCP analysis; contact Scientific Support or visit www.lonza.com/research for more information.

Performance of a 1.0X MDE® Gel for heteroduplex analysis

CFTR exon 10 mutations were analyzed in a 1X MDE® Gel (20 cm x 40 cm x 1 mm) run in 0.6X TBE Buffer at 800 volts for 3 hours. The voltage was increased to 1,000 volts for 18 hours (20,400 V/hr total). The gel was silver stained following a standard protocol.

<table>
<thead>
<tr>
<th>Lane 1: 100 bp ladder</th>
<th>Lane 2: F508/I506V (1 base substitution)</th>
<th>Lane 3: F508/F508 [homozygote]</th>
<th>Lane 4: F508/* (heterozygote)</th>
<th>Lane 5: F508* F508 [homozygote]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lane 6: F508/WT</td>
<td>Lane 7: F508*F508 [3 base deletion]</td>
<td>Lane 8: F508*F508 [3 base deletion]</td>
<td>Lane 9: F508/F508C [1 base substitution]</td>
<td>Lane 10: F508/F508C [1 base substitution]</td>
</tr>
<tr>
<td>Lane 11: F508/V</td>
<td>Lane 12: F508/I506V</td>
<td>Lane 13: F508/I506V</td>
<td>Lane 14: F508/I506V</td>
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</tr>
</tbody>
</table>


Ordering Information

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Size</th>
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<tbody>
<tr>
<td>50622</td>
<td>Kit</td>
<td>$514</td>
</tr>
<tr>
<td>50620</td>
<td>250 ml</td>
<td>$264</td>
</tr>
<tr>
<td>50630</td>
<td>60 μl</td>
<td>$151</td>
</tr>
<tr>
<td>50632</td>
<td>1.1 ml</td>
<td>$35</td>
</tr>
<tr>
<td>50535</td>
<td>2 x 250 μl</td>
<td>$146</td>
</tr>
<tr>
<td>50536</td>
<td>3 inch square</td>
<td>$58</td>
</tr>
<tr>
<td>50843</td>
<td>1 L</td>
<td>$20</td>
</tr>
<tr>
<td>50653</td>
<td>5 x 1 ml</td>
<td>$43</td>
</tr>
</tbody>
</table>

■ Applications
- Heteroduplex analysis
- SSCP analysis [8300 bp]

■ Storage Conditions
- MDE® Gel Solution: 18°C – 26°C
- MDE® Heteroduplex Kit: See components
- Heteroduplex Control DNA: 2°C – 8°C
- Triple Dye Loading Buffer: 2°C – 8°C
- GelStar® Nucleic Acid Gel Stain: -20°C
- AccuGENE® 10X TBE Buffer: 18°C – 26°C
- GelStar® Photographic Filter: 18°C – 26°C

Related Products
- Gel Slick® Solution 43
- GelBond® PAG Film 42
Protein Electrophoresis

Chapter 2
### Protein Gel Electrophoresis

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>47</td>
</tr>
<tr>
<td>PAGEr® Precast Gels for Protein Electrophoresis</td>
<td>48</td>
</tr>
<tr>
<td>PAGEr® Minigel Chamber</td>
<td>50</td>
</tr>
<tr>
<td>Protein Markers, Buffers, and Stains</td>
<td>51</td>
</tr>
<tr>
<td>IsoGel® Agarose and Precast Gels</td>
<td>56</td>
</tr>
<tr>
<td>for Isoelectric Focusing</td>
<td></td>
</tr>
<tr>
<td>Agarose for Protein Separation</td>
<td>58</td>
</tr>
<tr>
<td>ProSieve® 50 Acrylamide Gel Solution</td>
<td>60</td>
</tr>
<tr>
<td>GelBond® PAG Support Film</td>
<td>61</td>
</tr>
</tbody>
</table>
Lonza offers a versatile line of high performance protein electrophoresis products. Our precast gels, buffers, markers and stains are optimized to work together to provide the sharpest resolution and the greatest sensitivity, in the fastest, most convenient formats. Ask about our custom capabilities to create the precise format for your research requirements.

- PAGEr® Precast Gels
- ProSieve® Markers
- AccuGENE® Buffers
- SYPRO® Stains
PAGEr® Precast Gels for Protein Electrophoresis

Reliable, easy-to-use minigels

PAGEr® Precast Gels are easy-to-use protein minigels that offer sharper resolution, more consistent protein transfer, and longer shelf life than any other Tris-Glycine gel. PAGEr® Gels are easy-to-use and compatible with most minigel chambers.

- **Applications**
  - Western blotting
  - Denaturing and native protein electrophoresis
  - 2D electrophoresis

- **Performance and Quality Tests**
  - Every lot of PAGEr® Precast Gels is functionally tested and 100% guaranteed

- **PAGEr® Precast Gel comb formats**
  Comb configurations are designed for a range of sample volumes and throughputs, including multichannel pipette compatible formats.

- **Specifications**
  - Number of wells: 10 well
    - Recommended load volume: 32 μl
  - Number of wells: 12 well
    - Recommended load volume: 20 μl
  - Number of wells: 16 well
    - Recommended load volume: 14 μl
  - Number of wells: 17 well
    - Recommended load volume: 14 μl
  - Number of wells: 20 well
    - Recommended load volume: 550 μl sample, or 7 cm IPG strip, 12 μl marker
  - Number of wells: 8+1 well
    - Recommended load volume: 30 μl sample, or 12 μl marker
  - *Multichannel pipette compatible

Lonza offers over 70 format options for denatured and native protein separation over a wide molecular weight range, in an array of configurations in both 9 cm × 10 cm and 10 cm × 10 cm sizes to fit popular chambers. See chamber compatibility chart (below) to determine the right gel size for your system.

- **Razor sharp resolution** – Crisp separation of proteins 5 kDa – 250 kDa
- **Easy to use** – Marked sample lanes for easy loading and simple twist open design
- **Compatible** – 2 sizes to fit most chambers
- **Versatile** – Multiple well formats and gel concentrations
- **Tris-Glycine buffer** – Traditional Laemmli separation
- **Fresh** – We ship fresh gels every time for guaranteed performance

**Chamber Compatibility**

PAGEr® Precast Gels are available in 9 cm × 10 cm and 10 cm × 10 cm sizes and fit most standard mini-vertical systems.

Some chambers may require modifications for optimal fit with PAGEr® Precast Gels. See page 164 or contact Scientific Support for additional information.

<table>
<thead>
<tr>
<th>Standard Vertical Systems</th>
<th>PAGEr® Gels</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAGEr® Minigel Chamber</td>
<td>9 cm × 10 cm</td>
</tr>
<tr>
<td>10 cm × 10 cm gels</td>
<td></td>
</tr>
</tbody>
</table>

- Bio-Rad® MiniPROTEAN® II, MiniPROTEAN® 3 or Ready Gel® Cell Systems
  - Reverse the inner core gasket so the flat side faces outward

- Novex® XCell SureLock® Mini-Cell
  - Request the spacer for the XCell SureLock® Mini-Cell Chamber from Scientific Support, (Cat. No. 59900)

- FisherBiotech® Vertical MiniGel FBVE12L
  - Deep lower buffer chamber for the SE260'
  - Owl Separations Systems Wolverine™ PB2 Chamber comes with 2 sets of wedges. Use the thinner wedges for the PAGEr® Gold Gels.

- FisherBiotech® Vertical MiniGel FB-VE101
  - Owl Separations Systems Penguin™ Model PBDS
  - Request adapter for these chambers from Scientific Support, (Cat. No. 59902)

- Hoefer® Mighty Small [SE250]
  - 16 cm × 10 cm
  - Replace the buffer chamber with a ‘Deep lower buffer chamber for the SE260’, or order number 83-8148-7R, from GE Healthcare.

<table>
<thead>
<tr>
<th>Application</th>
<th>PAGEr® Gels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daiichi 2, 125 chambers</td>
<td>10 cm × 10 cm gels</td>
</tr>
</tbody>
</table>

- Novex® XCell II
  - 9 cm × 10 cm or 10 cm × 10 cm gels

- Hoefer® Mighty Small [SE260]
  - 9 cm × 10 cm or 10 cm × 10 cm gels

- EC 120 Mini Vertical Gel System
  - 9 cm × 10 cm or 10 cm × 10 cm gels

- Biomera® Mini V Chamber
  - 9 cm × 10 cm gels

- CBS Scientific MSV System, (10 cm × 8 cm units)
  - 9 cm × 10 cm gels

- Sigma-Aldrich Mini Techware
  - [11.3 cm × 10 cm units]
  - 10 cm × 10 cm gels

- Zaxis System 2000
  - 10 cm × 10 cm gels

- Hoefer® Mini VE
  - 10 cm × 10 cm gels
Selecting the Best Precast Gel

Ordering Information

PAGEr® Gold Tris-Glycine Precast Gels

<table>
<thead>
<tr>
<th>Gel Concentration/ Separation Range</th>
<th>Cassette size (cm)</th>
<th>2D well</th>
<th>10 well</th>
<th>12 well</th>
<th>16 well</th>
<th>17 well*</th>
<th>8 + 1 well*</th>
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<tbody>
<tr>
<td>4-12% gradient 25 – 250 kDa</td>
<td>9 x 10</td>
<td>58520</td>
<td>58522</td>
<td>58524</td>
<td>59524</td>
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<tr>
<td>5 – 200 kDa</td>
<td>10 x 10</td>
<td>58511</td>
<td>58505</td>
<td>58517</td>
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<td>58545</td>
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<tr>
<td>9-16% gradient 15 – 200 kDa</td>
<td>9 x 10</td>
<td>58519</td>
<td>58521</td>
<td>58523</td>
<td>58560</td>
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<td>10-20% gradient 5 – 150 kDa</td>
<td>9 x 10</td>
<td>58512</td>
<td>58506</td>
<td>58518</td>
<td>59518</td>
<td>59518</td>
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<tr>
<td>7.5%</td>
<td>10 x 10</td>
<td>58507</td>
<td>58501</td>
<td>58513</td>
<td>58542</td>
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<td>10%</td>
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<td>58509</td>
<td>58503</td>
<td>58515</td>
<td>58543</td>
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<td>15%</td>
<td>9 x 10</td>
<td>58510</td>
<td>58504</td>
<td>58516</td>
<td>58544</td>
<td>58550</td>
<td>59550</td>
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<tr>
<td>10 – 50 kDa</td>
<td>10 x 10</td>
<td>59510</td>
<td>59504</td>
<td>59516</td>
<td>59544</td>
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PAGEr® Gold Scouting Kit

<table>
<thead>
<tr>
<th>Cat. No.</th>
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<th>Price</th>
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<tbody>
<tr>
<td>58100</td>
<td>9 cm x 10 cm</td>
<td>Select 6 gels of any type</td>
<td>$70</td>
</tr>
<tr>
<td>59100</td>
<td>10 cm x 10 cm</td>
<td>Select 6 gels of any type</td>
<td>$70</td>
</tr>
</tbody>
</table>

*For added convenience, our 8+1 and 17-well comb formats can be loaded with a multichannel pipette.

### Gel concentration and size separation range

Lower concentrations are best for resolving large molecules and higher concentrations are best for resolving small molecules. Gradient gels are best for proteins that are unknown or occur over a wide molecular weight range.

### Percentage PAGEr® Precast Gel

Gels were run at 175 volts until the dye front reached the bottom of the gel (approx. 60 minutes). 8 μl – 10 μl of marker was loaded per lane (0.8 μg – 1 μg per band). Gels were stained with Coomassie® Brilliant Blue Stain.
PAGEr® Minigel Chamber

Absolute simplicity and optimal performance

PAGEr® Minigel Chamber

The PAGEr® Minigel Chamber is the easiest chamber you will ever use. The chamber is designed to provide optimized performance from PAGEr® Precast Gels and will also work with most other precast minigels. The simple, lock-in-place core design assures a tight, flat fit and eliminates the risk of buffer leaks. No need to remove the core – simply insert gels, close the clamps, fill with buffer and run. Runs one or two gels and accommodates a tank blotting module.

— Easy-to-use, lock-in-place core eliminates leaking and minimizes handling
— Perfect fit with 9 cm × 10 cm and 10 cm × 10 cm PAGEr® Gels
— Even electrical force ensures straight lanes
— Solid, robust construction
— Optimizes performance of PAGEr® Gels

PAGEr® Blot Module

The PAGEr® Blot Module works directly in the PAGEr® Minigel Chamber and provides exceptional blotting with a fast, simple protocol.

— Color-coded cassettes ensure proper orientation of the gel during transfer
— Transfer time – 90 minutes or less
— Hinged cassette design for easy assembly

The system can be purchased as a kit, including the PAGEr® Minigel Chamber and PAGEr® Blot Module, or components may be purchased separately.

■ Applications
  — SDS-PAGE electrophoresis
  — 2D electrophoresis
  — Tank blotting

■ Specifications

<table>
<thead>
<tr>
<th>Gel types:</th>
<th>Most standard precast minigels (casting apparatus not included)</th>
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<tbody>
<tr>
<td>Gel sizes:</td>
<td>9 cm × 10 cm (adapter included) and 10 cm × 10 cm</td>
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<tr>
<td>Chamber capacity:</td>
<td>Single gel [blank included], 2 gels, or blot cassettes</td>
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<tr>
<td>Buffer volume:</td>
<td>800 ml</td>
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■ Application Support
  — Tank Blotting PAGEr® Precast Gels, Section XI, page 174

Ordering Information

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<td>59905</td>
<td>PAGEr® Minigel Chamber</td>
<td>$417</td>
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<tr>
<td>59906</td>
<td>PAGEr® Blot Module</td>
<td>$256</td>
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<tr>
<td>59907</td>
<td>PAGEr® Minigel Chamber and Blot Module Kit*</td>
<td>$662</td>
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</table>

*Includes chamber, 2 blotting cassettes, and sponge pads (8/pack). Contact Science Support for information about replacement parts.

Performance of the PAGEr® Minigel Chamber

Markers and E. coli lysate run on a 9 cm × 10 cm PAGEr® Gel @ 200 V for 60 minutes in the PAGEr® Minigel Chamber. Samples from left to right: 1 & 2 ProSieve® Color Protein Marker; 3 – 8 E. coli lysate; 9 & 10 ProSieve® Protein Marker.

Related Products

| AccuGENE® Buffers | 52 |
| PAGEr® Precast Gels | 48 |
| ProSieve® Protein Markers | 51 |
| SYPRO® Protein Gel Stains | 53 |
ProSieve® Color Protein Marker

Sharp, accurate confirmation of protein transfer

ProSieve® Color Protein Markers are ideal for monitoring protein separation prior to staining and provide accurate confirmation of protein transfer in Western blotting.

— Convenient – Just add water and load
— Sharp – Multi-colored, readily identifiable band pattern for monitoring electrophoresis and confirming protein transfer

ProSieve® Color Protein Markers are a set of proteins and dyes for use as visible markers in SDS-PAGE gels. During electrophoresis, these markers help monitor the efficiency of separation. In Western blotting, they confirm transfer has occurred from the gel to the membrane. The proteins have been labeled with fluorescent dyes and are supplied as lyophilized solids containing the buffer salts and detergent found in the typical Laemmli buffer system.

ProSieve® Color Protein Markers contain 9 proteins with approximate masses of 10 kDa, 15 kDa, 20 kDa, 25 kDa, 40 kDa, 50 kDa, 80 kDa, 125 kDa, and 190 kDa. Each lot is individually calibrated against unstained ProSieve® Protein Markers.

Storage Conditions

-20°C

NOTE: Not recommended for accurate protein sizing. For sharp, accurate sizing, use ProSieve® Protein Markers (page 52).

ProSieve® Color Protein Markers performance vs. leading competitors

Markers were run on a Lonza 4-20% PAGE® Gold Precast Gel in Tris-Glycine SDS Buffer at 125 V for 75 minutes.

1. ProSieve® Color Protein Marker
2. ProSieve® Color Protein Marker
3. BenchMark® Prestained Protein Ladder (Invitrogen)
4. SeeBlue® Plus2 Pre-Stained Protein Standard (Invitrogen)
5. Multi-Mark® Multi-Colored Standard (Invitrogen)
6. ProSieve® Color Protein Marker
7. ProSieve® Color Protein Marker
8. Kaleidoscope® Prestained Standards (Bio-Rad)
10. High Range Color Markers (Sigma)
11. ProSieve® Color Protein Marker
12. ProSieve® Color Protein Marker

Ordering Information

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<td>100 μl</td>
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<tr>
<td>50550</td>
<td>500 μl</td>
<td>$123</td>
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Related Products

AccuGENE® Buffers
PAGE® Precast Gels
ProSieve® 50 Acrylamide Gel Solution

www.lonza.com/research
ProSieve® Protein Marker

Sharp, accurate sizing of proteins 5 kDa – 225 kDa

ProSieve® Protein Marker consists of a novel set of proteins designed for accurate sizing of protein samples in SDS-PAGE. The markers contain 10 proteins with exact masses of 5 kDa, 10 kDa, 15 kDa, 25 kDa, 35 kDa, 50 kDa, 75 kDa, 100 kDa, 150 kDa, and 225 kDa. The 50 kDa band is double-intensity for easy identification.

— Simple — Wide distribution of exact masses simplifies sample determination
— Accurate — Recombinant proteins do not contain oligosaccharides that can cause anomalous migration, heterogeneous “fuzzy” bands, and inaccurate size estimation

Performance of ProSieve® Protein Markers in various gel concentrations

Gels were run at 175 volts until the dye front reached the bottom of the gel (approx. 60 minutes). 8 μl – 10 μl of marker was loaded per lane (0.8 μg – 1 μg per band). Gels were stained with Coomassie® Brilliant Blue Stain.

AccuGENE® Electrophoresis Buffers

Optimum performance

AccuGENE® Electrophoresis Buffers are formulated to match PAGEr® Precast Gels. AccuGENE® Buffers for protein electrophoresis are prepared with high quality reagents and use 18 megOhm water. Products are filtered using a 0.2-micron filter.

— Reliable — Manufactured according to strict quality control standards to ensure lot-to-lot consistency
— Efficient — Ready-to-use solutions eliminate preparation time
— Flexible — Customized solutions are available to meet individual needs

Storage Conditions
18°C – 24°C

Ordering Information

Buffers for Protein Electrophoresis

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<tr>
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<td>$60</td>
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<tr>
<td>50881</td>
<td>4 L</td>
<td>$81</td>
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AccuGENE® 10X Tris-Glycine Buffer
0.25 M Tris base, 1.92 M Glycine

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<th>Size</th>
<th>Price</th>
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</thead>
<tbody>
<tr>
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<td>1 L</td>
<td>$60</td>
</tr>
<tr>
<td>50881</td>
<td>4 L</td>
<td>$81</td>
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</tbody>
</table>

AccuGENE® 10X Tris-Glycine SDS Buffer
0.25 M Tris base, 1.92 M Glycine, 1% SDS

<table>
<thead>
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<tbody>
<tr>
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<td>$60</td>
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<tr>
<td>50882</td>
<td>4 L</td>
<td>$81</td>
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</table>

Related Products

PAGEr® Minigel Chamber 50
PAGEr® Precast Gels 49
ProSieve® 50 Acrylamide Gel Solution 60
SYPRO® Protein Gel Stains are simple, sensitive alternatives to Coomassie® Brilliant Blue and silver stain for a diverse range of applications from 2D gel staining to staining gels prior to Western blotting.

— Exquisitely sensitive — Detection limits rival the best silver stains
— Fast and easy — Simple procedures require no complex fixation or destain
— Quantitative — Broad linear range over 3 orders of magnitude
— Versatile — Visualize with UV transilluminators, Dark Reader® Transilluminators, and laser scanners
— Compatible — With downstream processing such as mass spectrometry and microsequencing

Sensitivity of SYPRO® Stains compared to Coomassie® Brilliant Blue and silver stain

Serial dilutions of ProSieve® Protein Marker 50 kDa band on 12% PAGEr® Gold Precast Gels, stained and photographed as noted. Protein levels indicated in nanograms.

<table>
<thead>
<tr>
<th>Application</th>
<th>SYPRO® Ruby</th>
<th>SYPRO® Tangerine</th>
<th>SYPRO® Red</th>
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</thead>
<tbody>
<tr>
<td>High performance staining</td>
<td>■</td>
<td>■</td>
<td>■</td>
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<tr>
<td>Staining prior to Western blotting</td>
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<tr>
<td>2D Electrophoresis</td>
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<td>■</td>
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<tr>
<td>Edman microsequencing</td>
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<td>■</td>
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<tr>
<td>Mass spectrometry</td>
<td>■</td>
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<tr>
<td>Quantitation</td>
<td>■</td>
<td>■</td>
<td>■</td>
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<tr>
<td>Zymography</td>
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<tr>
<td>Electroelution</td>
<td>■</td>
<td>■</td>
<td>■</td>
</tr>
<tr>
<td>Membrane staining</td>
<td>■</td>
<td>■</td>
<td>■</td>
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<tr>
<td>Protein expression</td>
<td>■</td>
<td>■</td>
<td>■</td>
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<tr>
<td>Detection prior to Immunostaining</td>
<td>■</td>
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<td>■</td>
</tr>
<tr>
<td>PB cult to stain proteins</td>
<td>■</td>
<td>■</td>
<td>■</td>
</tr>
<tr>
<td>IEF Gels</td>
<td>■</td>
<td>■</td>
<td>■</td>
</tr>
</tbody>
</table>

Select the Best Stain for your Application

Fast, simple staining procedure

Fixation is required for staining 2D gels in SYPRO® Ruby Gel Stain. No wash step is necessary for SYPRO® Red or Tangerine Gel Stains.

Application Support

— Detection of Proteins in Polyacrylamide Gels, Section X, page 166
— SYPRO® Protein Gel Stains, Section X, page 167

Related Products

<table>
<thead>
<tr>
<th>Related Product</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAGEr® Minigel Chamber</td>
<td>50</td>
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<tr>
<td>PAGEr® Precast Gels</td>
<td>49</td>
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<tr>
<td>ProSieve® 50 Acrylamide Gel Solution</td>
<td>60</td>
</tr>
<tr>
<td>ProSieve® Protein Markers</td>
<td>52</td>
</tr>
</tbody>
</table>
**SYPRO® Ruby Protein Gel Stain**

The best stain for 2D gel analysis

SYPRO® Ruby Protein Gel Stain is a highly sensitive, simple to use fluorescent protein gel stain that can accurately quantitate protein expression levels and is compatible with standard fluorescent visualization systems and downstream identification techniques such as mass spectrometry.

- **Highly sensitive** – Rivals the best silver stain
- **Quantitative** – Broad linear range and consistent gel-to-gel staining
- **Fast** – Simple staining procedure saves time and money
- **High throughput** – Fast, easy staining of multiple gels
- **Versatile** – Detects difficult to stain proteins

**Storage Conditions**

18°C – 26°C

**Ordering Information**

<table>
<thead>
<tr>
<th>Cat. No.</th>
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<tr>
<td>50563</td>
<td>5 L</td>
<td>$964</td>
</tr>
</tbody>
</table>

Product licensed from Molecular Probes, Inc.
Photographic filter recommended (see page 55)

**SYPRO® Red Protein Gel Stain**

The fastest, easiest stain for detecting proteins

SYPRO® Red Protein Gel Stain is a fast, highly sensitive fluorescent protein gel stain that detects as little as 4 ng – 8 ng protein per band.

- **Fast** – Complete staining in less than 1 hour
- **Sensitive** – Five times more sensitive than Coomassie® Brilliant Blue stain
- **Simple** – No fixation or destaining required
- **Consistent** – Low protein-to-protein variability

Staining is easy – simply soak gels in a solution of 1X SYPRO® Red Stain in 7.5% acetic acid for 40 to 60 minutes. The stain is compatible with UV transilluminators, CCD cameras or laser scanners.

**Storage Conditions**

18°C – 26°C

**Ordering Information**

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Size</th>
<th>Price</th>
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<td>50542</td>
<td>10 × 50 μl</td>
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</tr>
<tr>
<td>50543</td>
<td>500 μl</td>
<td>$158</td>
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</tbody>
</table>

Product licensed from Molecular Probes, Inc.
Photographic filter recommended (see page 55)
SYPRO® Tangerine Protein Gel Stain

Ideal for staining gels prior to Western blotting

SYPRO® Tangerine Protein Gel Stain is a versatile, sensitive stain that can be used to visualize proteins prior to Western blotting.

- **Visualize proteins prior to transfer** – Does not interfere with protein activity or transfer
- **Safe** – No acids or organic solvents necessary
- **Sensitive** – Detects as little as 4 ng – 8 ng protein per band

The staining procedure is fast and simple and does not require the use of organic solvents; staining can be performed in saline or PBS solutions. Proteins can be used in zymography assays or analyzed by mass spectrometry.

**Storage Conditions**
18°C – 26°C

SYPRO® Ruby Protein Blot Stain

Fast, simple, sensitive stain for detecting proteins on blots

SYPRO® Ruby Protein Blot Stain offers sensitivity levels that rival colloidal stains. The stain is 60-times more sensitive than reversible stains like Ponceau S, and 30-times more sensitive than Amido Black or Coomassie® Brilliant Blue Stains.

- **Highly Sensitive** – Detects as little as 2 ng – 8 ng protein per band
- **Fast** – Simple staining procedure takes less than 1 hour
- **Compatible** – With fluorogenic, chemiluminescent and colorimetric detection techniques

**Storage Conditions**
18°C – 26°C

SYPRO® Protein Gel Stain Photographic Filter

For optimal detection sensitivity with black and white film photography

The SYPRO® Protein Gel Stain Photographic Filter is suitable for Polaroid® Camera Systems. The filter does not work with CCD camera systems. Check with the manufacturer for the appropriate filter. Recommended for use with all SYPRO® Protein Gel Stains.

**Ordering Information**

<table>
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<th>Cat. No.</th>
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<tr>
<td>SYPRO® Ruby Protein Blot Stain</td>
<td>50565</td>
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<tr>
<td>SYPRO® Protein Gel Stain Photographic Filter</td>
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Two identical SDS-PAGE gels were run with samples of protein molecular weight standards (leftmost lanes) and protein molecular weight standards mixed with decreasing amounts of *E. coli* β-glucuronidase and rabbit liver esterase. Gels were stained for total protein with SYPRO® Tangerine Protein Gel Stain, and for specific enzymatic activities. Both gels were first stained with SYPRO® Tangerine Protein Gel Stain (one gel shown, Panel A). One gel was stained with ELPI-97 β-d-glucuronidase substrate (E-6587) for the detection of β-glucuronidase activity (Panel B).

Molecular weight standards containing decreasing amounts of α-tubulin were run on an SDS-PAGE gel, blotted onto a PVDF membrane and stained with SYPRO® Ruby Protein Blot Stain.
IsoGel® Agarose and Precast IsoGel® Agarose IEF Plates

Isoelectric focusing for rapid separation of large proteins

Separation of proteins in complex mixtures for analytical resolution can be achieved by isoelectric focusing (IEF), in which proteins are separated based on their net charge (isoelectric point or pI) in the presence of a pH gradient. Agarose has distinct advantages over polyacrylamide gels for isoelectric focusing. Separation in agarose is more rapid, and agarose gels can be used to separate proteins up to 2,000 kDa. Lonza has developed two high quality products that are specifically designed and tested for their performance with IEF.

- IsoGel® Agarose is a highly purified agarose that is easy to prepare and produces a gel with high clarity and a less restrictive matrix than polyacrylamide
- IsoGel® Agarose IEF Plates are ready-to-use precast gels supported on GelBond® PAG Support Film, eliminating gel preparation time and providing easy handling throughout the IEF process

Advantages
- Safe — No toxic acrylamide required
- Fast — Shorter staining times
- Simple — Nontacky and easy to blot

Applications
- Isoelectric focusing
- Antibody separation and analysis
- Immunofixation directly in the gel
- Crossed immunoelectric focusing
- Direct tissue or preparative isoelectric focusing
- Protein blotting
- Immunodetection of proteins

Application Support
- Isoelectric Focusing of Proteins on Agraose Gels, Section XII, page 177

IsoGel® Agarose

Highly purified agarose for isoelectric focusing

- No measurable EEO — Manufacturing process minimizes fixed anions and mobile cations
- Versatile — Sufficiently rigid for casting in vertical tubes (e.g., O’Farrell gels), vertically molded or horizontally open cast thin gels

Applications
- Isoelectric focusing

Analytical Specifications

| Moisture: | ≤10% |
| Sulfate: | ≤0.20% |
| EEO (m-): | Not detectable |
| Gel strength (1.5%): | ≥500 g/cm² |
| IEF test: | Passes test |

Storage Conditions

18°C – 25°C

Reference


Application Support

- Isoelectric Focusing of Proteins on Agraose Gels, Section XII, page 177

Ordering Information

<table>
<thead>
<tr>
<th>Cat. No.</th>
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<tbody>
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<td>IsoGel® Agarose 50202</td>
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<td>$368</td>
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</tbody>
</table>

Larger package sizes are available upon request. Please inquire for pricing and availability.

Related Products

GelBond® PAG Support Film 42

1-800-638-8174 www.lonza.com/research
IsoGel® Products for Isoelectric Focusing

Easy handling — Each gel is supported on GelBond® PAG Support Film to provide dimensional stability throughout IEF processing.

Versatile — Convenient 125 mm × 100 mm gel size fits most horizontal IEF chambers.

Fast — Proteins can be quickly transferred from gel to membrane, stained in situ, or detected by antibodies within 1 hour.

Applications
- Isoelectric focusing
- Antibody separation and analysis

Performance and Quality Tests
- Each lot of IsoGel® Agarose IEF Plates is functionally tested; Certificate of Analysis available upon request.

Storage Conditions
- 2°C – 8°C for 12 months from the date of manufacture
- Accessories: 18°C – 26°C

Ordering Information

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<td>6 plates</td>
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<td>56014</td>
<td>Precast IsoGel® Agarose IEF Plate Accessory Pack Contains masks, 100 mm and 125 mm wicks and blotting paper for 6 plates</td>
<td>Sufl cient for 6 plates</td>
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<td>Precast IsoGel® Agarose IEF Plate Accessory Bulk Pack Contains 125 mm wicks and blotting paper for 6 plates</td>
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<td>56007</td>
<td>Precast IsoGel® Agarose IEF Blotting Paper</td>
<td>250 sheets</td>
<td>$222</td>
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</table>

Precast IsoGel® Agarose IEF Plates

Precast gels for the analysis of antibodies and proteins up to 2,000 kDa

Performance of IsoGel® Agarose IEF Plate

Separation of proteins in an IsoGel® Agarose IEF Plate, pH 3 – 10. Lanes 1 & 4: pl Marker (in-house). Lanes 2 & 3: Broad Range pl 4.45-9.6 marker [Bio-Rad®]. Lane 5: Hemoglobin, HB Type AFSC [PE Wallac]. 2.5 μl of each sample were loaded on the gel and prefocused at 1 watt for 10 minutes and focused at 2000 volts [max], 25 mA [max], 25 W [max] for 60 minutes on a GE MultiPhor® II Chamber at 10°C. The gel was stained with Crowle's stain.

Related Products

GelBond® PAG Support Film 42
Electrophoresis of proteins in agarose gels has distinct advantages compared to polyacrylamide for some applications. Agarose gels can easily and effectively separate high molecular weight proteins and protein complexes (>600 kDa).

- **Safe** – No toxic monomer solutions required
- **Efficient recovery** – High recovery yields with simple procedures
- **Flexible** – Gels can be made with standard Laemmli buffer systems

**Reference**


### Normal distribution of von Willebrand Factor multimers as separated on 1%, 2% or 3% SeaKem® HGT(P) Agarose gels

vWF is the largest protein in the human plasma, the basic repeating unit of which is a 556 kDa homodimer comprised of identical 278 kDa subunits disulfide-linked at the C-termini. Each band subfractionates into three bands at higher gel concentrations. Gels prepared with 200 mM Tris, 100 mM glycine pH 9.0 supplementing with 0.1% LiDS. Gels run for 20 min at 10°C using 250 V. Proteins were transferred to nitrocellulose membranes and sequentially probed with anti-human vWF antisera and alkaline phosphatase secondary antibody and visualized using an enhanced NBT-BCIP substrate.

Used by permission from Gary B. Smajekal, Cleveland State University, Cleveland, Ohio.

### Routine Protein Separation

<table>
<thead>
<tr>
<th>Agarose</th>
<th>Typical Application</th>
<th>Protein Size Range (kDa)</th>
<th>Gel Concentration</th>
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<tbody>
<tr>
<td>MetaPhor® Agarose</td>
<td>Protein electrophoresis</td>
<td>20 – 200</td>
<td>4%</td>
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<tr>
<td></td>
<td></td>
<td>150 – 300</td>
<td>3%</td>
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<tr>
<td></td>
<td></td>
<td>300 – 600</td>
<td>2%</td>
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<tr>
<td>SeaKem® Gold Agarose or</td>
<td>Protein electrophoresis</td>
<td>600 – 1,000</td>
<td>1.5%</td>
</tr>
<tr>
<td>SeaPlaque® Agarose</td>
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<td>1,000 – 5,000</td>
<td>1%</td>
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### Specialty Protein Separation

<table>
<thead>
<tr>
<th>Agarose</th>
<th>Typical Application</th>
<th>Separation based on isolectric point</th>
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<tbody>
<tr>
<td>SeaKem® HGT Agarose</td>
<td>Counter-Immunelectrophoresis</td>
<td>CIEP, Crossed-IEP</td>
</tr>
<tr>
<td>SeaKem® HGT(P) Agarose</td>
<td>Factor VIII/von Willebrand’s factor multimer separation</td>
<td></td>
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<tr>
<td>SeaKem® ME Agarose</td>
<td>Serum protein electrophoresis</td>
<td></td>
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<tr>
<td>SeaKem® HEEO Agarose</td>
<td>Immunoelectrophoresis of IgG and IgM</td>
<td></td>
</tr>
<tr>
<td>SeaKem® HE Agarose</td>
<td>Serum protein electrophoresis, IEP, Crossed-IEP, CIEP</td>
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## Agarose for Protein Separation

Continued

### Ordering Information

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<tr>
<td>50040</td>
<td>125 g</td>
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**SeaKem® HGT Agarose**
- High gel strength, high clarity agarose for use in counter-immunoelectrophoresis and crossed immunoelectrophoresis.
- Gelling temp. (1.5%): 42°C ± 1.5°C
- Moisture: 10%
- Sulfate: 0.30%
- EEO (–mr): 0.10
- Gel strength (1%): 800 g/cm²

<table>
<thead>
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</thead>
<tbody>
<tr>
<td>50050</td>
<td>125 g</td>
<td>$753</td>
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</table>

**SeaKem® HGT(P) Agarose**
- Used for separating Factor VIII/von Willenbrand's (VWF) factor multimers.
- Gelling temp. (1.5%): 42°C ± 1.5°C
- Moisture: 10%
- Sulfate: 0.20%
- EEO (–mr): 0.10

<table>
<thead>
<tr>
<th>Cat. No.</th>
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<tbody>
<tr>
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<tr>
<td>50020</td>
<td>125 g</td>
<td>$250</td>
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</table>

**SeaKem® HE Agarose**
- A high EEO agarose that provides enhanced resolution in immunoelectrophoresis, crossed-immunoelectrophoresis, counter-immunoelectrophoresis, and serum protein electrophoresis.
- Gelling temp. (1.5%): 36°C ± 1.5°C
- Moisture: 10%
- Sulfate: 0.20%
- EEO (–mr): 0.23 – 0.26
- Gel strength (1%): 650 g/cm²

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Size</th>
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<tbody>
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<tr>
<td>50010</td>
<td>125 g</td>
<td>$250</td>
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</table>

**SeaKem® HEEO Agarose**
- A very high EEO agarose useful in applications requiring significant cathodal migration, such as immunoelectrophoresis of IgG and IgM. May also be blended with lower EEO agarose to achieve a specific EEO value.
- Gelling temp. (1.5%): 36°C ± 1.5°C
- Moisture: 10%
- Sulfate: 0.20%
- EEO (–mr): 0.30 – 0.42
- Gel strength (1%): 650 g/cm²

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<td>50010</td>
<td>125 g</td>
<td>$327</td>
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**SeaKem® ME Agarose**
- An ideal alternative to polyacrylamide for serum protein electrophoresis.
- Gelling temp. (1.5%): 36°C ± 1.5°C
- Moisture: 10%
- Sulfate: 0.20%
- EEO (–mr): 0.10

<table>
<thead>
<tr>
<th>Cat. No.</th>
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<th>Price</th>
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</thead>
<tbody>
<tr>
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<td>50014</td>
<td>500 g</td>
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Larger package sizes are available upon request. Please inquire for pricing and availability.

### Analytical specifications

<table>
<thead>
<tr>
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<th>SeaKem® HGT</th>
<th>SeaKem® HGT(P)</th>
<th>SeaKem® HE</th>
<th>SeaKem® HEEO</th>
<th>SeaKem® ME</th>
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<td>Gelling temp. (1.5%):</td>
<td>42°C ± 1.5°C</td>
<td>42°C ± 1.5°C</td>
<td>36°C ± 1.5°C</td>
<td>36 ± 1.5°C</td>
<td>36 ± 1.5°C</td>
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<tr>
<td>Moisture:</td>
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<td>Sulfate:</td>
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<td>0.20%</td>
<td>0.20%</td>
<td>0.25%</td>
<td>0.20%</td>
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<tr>
<td>EEO (–mr):</td>
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<td>0.10</td>
<td>0.23 – 0.26</td>
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<tr>
<td>Gel strength (1%):</td>
<td>800 g/cm²</td>
<td>1,000 g/cm²</td>
<td>650 g/cm²</td>
<td>650 g/cm²</td>
<td>1,000 g/cm²</td>
</tr>
</tbody>
</table>

### Storage Conditions

18°C – 26°C

### Application Support

- Protein Separation in Agarose Gels, Section XIII, page 195

### Related Products

- AccuGENE® Buffers
- GelBond® PAG Support Film
- Precast IsoGel® Agarose IEF Plates

1-800-638-8174  www.lonza.com/research
ProSieve® 50 Acrylamide Gel Solution

Modified acrylamide formulation for high performance electrophoresis of large proteins

— **Gradient separation** — From easy-to-cast single concentration gels
— **Easy-to-handle** — Gels are more durable than standard acrylamide
— **Sharp resolution** — Resolves large proteins (>200 kDa)
— **Fast** — Shorter destaining times and faster protein mobility times
— **Low Background** — Even when used with silver stain

■ **Applications**
  — Protein gel electrophoresis

■ **Storage Conditions**
  18°C – 26°C

■ **Application Support**
  — Protein Separation in Polyacrylamide Gels, Section X, page 155

### Performance of ProSieve® 50 Acrylamide Gel Solution

<table>
<thead>
<tr>
<th>10% ProSieve® 50 Gel</th>
<th>10-20% gradient polyacrylamide gel</th>
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<tr>
<td><img src="image1.png" alt="Image of gel" /></td>
<td><img src="image2.png" alt="Image of gel" /></td>
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</tbody>
</table>

ProSieve® Protein Markers were loaded in lanes 1, 5, and 10; aqueous extract from *E. coli* bacterial cells was loaded in lanes 2, 6, and 9; Protein Markers, broad range were loaded in lanes 3 and 7; and 10 kDa Protein Ladder was loaded in lanes 4 and 8. Proteins were detected by Coomassie® Brilliant Blue Stain.

### Ordering Information

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<thead>
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<tr>
<td>50618</td>
<td>250 ml</td>
<td>$202</td>
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GelBond® PAG Support Film

Polyacrylamide support film

GelBond® PAG Support Film is a transparent, flexible polyester film designed to support polyacrylamide or MDE® Gels. The acrylamide monomers covalently attach to the coating on the film during the polymerization reaction. Gels remain permanently attached to the film through electrophoresis and all subsequent fixing, staining, destaining, and drying procedures.

— Reliable — Polyacrylamide gels retain their original dimensions during staining and after drying

— Durable — Gels, particularly thin ones, are easier to handle during staining, destaining, and drying when supported

— Convenient — Gel orientation can be recorded directly on the GelBond® PAG Support Film prior to casting

NOTE: Polyester films will not transmit light of less than 310 nm, and will fluoresce at higher wavelengths.

Applications

— Drying and support of polyacrylamide gels

Storage Conditions

18°C – 26°C, protect from light

Ordering Information

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<tr>
<th>Cat. No.</th>
<th>Size (mm)</th>
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<td>54723</td>
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<td>Hoefer® SE400, SE600, Bio-Rad® PROTEAN® II</td>
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<td>54727</td>
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<td>$289</td>
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</table>

Custom-cut GelBond® PAG Support Film is available on special request. Please inquire for pricing and availability.

Related Products

<table>
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<tr>
<th>Product</th>
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<tbody>
<tr>
<td>AccuGENE® Buffers</td>
<td>41</td>
</tr>
<tr>
<td>ProSieve® Protein Markers</td>
<td>51</td>
</tr>
<tr>
<td>SYPRO® Protein Stains</td>
<td>53</td>
</tr>
<tr>
<td>MDE® Solution</td>
<td>44</td>
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</table>
The names used to define areas of life science research are continually changing, but many of the basic questions researchers ask remain the same. Many of the techniques used remain the same techniques that have been applied for many years. Separation of nucleic acids and proteins by gel electrophoresis remains a fundamental tool of life science research, and Lonza continues to drive innovations that make this work faster, easier and more reliable.

Our company name has also changed over the years. But our high quality products and our commitment to our customers have not. For over four decades, we have developed and supplied the research community with the best products in the industry for separation, detection, modification and recovery of nucleic acids and proteins. We have compiled those years of experience into a new edition of The Sourcebook. In the following pages, you will find reference information and techniques to help you get maximum performance from our products, as well as your electrophoresis applications in general. We hope that you like our products and this manual becomes one of your preferred sources for all your electrophoresis needs.

We welcome your comments. Please contact us with your feedback at: scientific.support.eu@lonza.com
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Electrophoresis and Agarose

Q. What buffer conditions give me best resolution for agarose electrophoresis?

A. For small DNA fragments (<1,000 bp) when recovery is not necessary, we recommend the use of 1X TBE Buffer. For any given concentration of agarose, gels made with TBE Buffer give sharper bands than gels made with TAE Buffer. TBE results in better resolution for closely spaced DNA bands. For large DNA fragments (>15,000 bp), 1X TAE Buffer enhances separation of large DNA. Since TAE has a lower buffering capacity, it may be necessary either to recirculate the buffer, or periodically mix the buffer between the anodal and cathodal chambers when electrophoresing for an extended period of time. The time to buffer depletion can vary with the volts/hour and the size of chamber used.

Whichever buffer you use, the depth over the gel should be 3 to 5 mm deep. Less buffer, and you risk the chance of the gel drying out. Excessive buffer will decrease the resistance of the circuit between the anode and cathode, which results in a decreased voltage gradient through the gel. This causes inefficient DNA mobility, excessive heating, and band distortion.

Q. How should I cast my gels to get the best resolution?

A. The first concern is the thickness of the gel. We usually cast gels 3 mm to 4 mm thick. The gel volume needed can easily be estimated by measuring the surface area of the casting chamber, then multiplying by the gel thickness. Thinner gels can be cast on GelBond® Support Film, and/or cast in a vertical apparatus. The thickness of the comb in the direction of the electrical field can also profoundly affect the resolution. A thin comb (1 mm) will result in sharper DNA bands. With too thick a comb, the separated DNA bands will be quite broad.

Q. My DNA bands are sometimes wavy, but usually only in one or two lanes. What causes this?

A. Dried agarose on the comb teeth is a frequent cause of this problem. Prior to casting your gel, check the comb teeth for residual dried agarose. If not removed, this will attach to the newly cast agarose and fracture the well upon comb removal. This is usually not observable until the gel is on the transilluminator. Additionally, care must be taken during comb removal, particularly with low melting temperature agaroses. Well integrity may be maintained in these agaroses by pre-chilling the gel to 4°C for 30 minutes and/or by flooding the gel with cold buffer prior to removing the comb.

Q. How much DNA should I load per well?

A. The amount to load per well is variable. What is most important is how much DNA there is in the bands you wish to resolve. The least amount of DNA that can be consistently detected with ethidium bromide is about 10 ng. The most DNA you can have in a band and still get a sharp, clean band on an ethidium bromide stained gel is about 100 ng. These amounts will be less on gels stained with more sensitive stains such as GelStar® Stain. On a GelStar® Stained Gel it is possible to detect as little as 20 pg dsDNA.

The optimal amount of DNA to load in the well is calculated by the fraction of the total DNA which is in the band of interest. If you are unsure of how much DNA is present, load varying amounts in several lanes if possible.

To further increase the sharpness of the bands, use a Ficoll® based loading buffer such as Lonza DNA Loading Buffer (Cat. No. 50655) instead of sucrose-based or glycerol-based loading buffers. The use of lower molecular weight glycerol will allow DNA to stream up the sides of the well before electrophoresis which results in U-shaped bands. Loading buffer that is too high in ionic strength can cause the bands to be fuzzy. In the ideal situation, the DNA sample should be suspended in the same solution as the running buffer. If this is not possible, use a sample buffer with a lower ionic strength than the running buffer.
Electrophoresis and Agarose

Q. At what voltage should I run an agarose gel?
A. We recommend running agarose gels at 4 – 10 volts/cm (cm is determined by measuring the interelectrode distance, not the gel length) under normal horizontal electrophoretic conditions. If the voltage is too high, band streaking, especially for DNA >15 kb, may result. When the voltage is too low, the mobility of small (<1,000 bp) DNA is reduced and band broadening will occur due to diffusion.

MetaPhor® Agarose gels separate DNA optimally at 4.5-5 volts/cm in standard horizontal electrophoresis systems. Higher voltages result in a decrease in the resolution of DNA separation, mainly due to gel overheating.

Another special case is the separation of large (>15 kb) DNA fragments using conventional horizontal electrophoresis. The best separations in this instance are obtained at a voltage gradient of <5 volts/cm.

Q. What is the difference between NuSieve® 3:1 and NuSieve® GTG® Agaroses?
A. NuSieve® 3:1 Agarose is a standard melting temperature agarose. The resolution range for NuSieve® 3:1 Agarose is 50 bp – 1000 bp. NuSieve® 3:1 Agarose is designed for analytical electrophoresis; its high gel strength also makes it ideal for use in various blotting techniques.

NuSieve® GTG® Agarose is a low melting temperature agarose (≤65°C at 4%). The resolution range for this agarose is 50 bp – 1000 bp. NuSieve® GTG® Agarose is recommended for in-gel applications such as cloning or ligation and transformation.

GTG® stands for Genetic Technology Grade™. GTG® Grade Agarose is recommended for preparative DNA electrophoresis, or when further enzymatic manipulation of DNA is required. These agaroses are extensively tested to ensure maximum compatibility with standard molecular biology techniques.

Q. What is the difference between SeaKem® LE and SeaKem® GTG® Agaroses?
A. SeaKem® LE and SeaKem® GTG® Agaroses are both standard melting temperature agaroses. The resolution range for these agaroses is 100 bp to 23,000 bp.

SeaKem® LE Agarose is ideal for routine analysis of DNA. SeaKem® GTG® Agarose is a Genetic Technology Grade™ Agarose, specifically designed for preparative DNA electrophoresis.

Q. What is the difference between SeaPlaque® and SeaPlaque® GTG® Agaroses?
A. SeaPlaque® and SeaPlaque® GTG® Agaroses are both low melting temperature agaroses (≤65°C at 1.5%). The resolution range for these agaroses is 200 bp – 25,000 bp.

SeaPlaque® Agarose is recommended for preparative DNA electrophoresis. SeaPlaque® GTG® Agarose is a Genetic Technology Grade™ Agarose, recommended for direct enzymatic manipulation of nucleic acids in remelted agarose (in-gel reactions). It is also compatible with PCR and sequencing reactions carried out in the presence of the remelted gel.

Precast Agarose Gels

Q. Do I need to purchase a special chamber to use Reliant® and Latitude® Precast Gels?
A. Reliant® and Latitude® Precast Agarose Gels are designed to run in standard horizontal electrophoresis chambers. As long as there is room on the chamber platform for the gel, the chamber should be suitable. Measure the chamber platform and check against precast gel size to be sure. For example, the OWL® Centipede™ Chamber is ideal with the 1 cm x 2 cm Latitude® HT Precast Gels; the OWL® B1 EasyCast™ Chamber is good with Reliant® Precast Agarose Gels; and the Latitude® Chamber is perfect with Latitude® Midigels. Results with different chambers will vary depending on differences in chamber size and construction.

Q. Can I use the FlashGel® System for both DNA and RNA?
A. Yes. We offer FlashGel® Cassettes and Reagents for both DNA and RNA. The FlashGel® Dock may be used for both cassette types as there is no risk of contamination.

Q. Are the materials in the FlashGel® Cassette hazardous?
A. The stain in the FlashGel® Cassette is present at such low levels that it is not considered hazardous according to OSHA and EU hazard criteria. A copy of the MSDS is available online. The stain in the cassette is a potential mutagen. Wear gloves, safety glasses and a lab coat when handling. Use the same precautions when handling and disposing of the cassettes as you would ethidium bromide stained gels.
PAGE® Precast Gels

Q. Which PAGE® Precast Gels will fit my gel chamber?
A. PAGE® Precast Gels are available in 9 cm × 10 cm and 10 cm × 10 cm sizes and fit most standard mini-vertical systems. Some chambers may require modifications for optimal fit with PAGE® Precast Gels.

Q: Do Lonza PAGE® Precast Gels contain a stacking gel? What is the purpose of the stacking gel?
A. PAGE® Gold Precast Gels contain a 4% stacking gel, pH 8.6. The purpose of this stacking gel is to allow the proteins to accumulate and condense (i.e. stack) at the stacking/resolving gel boundary. This stacking effect results in superior resolution within the running gel.

Q. I would like to run a native or non-denaturing gel. What can I use?
A. PAGE® Precast Gels do not contain SDS or any other denaturing agents (e.g. DTT and β-ME). Additionally, you would use a Tris-Glycine Running Buffer that does not contain SDS.

Protein Electrophoresis

Q. How do I make the transfer, running, and sample buffers?
A. Tris-Glycine Gels (Tris-HCl Buffer System)

<table>
<thead>
<tr>
<th>Buffer Type</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Towbin Transfer Buffer (1X)</td>
<td>0.025 M Tris base</td>
</tr>
<tr>
<td>Running Buffer (1X)</td>
<td>25 mM Tris Base</td>
</tr>
<tr>
<td>Sample Buffer (1X)</td>
<td>62.5 mM Tris-HCl, pH 6.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Buffer Type</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.192 M Glycine</td>
<td>192 mM Glycine</td>
</tr>
<tr>
<td>0.05 – 0.1% SDS*</td>
<td>0.1% SDS*</td>
</tr>
<tr>
<td>20% Methanol</td>
<td>0.01% Bromophenol Blue</td>
</tr>
<tr>
<td>2.5% βME</td>
<td>(2-mercaptoethanol)*</td>
</tr>
</tbody>
</table>

NOTE: Limits are based on optimal detection methods for each stain.

Protein stain detection limits

<table>
<thead>
<tr>
<th>Protein Stain</th>
<th>Lower Detection Limit (Protein / Band)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coomassie® Blue Stain</td>
<td>30 ng</td>
</tr>
<tr>
<td>Silver Stain</td>
<td>2 ng</td>
</tr>
<tr>
<td>SYPRO® Red Protein Gel Stain</td>
<td>4 ng – 8 ng</td>
</tr>
<tr>
<td>SYPRO® Ruby Protein Gel Stain</td>
<td>2 ng – 8 ng</td>
</tr>
<tr>
<td>SYPRO® Tangerine Protein Gel Stain</td>
<td>4 ng – 8 ng</td>
</tr>
</tbody>
</table>
Q. **What is the best membrane to use for Western blotting?**

A. Use this table to find a suitable membrane.

<table>
<thead>
<tr>
<th>Nitrocellulose</th>
<th>PVDF</th>
<th>Nylon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrophobic binding</td>
<td>Hydrophobic binding</td>
<td>Hydrophobic and electrostatic binding</td>
</tr>
<tr>
<td>General purpose membrane</td>
<td>SDS tolerant</td>
<td>Stable if baked</td>
</tr>
<tr>
<td>Low background</td>
<td>High background</td>
<td>High background</td>
</tr>
<tr>
<td>Low strength</td>
<td>High strength</td>
<td>High strength</td>
</tr>
<tr>
<td>Becomes brittle if baked</td>
<td>Suitable for protein sequencing</td>
<td>Least suitable for Western transfer</td>
</tr>
</tbody>
</table>

Q. **What are the benefits of using agarose for protein gel electrophoresis?**

A. Protein electrophoresis in agarose gels is an alternative approach to using polyacrylamide gels and provides several benefits:

- Separate high molecular weight proteins (> 600 kDa)
- Easy to prepare and handle
- Efficient recovery of proteins
- Excised proteins can be used to immunize animals directly for antibody production
- Non-toxic
- Run gels using either a vertical or horizontal apparatus

**ProSieve® Protein Markers**

Q. In what applications do you recommend using the ProSieve® Color Protein Marker vs. the ProSieve® Protein Marker?

A. The ProSieve® Color Protein Markers are ideal for monitoring protein separation during electrophoresis and confirming protein transfer in Western blotting. They are not recommended for precise sizing of protein samples in SDS-PAGE. The ProSieve® Protein Markers are recommended for the most accurate sizing of protein samples in SDS-PAGE. These markers contain 10 proteins with exact masses of 5 kDa, 10 kDa, 15 kDa, 25 kDa, 35 kDa, 50 kDa, 75 kDa, 100 kDa, 150 kDa, and 225 kDa.
Section II: Preparation of Agarose Gels

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Agarose Types 77
Agarose Analytical Specifications 79
Suggested Agarose Concentrations & Dye Migration Information 80
Buffers for Electrophoresis 81
Dissolving Agarose 83
Casting Agarose Gels 85
Section II: Preparation of Agarose Gels

Agarose Selection Guide

Selecting the best agarose for your application can minimize opportunity for error, optimize results, and even reduce cost. Lonza offers a wide range of agarose types that are specifically engineered to optimize results by fragment size, sample type and application. The selection tools below will get you started. The following pages will guide you to the right concentration, buffer and marker to use for best performance in your experiment.

Choose the Agarose that is Right for You

Agarose and Compatible Techniques

<table>
<thead>
<tr>
<th>Recovery Method</th>
<th>SeaKem® LE</th>
<th>SeaKem® GTG®</th>
<th>SeaPlaque®</th>
<th>SeaPlaque® GTG®</th>
<th>NuSieve®</th>
<th>NuSieve® GTG®</th>
<th>MetaPhor®</th>
<th>SeaPrep®</th>
<th>InCert®</th>
<th>L.D. NA®</th>
</tr>
</thead>
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<tr>
<td>In-Gel Reactions</td>
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<td></td>
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<td>✓</td>
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<td>✓</td>
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<td>✓</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>Phenol/Chloroform</td>
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<td>✓</td>
<td>✓</td>
<td>✓</td>
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<td>✓</td>
</tr>
<tr>
<td>Electroelution</td>
<td></td>
<td></td>
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<td>✓</td>
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<tr>
<td>Freeze/Squeeze</td>
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<tr>
<td>Southern &lt;1 kb</td>
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<td>✓</td>
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<tr>
<td>Southern &gt;1 kb</td>
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<td>Northern &lt;1 kb</td>
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<td>Northern &gt;1 kb</td>
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<td>Viral Plaque Assays</td>
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<td>Preparation of Megabase Samples</td>
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<td>PFGE</td>
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<td>Cell Culture</td>
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<td>Encapsulation &amp; Embedding of Cells</td>
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<td>DNA Identity Testing</td>
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<tr>
<td>Comet Assays</td>
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<td>✓</td>
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</tr>
</tbody>
</table>

Our agarose is GUARANTEED DNase/RNase Free
Section II: Preparation of Agarose Gels

Agarose Types

Introduction

Nucleic acid applications take advantage of the specific properties of different agaroses and derivatized agaroses. The appropriate choice of agarose depends on the size of the DNA to be analyzed and any subsequent manipulations required. Gelling/melting temperatures, electroendosmosis and gel strength are all important factors in choosing the right agarose for your application. Refer to page 79 for analytical specifications of Lonza agarose.

Genetic Technology Grade™ (GTG®) Agaroses

Our Genetic Technology Grade™ (GTG®) Agarose Products are specially prepared and certified for demanding molecular biology applications for nucleic acids, including PCR amplified products. Lonza’s GTG® Agarose quality tests go beyond standard assays such as, DNase and RNase testing, to include enzymatic performance measurements. Our additional testing provides a more realistic index of overall product quality and reliability. You no longer need to screen agarose lots to find those that yield biologically active DNA.

- The following agaroses are GTG® Certified:
  - SeaKem® GTG® Agarose
  - SeaPlaque® GTG® Agarose (low melting temperature agarose)
  - NuSieve® GTG® Agarose (low melting temperature agarose)
  - SeaKem® Gold Agarose

- Lonza performs the following tests on GTG® Certified Agaroses:
  - DNA binding
  - DNase and RNase activity
  - DNA resolution
  - Gel background-gel exhibits low background fluorescence after ethidium bromide staining
  - In-gel cloning (low melt agarose)
  - In-gel restriction digestion (low melt agarose)
  - Restriction-ligation assay (SeaKem® GTG®)

Molecular Biology Grade Agaroses

Molecular biology grade agaroses are suitable for general analytical separation of DNA.

- The following agaroses are considered molecular biology grade agaroses:
  - MetaPhor® Agarose
  - SeaKem® LE Agarose
  - NuSieve® 3:1 Agarose
  - SeaPlaque® Agarose (low melting temperature agarose)

- Lonza screens our molecular biology grade agaroses for the following parameters:
  - DNA binding
  - DNase and RNase activity
  - Gel background staining

FDA Listing

Lonza agarose types are listed as Class 1 Medical Devices under registration number 1219614.
Section II: Preparation of Agarose Gels

Agarose Types — continued

DNA resolution examples

The photographs below show the different resolution properties of Lonza agaroses.

Separation of DNA markers in 1% SeaKem® GTG® and SeaPlaque® GTG® Agarose gels in 1X TBE Buffer [Prepared from AccuGENE® 10X TBE Buffer].
Lane A: Lonza's 50 - 2,500 bp DNA Marker (~0.25 ng/band)
Lane B: New England BioLabs Hind III digest of lambda DNA (0.125 mg/lane) 20 cm long gels were run at 6 V/cm for ~ 2.5 hrs. Gels were post stained using Lonza’s 1X GelStar® Nucleic Acid Gel Stain for 30 minutes. No destain.

Resolution Performance of Lonza agaroses for DNA <1 kb
Separation of DNA markers in 3% NuSieve® 3:1, NuSieve® GTG® and MetaPhor® Agarose gels in 1X TBE [Prepared from AccuGENE® 10X TBE Buffer].
Lane A: Lonza's 50 - 1,000 bp DNA Marker (~25 ng/band)
Lane B: New England BioLabs Msp I digest of pBR322 [0.125 mg/lane] 20 cm long gels were run at 6 V/cm for 2 hrs. & 20 mins. Gels were post stained using Lonza’s 1X GelStar® Nucleic Acid Gel Stain for 30 minutes. No destain.
Introduction

Lonza performs routine analytical testing on every lot of agarose to ensure lot-to-lot consistency for the critical characteristics that can impact functional performance. Specifications for properties such as gel strength, gelling temperature, electroendosmosis, and others are established at the optimal level for the target applications of the various agarose types. However, analytical specifications alone may not be sufficient for judging the best agarose for a specific application, nor are they the best means to compare agaroses from different vendors. The best way to find out if an agarose is right for your application, is to speak to our Scientific Support representatives, and test a sample of the recommended agarose type(s) based on your application. Lonza has extensive capabilities for modification of agarose properties to fit unique applications and requirements. Ask about our custom capabilities if one our standard products does not meet your needs.

Agarose Analytical Specifications

<table>
<thead>
<tr>
<th>Agarose</th>
<th>Melting Temperature</th>
<th>Gel Strength g/cm²</th>
<th>Gelling Temperature</th>
<th>EEO [-mr]</th>
<th>Moisture</th>
<th>Sulfate</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA &lt;1 kb</td>
<td>NuSieve® 3:1</td>
<td>≤90ºC at 4%</td>
<td>± 1,400 at 4%</td>
<td>32.5ºC – 38ºC at 4%</td>
<td>≤0.13</td>
<td>≤10%</td>
</tr>
<tr>
<td></td>
<td>MetaPhor®</td>
<td>≤75ºC at 3%</td>
<td>± 300 at 3%</td>
<td>≤35ºC at 3%</td>
<td>≤0.05</td>
<td>≤10%</td>
</tr>
<tr>
<td></td>
<td>NuSieve® GTG®</td>
<td>≤65ºC at 4%</td>
<td>±500 at 4%</td>
<td>≤35ºC at 4%</td>
<td>≤0.15</td>
<td>≤10%</td>
</tr>
<tr>
<td>DNA &gt;1 kb</td>
<td>SeaKem® LE</td>
<td>NA</td>
<td>±1,200 at 1%</td>
<td>36ºC ± 1.5ºC at 1.5%</td>
<td>0.09 – 0.13</td>
<td>≤10%</td>
</tr>
<tr>
<td></td>
<td>SeaKem® GTG®</td>
<td>NA</td>
<td>±1,200 at 1%</td>
<td>36ºC ± 1.5ºC at 1.5%</td>
<td>0.09 – 0.13</td>
<td>≤10%</td>
</tr>
<tr>
<td></td>
<td>SeaPlaque®</td>
<td>≤65ºC at 1.5%</td>
<td>±200 at 1%</td>
<td>26ºC – 30ºC at 1.5%</td>
<td>≤0.10</td>
<td>≤10%</td>
</tr>
<tr>
<td></td>
<td>SeaPlaque® GTG®</td>
<td>≤65ºC at 1.5%</td>
<td>±200 at 1%</td>
<td>26ºC – 30ºC at 1.5%</td>
<td>≤0.10</td>
<td>≤10%</td>
</tr>
<tr>
<td>PFGE</td>
<td>SeaKem® Gold</td>
<td>NA</td>
<td>±1,800 at 1%</td>
<td>34.5ºC – 37.5ºC at 1.5%</td>
<td>≤0.05</td>
<td>≤10%</td>
</tr>
<tr>
<td></td>
<td>InCert®</td>
<td>≤70ºC at 1.5%</td>
<td>±350 at 1%</td>
<td>26ºC – 30ºC at 1.5%</td>
<td>≤0.10</td>
<td>≤10%</td>
</tr>
<tr>
<td>Identity Testing</td>
<td>I.D.NA®</td>
<td>NA</td>
<td>±1,300 at 1%</td>
<td>36ºC ± 1.5ºC at 1.5%</td>
<td>≤0.10</td>
<td>≤10%</td>
</tr>
<tr>
<td>Protein Electrophoresis</td>
<td>SeaKem® ME</td>
<td>NA</td>
<td>±1,000 at 1%</td>
<td>36ºC ± 1.5ºC at 1.5%</td>
<td>0.16 – 0.19</td>
<td>≤10%</td>
</tr>
<tr>
<td></td>
<td>SeaKem® HE</td>
<td>NA</td>
<td>±650 at 1%</td>
<td>36ºC ± 1.5ºC at 1.5%</td>
<td>0.23 – 0.26</td>
<td>≤10%</td>
</tr>
<tr>
<td></td>
<td>SeaKem® HEE0</td>
<td>NA</td>
<td>±650 at 1%</td>
<td>36ºC ± 1.5ºC at 1.5%</td>
<td>≤0.30</td>
<td>≤10%</td>
</tr>
<tr>
<td></td>
<td>SeaKem® HGT</td>
<td>NA</td>
<td>±800 at 1%</td>
<td>42ºC ± 1.5ºC at 1.5%</td>
<td>≤0.10</td>
<td>≤10%</td>
</tr>
<tr>
<td>von Willenbrand’s Factor Separation</td>
<td>SeaKem® HGT(P)</td>
<td>NA</td>
<td>±1,000 at 1%</td>
<td>42ºC ± 1.5ºC at 1.5%</td>
<td>≤0.10</td>
<td>≤10%</td>
</tr>
<tr>
<td>Isoelectric Focusing</td>
<td>IsoGel®</td>
<td>NA</td>
<td>±500 at 1.5%</td>
<td>35ºC – 45ºC</td>
<td>Not Detectable</td>
<td>≤10%</td>
</tr>
<tr>
<td>Cell Culture</td>
<td>SeaPrep®</td>
<td>≤50ºC at 1%</td>
<td>±75 at 2%</td>
<td>8ºC – 12ºC at 0.8%</td>
<td>≤0.05</td>
<td>≤10%</td>
</tr>
</tbody>
</table>
## Preparation of Agarose Gels

### Suggested Agarose Concentrations and Dye Migration Information

#### Table 1: Suggested agaroses for particular applications

<table>
<thead>
<tr>
<th>Size Range (Base Pairs)</th>
<th>Agarose Type</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 – 800</td>
<td>MetaPhor® Agarose</td>
<td>High resolution analysis; 2% size differences</td>
</tr>
<tr>
<td>50 – 1,000</td>
<td>NuSieve® 3:1 Agarose</td>
<td>Analysis and blotting; 4% – 6% size differences resolved</td>
</tr>
<tr>
<td></td>
<td>NuSieve® GTG® Agarose</td>
<td>Analysis and blotting; In-gel; 6% size differences resolved</td>
</tr>
<tr>
<td>1,000 – 10,000</td>
<td>SeaKem® GTG® Agarose</td>
<td>Analysis and blotting; recovery required</td>
</tr>
<tr>
<td></td>
<td>SeaPlaque® GTG® Agarose</td>
<td>In-gel</td>
</tr>
<tr>
<td>10,000</td>
<td>SeaKem® Gold Agarose</td>
<td>Analysis</td>
</tr>
</tbody>
</table>

#### Table 2: Properties of TAE and TBE Buffer Systems

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Suggested Uses and Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAE Buffer</td>
<td>Use when DNA is to be recovered. Use for electrophoresis of large (&gt;12 kb) DNA. Low ionic strength. Low buffer capacity – recirculation may be necessary for extended electrophoretic times.</td>
</tr>
<tr>
<td>TBE Buffer</td>
<td>Use for electrophoresis of small (&lt;1 kb) DNA. Decreased DNA mobility. High ionic strength. High buffer capacity – no recirculation required for extended run times.</td>
</tr>
</tbody>
</table>

#### Table 3: Suggested agarose concentrations for DNA sizes

<table>
<thead>
<tr>
<th>Size Range (Base Pairs)</th>
<th>Final Agarose Concentration % (w/v)</th>
<th>1X TAE Buffer</th>
<th>1X TBE Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>SeaKem® LE and SeaKem® GTG® Agarose</td>
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<td>0.60</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>800 – 10,000</td>
<td>0.80</td>
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<tr>
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<td>300 – 7,000</td>
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<td>200 – 4,000</td>
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<td>100 – 3,000</td>
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</tr>
<tr>
<td>NuSieve® 3:1 Agarose</td>
<td>500 – 1,000</td>
<td>3.0</td>
<td>2.0</td>
</tr>
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<td>3.0</td>
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<td>10 – 100</td>
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<tr>
<td>MetaPhor® Agarose</td>
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<td>1.8</td>
</tr>
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<tr>
<td></td>
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<tr>
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</tr>
<tr>
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<td></td>
<td>150 – 700</td>
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<tr>
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<td>1,000 – 20,000</td>
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<tr>
<td></td>
<td>500 – 10,000</td>
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<td>—</td>
</tr>
<tr>
<td></td>
<td>400 – 6,000</td>
<td>1.0</td>
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</tr>
</tbody>
</table>

† TBE Buffer is not recommended for separation of DNA >12,000 bp.

#### Table 4: Migration of double-stranded DNA in relation to Bromophenol Blue (BPB) and Xylene Cyanol (XC) in agarose gels

<table>
<thead>
<tr>
<th>Buffer</th>
<th>1X TAE Buffer</th>
<th>1X TBE Buffer</th>
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</thead>
<tbody>
<tr>
<td>BPB</td>
<td>% Agarose</td>
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<tr>
<td>SeaKem® LE and SeaKem® GTG® Agarose</td>
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<td></td>
<td>11,000</td>
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<td></td>
<td>6,100</td>
<td>500</td>
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<td></td>
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<tr>
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<td>85</td>
<td>30</td>
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<td>85</td>
<td>&lt;20</td>
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<td>SeaKem® Gold Agarose</td>
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</tr>
<tr>
<td></td>
<td>1,500</td>
<td>250</td>
</tr>
</tbody>
</table>
Introduction

During electrophoresis, water is electrolyzed, which generates protons at the anode, and hydroxyl ions at the cathode. The cathodal end of the electrophoresis chamber then becomes basic and the anodal end acidic. The use of a buffering system is therefore required when charged molecules are electrophoresed through a separation medium. The two buffers commonly used for DNA electrophoresis are Tris-acetate with EDTA (TAE; 40 mM Tris-acetate, 1 mM EDTA) and Tris-borate with EDTA (TBE; 89 mM Tris-borate, 2 mM EDTA). Because the pH of these buffers is neutral, the phosphate backbone of DNA has a net negative charge and migrates towards the anode.

Properties of TAE and TBE Buffer Systems

Despite the apparent similarity of TAE and TBE Buffers, each has different properties which make it best suited for different applications (see table below).

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Suggested Uses and Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAE</td>
<td>Use when DNA is to be recovered Use for electrophoresis of large (&gt;12 kb) DNA Low buffering capacity – recirculation may be necessary for extended electrophoretic times (&gt;6 hours)</td>
</tr>
<tr>
<td>TBE</td>
<td>Use for electrophoresis of small (&lt;1 kb) DNA Increased resolution of small (&lt;1 kb) DNA Decreased DNA mobility High buffering capacity – no recirculation required for extended run times</td>
</tr>
</tbody>
</table>

Lanza offers ready-to-go buffers for electrophoresis. Refer to AccuGENE® Buffer products on page 41.

DNA Electrophoresis in TAE or TBE Buffer

When DNA will not be recovered, either 1X TAE or TBE (1X or 0.5X) Buffer is suitable for use when the DNA is less than 12 kb to 15 kb. For larger DNA, the best buffer to use for electrophoresis is TAE in combination with a low field strength (1 -2 V/cm). During these extended electrophoretic runs, larger apparent gel porosity, lower EEO and low field strength decrease the tendency of large DNA to smear.

TBE Buffer is preferred for separation of small DNA (<1 kb) when DNA recovery is not required. TBE Buffer’s interaction with agarose results in a smaller apparent pore size. The tighter gel reduces the broadening of DNA bands due to dispersion and diffusion.
Section II: Preparation of Agarose Gels

Buffers for Electrophoresis — continued

Buffer Depth

Whichever buffer is used, the depth over the gel in a horizontal electrophoretic system should be 3 mm - 5 mm. Excessive buffer depth will decrease DNA mobility, promote band distortion and can cause excessive heating within the system. Less buffer and the gel may dry out during electrophoresis.

The photograph below depicts the effect of buffer depth on DNA electrophoresis. The DNA mobility in the gel with a 10 mm buffer overlay is slower than the gel with a 3 mm buffer overlay. In the *Hae* III digested *φ*X174 marker, the 281/271 bands are starting to resolve in the gel with the 3 mm buffer overlay whereas in the gel with the 10 mm buffer overlay they are not.

![Buffer Depth Diagram](image)

Other Buffering Systems

Tris-phosphate Buffer (TPE) may also be used for DNA electrophoresis. Like TBE Buffer, TPE has a high buffering capacity, and will not interfere with DNA recovery procedures. However, TPE can not be used when recovered DNA will be used in a phosphate-sensitive reaction.

10X Tris-phosphate Stock [TPE]

\(1X=90 \text{ mM Tris base, } 90 \text{ mM phosphoric acid, } 2 \text{ mM EDTA}\)

- 108.0 g Tris base
- 15.5 ml 85% Phosphoric acid
- 7.44 g Na₂EDTA • 2H₂O

To 1 liter with distilled water

Alkaline electrophoresis buffer is used for the analysis of single-stranded DNA.

Buffer Preparation

Alternatively, Lonza offers AccuGENE® TBE Buffer in 5X and 10X stock solutions and AccuGENE® TAE Buffer in 10X and 50X stock solutions.

50X TAE (Tris-acetate) stock

\(1X=40 \text{ mM Tris base, } 40 \text{ mM acetic acid, } 1 \text{ mM EDTA}\)

- 242.0 g Tris base
- 57.1 ml Glacial acetic acid
- 18.6 g Na₂EDTA • 2H₂O

To 1 liter with distilled water

Buffer Preparation

Alternatively, Lonza offers AccuGENE® TBE Buffer in 5X and 10X stock solutions and AccuGENE® TAE Buffer in 10X and 50X stock solutions.

5X TBE (Tris-borate) stock

\(0.5X=45 \text{ mM Tris-borate, } 1 \text{ mM EDTA}\)

- 54.0 g Tris base
- 27.5 g Boric acid
- 3.72 g Na₂EDTA • 2H₂O

To 1 liter with distilled water

Effects of buffer depletion and development of a pH gradient can be reduced by recirculating the buffer. This is usually necessary only when electrophoresis is done for extended times or the electrophoresis buffer has a low buffering capacity.

Buffer Depletion

The rate of buffer depletion is influenced by the buffer used and its buffering capacity. Evidence of buffer depletion is gel melting, smearing of DNA and/or overheating. A 0.5X TBE Buffer has greater buffering capacity than a 1X TAE Buffer at the pH used because the pKₐ of borate is closer to the initial Buffer pH than that of acetate. Standard-sized electrophoresis chambers (15 cm x 30 cm) with a 1.5 L to 2 L capacity will tolerate 40 to 50 Watt hours before buffer depletion, and buffer depletion will not occur in mini-electrophoresis chambers for 10 to 13 Watt hours. Consult the electrophoresis chamber manufacturer for specific values.

Separation of DNA markers in a 3% NuSieve® 3:1 Agarose gel prepared and run in 1X TBE Buffer.

Lane A: DNA marker 50 bp - 1,000 bp DNA Marker (Lonza); 0.4 μg/lane.

Lane B: *Hae* III digested *φ*X174 DNA (New England Biolabs); 0.5 μg/lane.

The gels were run at 5 V/cm until the bromophenol blue tracking dye was 1 cm from the bottom of each gel.

![Separation of DNA Markers](image)
Section II: Preparation of Agarose Gels

Dissolving Agarose

Introduction

Agarose undergoes a series of steps when it is dissolved; dispersion, hydration and melting/dissolution.

Dispersion

Dispersion simply refers to the separation of the particles by the buffer without clumping. Clumping occurs when the agarose starts to dissolve before it is completely dispersed, coating itself with a gelatinous layer which inhibits the penetration of water and keeps the powder inside from dispersing. Dispersion then becomes a long process.

Hydration

Hydration is the surrounding of agarose particles by a solution (e.g., water or running buffer). Problems are sometimes encountered with hydration when using a microwave oven to dissolve agarose. In part, this occurs because hydration is time dependent and microwave ovens bring the temperature up rapidly. The problem is exacerbated by the fact that the agarose is not being agitated to help dilute the highly concentrated solution around each particle and dissolution is slowed.

Melting and Dissolution

The final stage in dissolving the agarose is the melting and dissolution. Melting can be done in either a microwave oven or on a hot plate. As the particles hydrate, they become small, highly concentrated gels. Since the melting temperature of a standard agarose gel is about 93°C, merely heating a mixture to 90°C will not completely dissolve agarose. Even low melting temperature agaroses should be boiled to ensure that all the agarose is fully dissolved. Microwave instructions for agarose preparation for gel concentrations of ≤2% w/v

Microwave Instructions for Gel Concentrations <2%w/v

1. Choose a beaker that is 2 - 4 times the volume of the solution.
2. Add room temperature 1X or 0.5X buffer and a stir bar to the beaker.
3. Sprinkle in the premeasured agarose powder while the solution is rapidly stirred.
4. Remove the stir bar if not Teflon® coated.
5. Weigh the beaker and solution before heating.
6. Cover the beaker with plastic wrap.
7. Pierce a small hole in the plastic wrap for ventilation.
8. Heat the beaker in the microwave oven on HIGH power until bubbles appear.
   - **Caution:** Any Microwave solution may become superheated and foam over when agitated.
9. Remove the beaker from the microwave oven.
10. GENTLY swirl the beaker to resuspend any settled powder and gel pieces.
11. Reheat the beaker on HIGH power until the solution comes to a boil.
12. Hold at boiling point for 1 minute or until all of the particles are dissolved.
13. Remove the beaker from the microwave oven.
   - **Caution:** Use oven mits when removing beaker from microwave, as container will be hot and may cause burns.
14. GENTLY swirl the beaker to mix the agarose solution thoroughly.
15. After dissolution, add sufficient hot distilled water to obtain the initial weight.
16. Mix thoroughly.
17. Cool the solution to 60°C prior to casting.

Materials

- Microwave oven or hot plate
- Beaker that is 2 - 4 times the volume of the solution
- Teflon®-coated magnetic stir bar
- Magnetic stir plate
- Plastic wrap
- Oven mitts or other heat protection for hands

Reagents

- Distilled water
- 1X TAE, 1X TBE or 0.5X TBE Electrophoresis Buffer
- Agarose powder

   - **Caution:** Always wear eye protection, and guard yourself and others against scalding solutions.
Section II: Preparation of Agarose Gels

Dissolving Agarose — continued

**Microwave instructions**

For agarose preparation for gel concentrations ≥ 2% w/v

1. Choose a beaker that is 2 - 4 times the volume of the solution.
2. Add room temperature or chilled buffer (for MetaPhor® and NuSieve® GTG® Agarose) and a stir bar to the beaker.
3. Sprinkle in the premeasured agarose powder while the solution is rapidly stirred to prevent the formation of clumps.
4. Remove the stir bar if not Teflon® coated.
5. Soak the agarose in the buffer for 15 minutes before heating. This reduces the tendency of the agarose solution to foam during heating.
6. Weigh the beaker and solution before heating.
7. Cover the beaker with plastic wrap.
8. Pierce a small hole in the plastic wrap for ventilation.
   - For agarose concentrations >4%, the following additional steps will further help prevent the agarose solution from foaming during melting/dissolution:
   8a. Heat the beaker in the microwave oven on MEDIUM power for 1 minute.
   8b. Remove the solution from the microwave.
   8c. Allow the solution to sit on the bench for 15 minutes.
9. Heat the beaker in the microwave oven on MEDIUM power for 2 minutes.
   - **Caution:** Any microwaved solution may become superheated and foam over when agitated.
10. Remove the beaker from the microwave oven.
   - **Caution:** Use oven mits when removing beaker from microwave, as container will be hot and may cause burns.
11. GENTLY swirl to resuspend any settled powder and gel pieces.
12. Reheat the beaker on HIGH power for 1 - 2 minutes or until the solution comes to a boil.
13. Hold at the boiling point for 1 minute or until all of the particles are dissolved.
14. Remove the beaker from the microwave oven.
15. GENTLY swirl to mix the agarose solution thoroughly.
16. After dissolution, add sufficient hot distilled water to obtain the initial weight.
17. Mix thoroughly.
18. Cool the solution to 60°C prior to gel casting.

**Hot plate instructions for preparing agarose**

1. Choose a beaker that is 2 - 4 times the volume of the solution.
2. Add room temperature or chilled buffer (for MetaPhor® or NuSieve® GTG® Agarose) and a stir bar to the beaker.
3. Sprinkle in the premeasured agarose powder while the solution is rapidly stirred to prevent the formation of clumps.
4. Weigh the beaker and solution before heating.
5. Cover the beaker with plastic wrap.
6. Pierce a small hole in the plastic wrap for ventilation.
7. Maintain gentle boiling until the agarose is dissolved (approximately 5 -10 minutes).
8. Add sufficient hot distilled water to obtain the initial weight.
9. Mix thoroughly.
10. Cool the solution to 60°C prior to casting.

**Materials**

- Microwave oven or hot plate
- Beaker that is 2 - 4 times the volume of the solution
- Teflon®-coated Magnetic Stir Bar
- Magnetic stir plate
- Plastic wrap

**Reagents**

- Distilled water
- 1X TAE, 1X TBE or 0.5X TBE Electrophoresis Buffer
- Agarose powder

**Caution:** Always wear eye protection, and guard yourself and others against scalding solutions.
Introduction

For optimal resolution, cast horizontal gels 3 mm - 4 mm thick (see figure below). The volume of gel solution needed can be estimated by measuring the surface area of the casting chamber, then multiplying by the gel thickness. Thinner gels can be cast on GelBond® Film and/or in a vertical electrophoresis apparatus.

The photographs below depict the effect of gel depth on DNA electrophoresis. Gel thickness has a profound effect on the resolution of smaller fragments. The smaller DNA fragments in the 10 mm thick gel are fuzzy, whereas in the 3 mm thick gel the resolution is sharp throughout the gel. There is also a higher background staining in gels thicker than 5 mm.

Separation of DNA markers in 1% SeaKem® GTG® Agarose gels prepared and run in 1X TBE Buffer.
Lane A: Hind III digest of lambda DNA (Boehringer Mannheim); 0.1 μg/lane.
Lane B: Hae III digested φX174 DNA (New England Biolabs); 0.5 μg/lane. 20 cm long gels were run at 6 V/cm for 2 hours 15 minutes (10 mm thick gel) and 2 hours 10 minutes (3 mm thick gel).

Lonza offers a wide range of precast gels.
Refer to page 17 for information.

Horizontal gel casting instructions

1. Allow the agarose solution to cool to 60°C.
2. While the agarose solution is cooling:
   2a. Assemble the gel casting tray.
   2b. Level the casting tray prior to pouring the agarose solution.
   2c. Check the comb(s)* teeth for residual dried agarose. Dried agarose can be removed by scrubbing the comb teeth with a lint-free tissue soaked in hot distilled water.
   2d. Allow a small space (approximately 0.5 mm - 1 mm) between the bottom of the comb teeth and the casting tray.
3. Pour the agarose solution into the gel tray.
4. Replace the comb(s).
5. Allow the agarose to gel at room temperature for 30 minutes.
6. Low melting temperature agaroses and MetaPhor® Agarose require an additional 30 minutes of gelling at 4°C to obtain the best gel handling. The additional cooling step is essential for obtaining fine resolution in MetaPhor® Agarose.
7. Once the gel is set, flood with running buffer.
8. Slowly remove the comb.
9. Place the gel casting tray into the electrophoresis chamber.
10. Fill the chamber with running buffer until the buffer reaches 3 mm - 5 mm over the surface of the gel.
11. Gently flush the wells out with electrophoresis buffer using a Pasteur pipette to remove loose gel fragments prior to loading the samples.
12. Load DNA and electrophorese.

*The thickness of the comb in the direction of the electric field can affect the resolution. A thin comb (1 mm) will result in sharper DNA bands. With a thicker comb, more volume can be added to the well but the separated DNA bands may be broader.

Materials
- Horizontal electrophoresis apparatus
- Combs
- Pasteur pipette

Reagents
- Agarose solution
- Electrophoresis buffer
Vertical Gel Casting Instructions

Follow the steps below to cast a vertical agarose gel. This protocol is divided into the following segments:

- Cassette assembly
- Cassette sealing
- Casting the gel
- Preparing for electrophoresis

Cassette assembly

Unlike polyacrylamide gels, agarose gels do not adhere to glass plates and may slide out during electrophoresis. To prevent this from happening, frosted glass plates or plastic plates can be used.

Follow the steps below to assemble the glass plates.

1. Use clean glass plates. Clean with soap and water, rinse with distilled water and dry.
2. Wipe the plates with ethanol and a lint-free tissue.
3. Place two side spacers on the back plate. Follow the steps below if using glass plates.
   3a. For 1 mm thick standard size gels, cut a strip of Whatman® 3MM Chromatography paper (1 mm thick and 5 - 10 mm wide) long enough to fit between the two spacers.*
   3b. Wet with running buffer.
   3c. Place at the bottom of the back plate in contact with the spacers on each side (see Figure 1, page 87).
4. Put on the front plate.
5. Clamp the glass plates together.
6. Use the manufacturer’s casting apparatus or seal the cassette against leaks with silicone tubing or tape.

*Alternatively, use GelBond® Film as a support for the gel, which obviates the need for the use of Whatman® 3MM Chromatography paper to hold the gel in the cassette during electrophoresis (see Chapter IX). GelBond® Film is put into the casting cassette and the gel attaches to the film during the gelling process. After electrophoresis, the gel may be dried down on the GelBond® Film and kept as a permanent record. However, GelBond® Film blocks UV light below 300 nm and exhibits background fluorescence. To overcome these problems, gels cast on GelBond® Film may be photographed inverted (gel side down) on the UV light box. Background fluorescence can be screened out by using red, orange (Wratten® #22 or #25 gelatin filter) and UV filters (Wratten® #2B gelatin filter).

Materials

- Vertical electrophoresis apparatus
- Combs and side spacers
- Whatman® 3MM Chromatography Paper
- Clamps
- Silicone tubing or electrical tape
- Two 60 ml syringes with 16-gauge needles
- Heat gun or 55°C oven
- Scalpel or razor blade

Reagents

- Agarose solution
- Electrophoresis buffer
Cassette Sealing

Follow one of the methods below to seal the cassette prior to casting the gel.

Silicone tubing method

1. Use silicone tubing which is the same diameter as the spacer thickness.
2. Cut a piece long enough to extend along the bottom and up both sides of the cassette.
3. Place the tubing across the bottom of the back plate below the blotting paper strip (see Figure 2).
4. Place the top plate over the bottom plate.
5. Clamp the glass plates together at the bottom.
6. Run the tubing up either side of the plates and finish clamping the plates together (see Figure 3).

Tape method

1. Place the top plate over the bottom plate.
2. Tape the sides of the cassette with separate pieces of tape.
3. Tape the bottom of the cassette with a separate piece of tape. This way the tape on the bottom can be removed for electrophoresis without disturbing the tape at the sides of the gel (see Figure 4).
4. Clamp the plates together.

Casting a Vertical Agarose Gel

1. Prepare agarose solution as described previously.
2. Pre-warm the assembled cassette and a 60 ml syringe for 15 minutes by placing in a 55°C oven or by using a heat gun.
3. Cool the dissolved agarose to 60°C.
4. Pour into a pre-warmed 60 ml syringe fitted with a 16-gauge needle.
5. Wedge the needle tip between the plates in the upper corner of the cassette with the needle opening directed toward the back plate (see Figure 5).
6. Inject the agarose solution at a moderate, steady rate. Keep a constant flow to prevent air bubble formation (see Figure 5).
7. Angle the cassette while pouring so the agarose solution flows down one side spacer, across the bottom and up the other side.
8. Fill until the agarose solution goes just above the glass plates.
9. Insert one end of the comb, then slowly insert the rest of the comb until the teeth are at an even depth. Insert the comb into the agarose to the minimal depth necessary to accommodate your samples (see Figure 6, page 88).
10. Place extra clamps on the side of the glass plates to hold the comb in place.
11. Cool the gel at room temperature for 15 minutes.
12. Place the gel at 4°C for 20 minutes.
13. Remove the clamps at the top of the gel.
14. Remove any excess agarose with a scalpel or razor blade.
15. Squirt running buffer in the spaces between the comb and the gel.
16. Slowly and gently lift the comb straight up. Allow air or buffer to enter the well area to release the vacuum which forms between the agarose and the comb.
17. The wells can be further cleaned by flushing with running buffer.
18. The gel can be stored overnight in a humidity chamber or in a sealed bag with a buffer-dampened paper towel.

Preparing for electrophoresis

1. Remove the silicone tubing or tape at the bottom of the cassette

**NOTE:** If you have placed Whatman® 3MM Chromatography Paper between the plates at the bottom, it is not necessary to remove it as it will not interfere with electrophoresis.

2. Place the cassette into the chamber at an angle to minimize the number of bubbles which can collect in the well area.
3. Rinse out well area with a syringe.

**NOTE:** Since agarose does not adhere well to glass, leave as many clips in place as possible. For some electrophoresis chambers, it is helpful to seal the spacers at the top of the gel with molten agarose.
Section III: Loading and Running DNA in Agarose Gels

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</tbody>
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Section III: Loading and Running DNA in Agarose Gels

DNA Loading

Introduction

The amount of DNA to load per well is variable. Most important are the quantities of DNA in the bands of interest.

Optimal DNA loading amount

The amount of DNA that may be loaded on a gel depends on several factors:

— Well volume

— Fragment size: The capacity of the gel drops sharply as the fragment size increases, especially over a few kilobases

— Distribution of fragment sizes

— Voltage gradient: Higher voltage gradients are better suited to DNA fragments <1 kb, lower voltages are better suited to fragments >1 kb

— Detection Method: The least amount of dsDNA in a single band that can be reliably detected with ethidium bromide is approximately 10 ng, with GelStar® Nucleic Acid Gel Stain is approximately 20 pg and with SYBR® Green I Stain is 60 pg

Overloaded DNA results in trailing and smearing, a problem that will become more severe as the size of the DNA increases.

The photograph below shows the effect of overloaded and underloaded DNA on an agarose gel. Where samples are loaded in excess (0.5 μg/lane), you can see band broadening and smearing of the larger molecular weight fragments. Where samples are underloaded, you lose the small molecular weight fragments. The optimum loading level for the marker used in the photograph is 0.1 μg/lane.

The optimal amount of DNA to load in the well may be calculated by the fraction of the total DNA which is in the band of interest, represented by the following:

\[
\frac{\text{Fragment of interest (kbp)}}{\text{Size of DNA sample (kbp)}} \times 100 = \% \text{ DNA in band of interest}
\]

**NOTE:** The most DNA compatible with a clean sharp band is approximately 100 ng.

For example:
The size of your DNA sample is 48.5 kbp and when run on the gel 8 fragments are separated. Your fragment of interest is 2.3 kbp.

**Calculation:**

\[
\frac{2.3 \text{ kbp}}{48.5 \text{ kbp}} \times 100 = 4.7\% \text{ DNA in fragment of interest}
\]

If you load 1 μg of DNA, then 4.7% of the 1 μg of loaded sample will appear in your fragment of interest (47 ng).

Separation of DNA markers in a 1% SeaKem® GTG® Agarose gel prepared and run in 1X TBE Buffer. Hind III digested lambda DNA (Boehringer Mannheim) was loaded from left to right at 0.025, 0.05, 0.1, 0.2 and 0.5 μg/lane. 20 cm long gels were run at 6 V/cm for 2 hours, 5 minutes.
Section III: Loading and Running DNA in Agarose Gels

Loading Buffers

Introduction

Gel loading buffers serve three purposes in DNA electrophoresis:

- Increase the density of the sample: This ensures that the DNA will drop evenly into the well
- Add color to the sample: Simplifies loading
- Add mobility dyes: The dyes migrate in an electric field towards the anode at predictable rates. This enables one to monitor the electrophoretic process

Loading buffers

At least five loading buffers are commonly used for agarose gel electrophoresis. They are prepared as six-fold concentrated solutions. If needed, 10X solutions of each buffer can also be prepared. Alkaline loading buffer is used when performing alkaline gel electrophoresis.

<table>
<thead>
<tr>
<th>Loading Buffer</th>
<th>6X Recipe</th>
<th>Storage Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose Based</td>
<td>40% (w/v) Sucrose 0.25% Bromophenol Blue 0.25% Xylene cyanol FF</td>
<td>4°C</td>
</tr>
<tr>
<td>Glycerol Based</td>
<td>30% Glycerol in distilled water 0.25% Bromophenol Blue 0.25% Xylene cyanol FF</td>
<td>4°C</td>
</tr>
<tr>
<td>Ficoll® Based</td>
<td>15% Ficoll® (Type 400) Polymer in distilled water 0.25% Bromophenol Blue 0.25% Xylene cyanol FF</td>
<td>room temperature</td>
</tr>
<tr>
<td>Alkaline</td>
<td>300 mM NaOH 6 mM EDTA 18% Ficoll® (Type 400) Polymer in distilled water 0.15% Bromocresol Green 0.25% Xylene cyanol FF</td>
<td>4°C</td>
</tr>
</tbody>
</table>

Ficoll® based loading buffers

To increase the sharpness of DNA bands, use Ficoll® (Type 400) Polymer as a sinking agent instead of glycerol. The use of the lower molecular weight glycerol in the loading buffer allows DNA to stream up the sides of the well before electrophoresis has begun and can result in a U-shaped band. In TBE gels, glycerol also interacts with borate which can alter the local pH.

Sample preparation

Loading buffer that is too high in ionic strength causes bands to be fuzzy and migrate through the gel at unpredictable rates. Ideally, the DNA sample should be resuspended in the same solution as the running buffer. If this is not possible, use a sample buffer with a lower ionic strength than the running buffer.

The photograph below shows the effect of high salt concentrations in loading buffers on DNA resolution.

Separation of Hind III digested lambda DNA (Invitrogen, Inc.) marker (5 μg/lane) in a 1% SeaKem® GTG® Agarose gel prepared and run in 1X TAE Buffer. 20 cm long gels were run at 6 V/cm for 2 hours. The sample buffer was mixed with varying amounts of NaCl to obtain different final salt concentrations.

Lane 1: 4 M NaCl, Lane 2: 3 M NaCl, Lane 3: 1 M NaCl, Lane 4: 0.5 M NaCl, Lane 5: 0.25 M NaCl and Lanes 7 - 9: No addition of NaCl.

Lonza offers ready-to-use DNA loading buffers. Refer to page 41 for information.
Section III: Loading and Running DNA in Agarose Gel

Optimal Voltage and Electrophoretic Times

Optimal voltage

The distance used to determine voltage gradients is the distance between the electrodes, not the gel length. If the voltage is too high, band streaking, especially for DNA Ø12 kb - 15 kb, may result. When the voltage is too low, the mobility of small (≤1 kb) DNA is reduced and band broadening will occur due to dispersion and diffusion.

The photographs below show the effect of voltage on small DNA. The small fragments on the gel run at 1 V/cm show severe band broadening and fuzziness.

The gel run under 5 V/cm has sharp bands both in the small fragments and the larger fragments. Buffer also plays a role in band sharpness.

The photographs below show the effect of voltage and buffer on large DNA. When large DNA is subjected to very high voltage, smearing occurs.

Voltage table

The table below provides a quick reference for optimal voltage for DNA electrophoresis.

<table>
<thead>
<tr>
<th>Size</th>
<th>Voltage</th>
<th>Recovery</th>
<th>Analytical</th>
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</thead>
<tbody>
<tr>
<td>≤1 kb</td>
<td>5 V/cm</td>
<td>TAE</td>
<td>TBE</td>
</tr>
<tr>
<td>1 kb to 12 kb</td>
<td>4 - 10 V/cm</td>
<td>TAE</td>
<td>TAE/TBE</td>
</tr>
<tr>
<td>&gt;12 kb</td>
<td>1 - 2 V/cm</td>
<td>TAE</td>
<td>TAE</td>
</tr>
</tbody>
</table>

NOTE: MetaPhor® Agarose can also be run at very high voltages to achieve 1% - 2% resolution. See “Fast Running Protocols for High Resolution in MetaPhor® Agarose Gels,” this chapter.

Optimal electrophoretic time

The gel should be run until the band of interest has migrated 40% - 60% down the length of the gel (see the Dye Mobility Table). Band broadening resulting from dispersion and diffusion results in a decrease in resolution in the lower third of the gel. Resolution may also be decreased in smaller gels, since longer electrophoretic runs result in greater separation between two fragments.

Separation of Gensura’s 5 kb and 2 kb DNA ladders in 0.5% SeaKem® LE Agarose gels. Odd numbered lanes are 5 kb ladders; even numbered lanes are 2 kb ladders. Lane 1: 5 ng/band; Lane 2: 5 ng/band; Lane 3: 20 ng/band; Lane 4: 20 ng/band; Lane 5: 60 ng/band; Lane 6: 60 ng/band.

Gel 1: 20 cm long gel run at 8 V/cm in 1X TBE Buffer without recirculation.

Gel 2: 21 cm long gel run at 1 V/cm in 1X TAE Buffer with recirculation.

The FlashGel® System (pages 18-25) enables very fast (5 minute) high voltage separation of DNA fragments 10 bp to 10 kb.
Section III: Loading and Running DNA in Agarose Gels

Fast Running Protocols for High Resolution in MetaPhor® Agarose Gels

Introduction

The protocols in the following section describe how to increase resolution to a 1% size difference with DNA between 100 bp and 500 bp, and decrease your electrophoretic time to 1.5 hours.

A standard horizontal submarine gel apparatus can be used to achieve resolution which is comparable to polyacrylamide gels at ≤8%. MetaPhor® Agarose can also be used to achieve similar resolution in a standard vertical gel electrophoretic system.

The photographs below show the resolution achieved in 4% MetaPhor® Agarose gels, using the same markers in three different formats.

Electrophoretic conditions

<table>
<thead>
<tr>
<th></th>
<th>Vertical Format</th>
<th>Rapid Horizontal Format</th>
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</thead>
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<tr>
<td>Resolution</td>
<td>1% (≤500 bp)</td>
<td>1% (≤500 bp)</td>
</tr>
<tr>
<td>Run Time</td>
<td>1 - 1.5 hours</td>
<td>1.5 hours</td>
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<tr>
<td>Gel Concentration</td>
<td>3% - 4%</td>
<td>3% - 4%</td>
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<tr>
<td>Gel Length</td>
<td>16 cm - 26 cm</td>
<td>20 cm</td>
</tr>
<tr>
<td>Gel Thickness</td>
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<td>3 mm</td>
</tr>
<tr>
<td>Gel Buffer</td>
<td>1X TAE or 1X TBE</td>
<td>1X TAE or 1X TBE</td>
</tr>
<tr>
<td>Running Buffer</td>
<td>1X TAE or 1X TBE</td>
<td>0.5X TAE or 0.5X TBE</td>
</tr>
<tr>
<td>TBE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Voltage*</td>
<td>17 V/cm</td>
<td>17 V/cm</td>
</tr>
<tr>
<td>Temperature</td>
<td>ambient</td>
<td>15°C**</td>
</tr>
</tbody>
</table>

*V/cm is determined by the total voltage divided by the interelectrode distance in cm.
**Circulate electrophoresis buffer with a recirculator-chiller water bath.

Materials

- Horizontal electrophoresis chamber to accommodate a 20 cm long gel or vertical electrophoresis chamber
- Power supply
- Recirculator-chiller water bath

Reagents

- Electrophoresis buffer (TAE or TBE)
- MetaPhor® Agarose
- GelStar® or SYBR® Green Nucleic Acid Gel Stains or ethidium bromide solution

Caution: Materials and methods shown here present hazards to the user and the environment. Refer to the safety information on page 210 before beginning these procedures.

Fast running protocol for horizontal gels

NOTE: This protocol cannot be used with only a peristaltic pump in the cold room; the gel will melt.

1. Prepare a 3 - 4% MetaPhor® Agarose gel in 1X electrophoresis buffer.
2. Cast a 3 mm thick, 20 cm long agarose gel.
3. Allow the gel to solidify at room temperature.
4. Place at 4°C for 30 minutes.
5. Place the gel in the electrophoresis chamber.
6. Use 0.5X TBE or 1X TAE Running Buffer in the electrophoresis chamber.
7. Add the running buffer to a depth of 3 mm over the surface of the gel.
8. Load 20 ng - 50 ng of DNA. If this cannot be estimated, load varying amounts of the sample. The sharpest bands are seen with small (5 μl - 10 μl) loading volumes.
9. Run the gel at 17 V/cm (interelectrode distance).
10. When the sample has left the well and moved into the gel, begin recirculating the electrophoresis buffer.
11. Chill and circulate the electrophoresis buffer with a recirculator-chiller water bath.
12. Run the gel for approximately 1.5 hours.
Troubleshooting

The fast running protocol will not work if the buffer is not chilled or recirculated. The photograph to the right shows the effect of DNA resolution on running the gel in a cold room (4°C), without buffer recirculation.

Fast running protocol for vertical gels

1. Cast a vertical agarose gel in 1X electrophoresis buffer following the Vertical Gel Casting Instructions on page 86.
2. Carefully flush the wells with running buffer.
3. Load 20 ng - 50 ng of DNA in the band of interest. If this cannot be estimated, load varying amounts of the sample. The sharpest bands are seen with a small (5 μl - 10 μl) loading volume.
4. Run the gel at 17 V/cm interelectrode distance.
5. Run the gel approximately 1 - 1.5 hours.

NOTE: The gel melted and was allowed to resolidify prior to staining.

References

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### Section IV: Detection and Sizing of DNA in Agarose Gels

#### Guide to Lonza Ladders and Markers

**Size Range** (Bold numbers indicate brighter bands)

<table>
<thead>
<tr>
<th>Size Range</th>
<th>20 bp</th>
<th>20 bp Ext</th>
<th>100 bp</th>
<th>100 bp Ext</th>
<th>Tandem</th>
<th>500 bp</th>
<th>Quant Ladder</th>
<th>Rev Quant Ladder</th>
<th>50 bp-1000 bp</th>
<th>50 bp-2500 bp</th>
<th>1 kb-10 kb</th>
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<td>20 bp – 500 bp</td>
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<td>100 bp – 1,000 bp</td>
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<td>500 bp – 8 kb</td>
<td>100 bp – 1,000 bp</td>
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<td>50 bp – 1,000 bp</td>
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<td>100 bp</td>
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</table>

**Contact Information**

1-800-638-8174  
www.lonza.com/research
Section IV: Detection and Sizing of DNA in Agarose Gels

Guide to Lonza Ladders and Markers — continued

FlashGel® DNA and RNA Markers

<table>
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FlashGel® System Markers and Ladders

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Section IV: Detection and Sizing of DNA in Agarose Gels

Detecting DNA with GelStar®, SYBR® Green I or II Nucleic Acid Gel Stains

Introduction

GelStar® and SYBR® Green Nucleic Acid Gel Stains are highly sensitive fluorescent stains for detecting nucleic acids in agarose gels. Unlike ethidium bromide, these stains fluoresce only upon binding to nucleic acids. Background staining is minimal and destaining of gels is not required. GelStar® Stain gives high detection sensitivity for double-stranded or single-stranded DNA and RNA. SYBR® Green I Stain exhibits a preferential affinity for double-stranded nucleic acids and SYBR® Green II Stain is most sensitive for single-stranded nucleic acids.

Advantages

High sensitivity – 25 - 100 times more sensitive than ethidium bromide

Flexible – Add GelStar® Stain directly to the agarose solution or post-stain your gel with GelStar® or SYBR® Green Stains

Fast – No destaining required

The table below compares the detection sensitivities of several commonly used staining methods. Samples detected with SYBR® Green Stains were post-stained with the dye. Samples detected with Ethidium Bromide or GelStar® Stain were detected by in-gel staining. Detection limits were determined by using DNA samples that were serially diluted and by recording the lowest amount that gave a visible band on photographs. Photographic conditions were varied as needed to use the longest exposure time possible without increasing gel background to an unacceptable level.

<table>
<thead>
<tr>
<th>Stain and Method</th>
<th>ssDNA</th>
<th>dsDNA</th>
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</thead>
<tbody>
<tr>
<td>GelStar® Stain – in gel</td>
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<td>20 pg</td>
</tr>
<tr>
<td>Ethidium bromide, no destain</td>
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<td>350 pg</td>
</tr>
<tr>
<td>Ethidium bromide, destain</td>
<td>350 pg</td>
<td>100 pg</td>
</tr>
<tr>
<td>SYBR® Green I or II Stain</td>
<td>60 pg</td>
<td>20 - 30 pg</td>
</tr>
</tbody>
</table>

Materials

- Clear polypropylene container (e.g., Rubbermaid® Recycling #5 Plastics)
- GelStar® Photographic Filter (Wratten® #9 equivalent) or SYBR® Green Photographic Filter (Wratten® #15 equivalent)
- Microcentrifuge
- UV transilluminator, Dark Reader® Transilluminator (Clare Chemical Research, Inc.) or CCD imaging system

Reagents

- Buffer between pH 7.5 - 8.5 (TAE, TBE or TE)
- GelStar® or SYBR® Green I or II Stain stock solution

Caution: Materials and methods shown here present hazards to the user and the environment. Refer to the safety information on page 210 before beginning these procedures.

The photographs below demonstrate the detection sensitivity of GelStar® Nucleic Acid Gel Stain when used in-gel or when gels have been post-stained.

The FlashGel® System includes gel cassettes pre-stained with a similar high sensitivity stain. Refer to page 18-25.
Section IV: Detection and Sizing of DNA in Agarose Gels

Detecting DNA with GelStar®, SYBR® Green I or II Nucleic Acid Gel Stains — continued

Tips for staining gels with GelStar® or SYBR® Green I Stain

Follow the guidelines below to increase the detection sensitivity of GelStar® or SYBR® Green Stains:

- New clear polypropylene containers (e.g., Rubbermaid® Recycling #5 Plastics) should be obtained for use with GelStar® and SYBR® Green Stains; when stored in the dark in polypropylene containers, the diluted stain can be used for up to 24 hours and will stain 2 to 3 gels with little decrease in sensitivity; the containers should be rinsed with distilled water (do not use detergents) after each use and dedicated to GelStar® or SYBR® Green Stain use only.

- These stains bind to glass and some non-polypropylene (polystyrene) plastics resulting in reduced or no signal from the nucleic acid.

- A 1X working solution of GelStar® or SYBR® Green Stain should be prepared just prior to use from the 10,000X stock solution by diluting in a pH 7.5 to 8.5 buffer (e.g., TAE, TBE or TE).

- Agarose gels should be cast no thicker than 4 mm. As gel thickness increases, diffusion of the stain into the gel is decreased, lowering the efficiency of DNA detection.

- Optimal sensitivity for GelStar® and SYBR® Green Stains is obtained by using the appropriate photographic filters for each stain.

- GelStar® Stain: Wratten® or Tiffen® #9 Filter

- SYBR® Green Stains: Wratten® or Tiffen® #15 Filter

- We do not recommend photographing gels with a 254 nm transilluminator; an outline of the UV light source may appear in photographs; a filter that will allow a 525 nm transmission and exclude infrared light is required.

Procedure for staining DNA with GelStar® or SYBR® Green Stains

For optimal resolution, sharpest bands and lowest background, stain the gel with GelStar® or SYBR® Green Stain following electrophoresis. Alternatively, GelStar® Stain can be included in the agarose gel. It is not recommended to include SYBR® Green Stains in the agarose gel. When the dye is incorporated into the agarose, the gel is more sensitive to DNA overloading, and the electrophoretic separation of DNA may not be identical to that achieved with ethidium bromide.

The photograph below demonstrates the effect of adding SYBR® Green I Stain to the agarose.

![SYBR® Green Gel Stain](image)

DNA markers were separated on 1% SeaKem® Gold Agarose gels 10-cm-long, 3 mm thick, run in 1X TBE Buffer for 1 - 1.5 hours. SYBR® Green I Stain was diluted 1:10,000 and added directly to the agarose or the gel was post stained for 30 minutes in a 1:10,000 dilution of SYBR® Green Stain in Buffer. Lanes 1 - 4: BstE II digest of lambda DNA; 1:2 dilutions with an initial loading of 25 ng/lane. Lane 5: 1 kb DNA ladder (Invitrogen, Inc.); 200 ng/lane [SYBR® Green I stained gel]; 1 μg/lane [Ethidium bromide stained gel]. Lane 6: Lonza’s 1-10 kb DNA Ladder; 100 ng/lane. Lane 7: Gensura’s 1 kb DNA Ladder; 100 ng/lane. Lane 8: Lonza’s 100 bp DNA Ladder; 100 ng/lane.
Follow the steps below to stain DNA after electrophoresis

1. Remove the concentrated stock solution of GelStar® or SYBR® Green Stain from the freezer and allow the solution to thaw at room temperature.
2. Spin the solution in a microcentrifuge to collect the dye at the bottom of the tube.
3. Dilute the 10,000X concentrate to a 1X working solution (1 μl per 10 ml), in a pH 7.5 - 8.5 buffer, in a clear plastic polypropylene container. Prepare enough staining solution to just cover the top of the gel.
4. Remove the gel from the electrophoresis chamber.
5. Place the gel in staining solution.
6. Gently agitate the gel at room temperature.
7. Stain the gel for 15 - 30 minutes.
   The optimal staining time depends on the thickness of the gel, concentration of the agarose, and the fragment size to be detected. Longer staining times are required as gel thickness and agarose concentration increase.
8. Remove the gel from the staining solution and view with a 300 nm UV transilluminator, CCD camera or Dark Reader® Transilluminator (Clare Chemical Research, Inc.). GelStar® and SYBR® Green Stained Gels do not require destaining. The dye's fluorescence yield is much greater when bound to DNA than when in solution.

Follow this procedure when including GelStar® Stain in the agarose gel.

1. Remove the concentrated stock solution of GelStar® Stain from the freezer and allow the solution to thaw.
2. Spin the solution in a microcentrifuge tube.
3. Prepare the agarose solution (see pages 83-84).
4. Once the agarose solution has cooled to 70°C, add the stain by diluting the stock 1:10,000 into the gel solution prior to pouring the gel (1 μl per 10 ml).
5. Slowly swirl the solution.
6. Pour the gel into the casting tray (see page 85).
7. Load your DNA onto the gel.
8. Run the gel.
9. Remove the gel from the electrophoresis chamber.
10. View with a 300 nm UV transilluminator, CCD camera or Dark Reader® Transilluminator (Clare Chemical Research, Inc.). GelStar® Stained gels do not require destaining. The dye's fluorescence yield is much greater when bound to DNA than when in solution.

Staining vertical gels with GelStar® and SYBR® Green Stains

Incorporating GelStar® and SYBR® Green Stains into the gel or prestaining the DNA for use in a vertical format is not recommended. The dye binds to glass or plastic plates and DNA may show little to no signal. Gels should be post-stained as described in the previous section.

Follow this procedure when staining vertical gels with GelStar® or SYBR® Green Stain

1. Remove the concentrated stock solution of GelStar® or SYBR® Green Stain from the freezer and allow the solution to thaw at room temperature.
2. Spin the solution in a microcentrifuge to collect the dye at the bottom of the tube.
3. Dilute the 10,000X concentrate to a 1X working solution, in a pH 7.5 - 8.5 buffer, in a clear plastic polypropylene container. Prepare enough staining solution to just cover the top of the gel.
4. Remove the gel from the electrophoresis chamber.
5. Open the cassette and leave the gel in place on one plate.
6. Place the plate, gel side up in a staining container.
7. Gently pour the stain over the surface of the gel.
8. Stain the gel for 5 - 15 minutes.
9. View with a 300 nm UV transilluminator, CCD camera or Dark Reader® Transilluminator (Clare Chemical Research, Inc.). GelStar® or SYBR® Green Stained gels do not require destaining. The dye's fluorescence yield is much greater when bound to DNA than when in solution.
Section IV: Detection and Sizing of DNA in Agarose Gels

Detecting DNA with GelStar®, SYBR® Green I or II Nucleic Acid Gel Stains — continued

Visualization by Photography

Gels stained with GelStar® and SYBR® Green Stains exhibit negligible background fluorescence, allowing long film exposures when detecting small amounts of DNA. Use the appropriate photographic filter for the stain you are using.

The table below provides suggested film types and photographic conditions:

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<th>Exposure time</th>
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<tr>
<td>Type 55</td>
<td>4.5</td>
<td>15 - 45 seconds</td>
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Visualization by image capture system

For the best results and optimal sensitivity, visualize GelStar® Stained Gels on The Dark Reader® Transilluminator (Clare Chemical Research, Inc.) GelStar® and SYBR® Green Stains are compatible with most CCD and video imaging systems. Due to variations in the filters for these systems, you may need to purchase a new filter. Lonza does not sell filters for this type of camera. Contact your systems manufacturer and using the excitation and emission information listed, they can guide you to an appropriate filter.

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<th>Stain</th>
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<td>SYBR® Green I Stain</td>
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<td>SYBR® Green II Stain</td>
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Application notes

— The fluorescent characteristics of GelStar® and SYBR® Green Stains make them compatible with argon ion lasers.

— These stains are removed from double-stranded DNA by standard procedures for ethanol precipitation of nucleic acids.

— Gels previously stained with ethidium bromide can subsequently be stained with GelStar® or SYBR® Green Stain following the standard protocol for post-staining. There will be some decrease in sensitivity when compared to a gel stained with only GelStar® or SYBR® Green Stain.

— The inclusion of GelStar® and SYBR® Green Stains in cesium chloride density gradient plasmid preparations is not recommended. The effect of the dye on the buoyant density of DNA is unknown.

— These stains do not appear to interfere with enzymatic reactions.

— We recommend the addition of 0.1% to 0.3% SDS in the prehybridization and hybridization solutions when performing Southern blots on gels stained with these dyes.

— Double-stranded DNA-bound GelStar® or SYBR® Green Stain fluoresces green under UV transillumination. Gels that contain DNA with single-stranded regions may fluoresce orange rather than green.

Decontamination

Staining solutions should be disposed of by passing through activated charcoal followed by incineration of the charcoal. For absorption on activated charcoal, consult Sambrook, et al., pp. 6.16 - 6.19, [1989]. Follow state and local guidelines for decontamination and disposal of Nucleic Acid staining solutions.
Section IV: Detection and Sizing of DNA in Agarose Gels

Detecting DNA with Ethidium Bromide

Introduction

Ethidium bromide is a fluorescent dye which detects both single- and double-stranded DNA. However, the affinity for single-stranded DNA is relatively low compared to double-stranded DNA. Ethidium bromide contains a planar group which intercalates between the bases of DNA and, when bound to DNA, results in an increase in fluorescence yield. Ethidium bromide-stained DNA is detected by ultraviolet radiation. At 254 nm, UV light is absorbed by the DNA and transmitted to the dye; at 302 nm, and 366 nm, UV light is absorbed by the bound dye itself. In both cases, the energy is re-emitted at 590 nm in the red-orange region of the visible spectrum.

Procedure

For optimal resolution, sharpest bands and lowest background, stain the gel with ethidium bromide following electrophoresis.

Ethidium bromide can also be included in the gel and electrophoresis buffer (0.5 μg/ml) with only a minor loss of resolution. The electrophoretic mobility of DNA will be reduced by approximately 15%.

Follow the steps below to stain DNA after electrophoresis

1. Prepare enough working solution of ethidium bromide. (0.5 - 1 μg/ml of ethidium bromide in distilled water or gel buffer) to cover the surface of the agarose gel.
2. Remove the gel from the electrophoresis chamber.
3. Submerge the gel for 20 minutes in the ethidium bromide solution.
4. Remove the gel from the solution.
5. Submerge the gel for 20 minutes in a new container filled with distilled water.
6. Repeat in fresh distilled water.
7. Gels can be viewed with a hand-held or tabletop UV light. For gel concentrations of 4% or greater, these times may need to be doubled. If after destaining the background is still too high, continue to destain.

Follow the steps below when including ethidium bromide in the agarose gel

1. Prepare agarose solution (see Section II).
2. While the agarose solution is cooling, add ethidium bromide to a final concentration of 0.1 to 0.5 μg/ml to the solution.
3. Gently swirl the solution.
4. Pour the gel into the casting tray.
5. Add ethidium bromide to the running buffer to a final concentration of 0.5 μg/ml.
6. Load and run the gel (see Section III).
7. Destain the gel by submerging the gel in distilled water for 20 minutes.
8. Repeat in fresh distilled water.
9. Gels can be viewed with a hand-held or tabletop UV light during or after electrophoresis.

Decontamination of ethidium bromide solutions

Decontamination of ethidium bromide solutions is described in Sambrook, et al., pp. 6.16 - 6.17 (1989). Follow local guidelines and regulations for ethidium bromide decontamination and disposal.

Caution: Materials and methods shown here present hazards to the user and the environment. Refer to the safety information on page 210 before beginning these procedures.
Section IV: Detection and Sizing of DNA in Agarose Gels

High Sensitivity Detection using The FlashGel® System

Introduction

The FlashGel® System uses a proprietary stain that is 5-20 times more sensitive than ethidium bromide stain. Samples prepared at DNA concentrations one-fifth of the concentration typically required for ethidium bromide stained gels will clearly resolve on FlashGel® Cassettes. DNA levels of 5 ng per band or more are visible on the lighted FlashGel® Dock under most ambient light conditions. DNA levels as low as 0.1 ng per band can be detected on gel images and photos. DNA levels can be adjusted to provide best performance depending upon the image analysis system used (Figure 1).

Because the system is so sensitive, a load volume of 5 μl or less is recommended for best performance. Samples may be diluted with FlashGel® Loading Dye (for best results), or with water or other common buffers (e.g., TE Buffer) before adding a loading dye. See the table in Figure 2 for examples of sample and marker dilutions in a FlashGel® Cassette compared to an ethidium bromide stained agarose gel. The ethidium stained gel required at least 4 times more sample than the FlashGel® Cassette. One-tenth the concentration of the FlashGel® DNA Marker and QuantLadder, and one-twentieth the concentration of the PCR products were required for good sensitivity of detection on FlashGel® Cassettes compared to ethidium bromide gel.

Figure 1: DNA concentration detectable with the FlashGel® System

![DNA concentration detectable with the FlashGel® System](image)

1.2% FlashGel® Cassettes, 12+1 well format. 275 volts for 7 minutes. DNA concentrations are ng/band Lanes 1-12: Sample dilution series of 400 bp & 1,500 bp purified fragments (BioVentures) Lanes 13: FlashGel® DNA Marker. Photographed on UV Transilluminator or Dark Reader® Transilluminator. Dark Reader® Transilluminator Gel illuminated on the Dark Reader® with Dark Reader® orange filter in place. Photographed with CCD imager (EtBr filter in place) at 2 second exposure. UV transilluminator: Gel illuminated on UV transilluminator. Photographed with CCD imager (EtBr filter in place) at 2 second exposure.

<table>
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<tr>
<th>Lane Nos.</th>
<th>Sample Type</th>
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<td></td>
<td>FlashGel® Cassette</td>
<td>Ethidium Bromide Gel</td>
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<tr>
<td>Lanes 1 &amp; 7</td>
<td>FlashGel® DNA Marker</td>
<td>5 μl 1:5 dilution</td>
</tr>
<tr>
<td>Lanes 2 &amp; 8</td>
<td>FlashGel® QuantLadder</td>
<td>5 μl 1:5 dilution</td>
</tr>
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<td>Lanes 3 &amp; 9</td>
<td>Lonza 100 bp Ladder</td>
<td>3 μl 1:15 dilution</td>
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<tr>
<td>Lanes 4 &amp; 10</td>
<td>Lonza 50-2500 bp Marker</td>
<td>3 μl 1:5 dilution</td>
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<td>Lanes 5 &amp; 11</td>
<td>285 bp β-Actin PCR</td>
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<td>Lanes 6 &amp; 12</td>
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<td>5 μl 1:50 dilution</td>
</tr>
<tr>
<td>Lane 13</td>
<td>FlashGel® DNA Marker</td>
<td>5 μl 1:5 dilution</td>
</tr>
</tbody>
</table>
Section IV: Detection and Sizing of DNA in Agarose Gels

High Sensitivity Detection using The FlashGel® System — continued

Refer to The FlashGel® System Protocol for complete user instruction, safety and environmental precautions.

Some components and technology of the FlashGel® System are sold under licensing agreements. The nucleic acid stain in this product is manufactured and sold under license from Molecular Probes, Inc., and the FlashGel® Cassette is sold under license from Invitrogen IP Holdings, Inc, and is for use only in research applications or quality control, and is covered by pending and issued patents. The FlashGel® Dock technology contains Clare Chemical Research, Inc. Dark Reader® Transilluminator technology and is covered under US Patents 6,198,107, 6,512,236; and 6,914,250. The electrophoresis technology is licensed from Temple University and is covered under US Patent 6,905,585.

References

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In-Gel Reactions

Overview

Introduction
A variety of enzymatic reactions can be performed in the presence of agarose. In-gel reactions are an alternative approach to standard DNA recovery techniques and provide a multitude of benefits. The use of in-gel reactions will not only save time, but eliminate potential sample loss during DNA recovery from an agarose gel.

Advantages
— Saves time otherwise used for recovery
— Avoids recovery losses
— Avoids recovery damage, especially shearing of higher molecular weight DNA

Applications
Enzymes active in the presence of low melting temperature agaroses include:
— Alkaline phosphatase
— BAL 31 nuclease
— DNA polymerase I
— Klenow fragment
— Restriction endonucleases
— Reverse transcriptases
— T4 DNA ligase
— T4 DNA polymerase
— T4 polynucleotide kinase
— T7 DNA ligase
— T7 DNA polymerase (Sequenase® Polymerase)
— Taq DNA polymerase

Compatible agaroses
In-gel reactions require the use of a low melting temperature (65°C) agarose. Lonza has developed two high quality Genetic Technology Grade™ (GTG®) Agaroses for this application, specifically NuSieve® GTG® and SeaPlaque® GTG® Agarose. These agaroses are specifically designed and tested for compatibility with in-gel reactions.
— NuSieve® GTG® Agarose is the choice for separation of nucleic acids ≤1,000 bp with a resolving power of 4% - 6% difference in DNA size
— SeaPlaque® GTG® Agarose is the choice for separation of large nucleic acid fragments >1,000 bp

The figures below demonstrate the efficiency of in-gel cloning in our low melting temperature agaroses versus sample recovery and also the consistency between different agarose lots.

Efficiency of In-Gel Cloning from SeaPlaque® GTG® Agarose

Two lanes of Bst E II digest of lambda DNA were electrophoresed in a 1% SeaPlaque® GTG® Agarose gel prepared in 1X TAE. The 2.3 kb band was excised from each lane. One sample was ligated directly to pUC 19 in the presence of remelted agarose (In-gel) and the other sample was purified using a chaotrope-based recovery protocol (Recovered). Transformation into DH5α® Cells was done following standard protocols.

Efficiency of In-Gel Cloning from NuSieve® GTG® Agarose

A 377 bp fragment was electrophoresed in three separate lots of a 3% NuSieve® GTG® Agarose gel in 1X TAE Buffer then ligated to M13 in the presence of remelted agarose. Transformation into DH5α® Cells was done in remelted agarose following standard protocols. Results 1, 2 and 3 are from three separate lots of NuSieve® GTG® Agarose.
Section V: In-Gel Reactions

Tips for Increasing the Efficiency of In-Gel Reactions

Electrophoretic tips

— Use a low EDTA (0.1 mM) 1X TAE Buffer; increases the availability of Mg\(^{2+}\), a necessary cofactor in many enzymatic reactions

— Briefly stain the gel after electrophoresis with GelStar\textsuperscript{®} or SYBR\textsuperscript{®} Green I Nucleic Acid Gel Stain or ethidium bromide; the presence of ethidium bromide during electrophoresis can result in the degradation of DNA

— Visualize your DNA with a UV light source that is \(\lambda \leq 300\) nm. Short UV wavelength light can damage DNA; alternatively, DNA can be detected using Clare Chemical’s Dark Reader\textsuperscript{®} Transilluminator

— Minimize exposure of DNA to UV light to less than 1 minute

— The addition of 1 mM guanosine or cytidine to the gel and electrophoresis buffer is effective in protecting DNA against UV-induced damage; the nucleosides will not impede electrophoresis, detection or purification of DNA.

— Limit the amount of DNA in the band of interest to 100 ng if possible; more DNA may cause smearing and a wide band will result in an unacceptably large piece of gel to be excised and melted.

Reaction tips

— Melt the gel slice at 65°C - 70°C. Do not exceed 70°C as this may cause melting of your DNA

— Thoroughly mix the reaction components at 37°C - 40°C; this ensures proper mixing of DNA with reaction components

— Do not exceed 0.5% SeaPlaque\textsuperscript{®} GTG\textsuperscript{®} or 1.5% NuSieve\textsuperscript{®} GTG\textsuperscript{®} Agarose in the reaction mix

— Increase the efficiency of blunt end ligations; the addition of larger amounts of vector, insert DNA and T4 DNA ligase or the addition of 5% (w/v) PEG-8000 to the ligation mixture and incubating the ligation reaction mixture for a longer time, can increase the efficiency of the reaction; an alternative strategy is to add linkers with sticky ends to the insert DNA

— When transforming cells, dilute the melted ligation reaction mixture five-fold before adding to cells

— When transforming bacterial cells, dilute the agarose containing the ligated DNA further with warmed (40°C) TAE or sterile distilled water before adding the ligation reaction to the competent cells; it is better to add 10 μl of a five-fold diluted mixture than 2 μl of a more concentrated reaction mixture; by doing this, it is less likely that your ligated DNA will be trapped in the agarose when it is added to the chilled cells; also be sure that you do not add excessive DNA to the competent cells which can decrease the transformation efficiency

Effect of Agarose Concentration and Buffer on In-Gel PCR

![Graph of Agarose Concentration and Buffer](image)

A 500 bp PCR product was separated on 4% NuSieve\textsuperscript{®} GTG\textsuperscript{®} Agarose in either 1X TAE or 1X TBE Buffer. Following electrophoresis, the PCR product was reamplified with the same primers as in the original amplification. Relative efficiency was measured by comparing the products on an agarose gel.

Effect of UV Irradiation on In-Gel Cloning

![Graph of UV Irradiation](image)

A 2 kb fragment, separated in 1% SeaPlaque\textsuperscript{®} GTG\textsuperscript{®} Agarose and stained with ethidium bromide, was exposed to 300 nm UV irradiation for different lengths of time before excision from the gel, ligation into pBR322, and bacterial cell transformation. The mean of the number of white colonies per microgram vector on two plates is given.

Reference

Section V: In-Gel Reactions

Cloning in the Presence of Agarose

Timetable

2. (Day 2) Ligation reaction. Transformation reaction.
3. (Day 3) Assess the results obtained from the ligation-transformation reaction.

Tips

— Use 1X TAE Buffer with 0.1 mM EDTA.
— Electrophorese the DNA without ethidium bromide in the gel.
— Minimize the exposure time of the DNA to less than 1 minute under UV light.
— Do not exceed a final concentration of 0.5% SeaPlaque® GTG® or 1.5% NuSieve® GTG® Agarose in the reaction mixture.
— Dilute the agarose solution when running in-gel transformations. Do not exceed >0.02% agarose in the transformation reaction.

NOTE: Electroporation can not be used with in-gel ligation/transformation procedures.

Effect of Agarose Concentration on Transformation

<table>
<thead>
<tr>
<th>Agarose Concentration (%)</th>
<th>No. of Recombinants Per g Vector</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>4.0x10^7</td>
</tr>
<tr>
<td>0.02</td>
<td>3.0x10^7</td>
</tr>
<tr>
<td>0.03</td>
<td>2.0x10^7</td>
</tr>
<tr>
<td>0.04</td>
<td>1.0x10^7</td>
</tr>
<tr>
<td>0.05</td>
<td>0.5x10^7</td>
</tr>
<tr>
<td>0.06</td>
<td>0x10^7</td>
</tr>
<tr>
<td>0.07</td>
<td>0x10^7</td>
</tr>
<tr>
<td>0.08</td>
<td>0x10^7</td>
</tr>
</tbody>
</table>

Aliquots of agarose solutions [0.25%, 0.5%, 1.0% and 2.0% in 1X TAE] were added to pUC19 monomer DNA which had been placed in labeled tubes. Aliquots [6 μl=0.01 ng DNA] of the samples containing agarose were added to 100 μl of [Invitrogen, Inc.] frozen competent cells on ice. The agarose concentrations shown are those in the tubes of competent cells after samples were added. Transformations were carried out following standard protocol. Points shown on the graph represent the mean of three transformations.

Materials

— Sterile microcentrifuge (1 ml) and polypropylene tubes [17 mm x 100 mm]
— Horizontal electrophoresis chamber
— Scalpel or razor blade
— Heating block or water bath
— Ice bucket and ice

Reagents

— SeaPlaque® GTG® or NuSieve® GTG® Agarose
— GelStar® or SYBR® Green I Nucleic Acid Gel Stain or ethidium bromide
— Distilled water
— T4 DNA ligase
— Competent cells
— Restriction enzymes
— Calf intestine alkaline phosphatase
— 1X TAE [0.1 mM EDTA] Gel and Running Buffer
— Reagents for phenol extraction [Section VI]
— 10 mM Tris-HCl, pH 7.5
— Ethanol
— 10X T4 ligation buffer

500 mM Tris-HCl, pH 7.5; 100 mM MgCl₂ 50 mM Dithiothreitol; 10 mM ATP pH 7.6; 200.0 μg/μl nuclease-free BSA (bovine serum albumin)

Caution: Materials and methods shown here present hazards to the user and the environment. Refer to the safety information on page 210 before beginning these procedures.
Section V: In-Gel Reactions

Cloning in the Presence of Agarose — continued

### Preparing the vector

1. Cut vector with appropriate restriction enzymes.
2. Dephosphorylate vector with calf intestine alkaline phosphatase.
3. Phenol extract vector.
4. Ethanol precipitate vector.

### Preparing the insert

1. Cut DNA to be used as insert with appropriate restriction enzymes.
2. Electrophorese DNA (100 ng) in a low melting temperature agarose gel prepared in 1X TAE Buffer (0.1 mM EDTA).
3. Briefly stain DNA with GelStar® or SYBR® Green I Nucleic Acid Gel Stain or ethidium bromide.
4. Excise gel band containing insert and place in a sterile, preweighed tube.
5. Estimate the volume of the gel slice based upon the weight of the slice (e.g., 100 mg = 100 μl).
6. Determine the concentration of the DNA in the gel slice by assuming 100% recovery of DNA in the slice.
7. Store excised band at 4°C until ready for use.
8. Prepare agarose plates with the appropriate selective media for the next day’s use.

### Ligation reaction

1. Remelt the agarose slice by heating to 68°C for 10 minutes.
2. Place the remelted gel slice at 37°C until needed.
3. For a final reaction mixture of 50 μl, add the following components in the following order:

<table>
<thead>
<tr>
<th>Volume</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 μl</td>
<td>10 mM Tris-HCl, pH 7.5 or distilled H.O</td>
</tr>
<tr>
<td>5 μl</td>
<td>10X T4 DNA ligation buffer</td>
</tr>
<tr>
<td>1 μl</td>
<td>Vector (amount sufficient to obtain a molar ratio of insert to vector of 3 - 4:1)</td>
</tr>
<tr>
<td>1 μl</td>
<td>T4 DNA ligase (between 1 - 2 units)</td>
</tr>
</tbody>
</table>

4. Mix the components gently with a pipette.
5. Add up to 25 μl of the remelted agarose gel slice [25 ng of insert DNA]. Do not exceed a final concentration of 0.5% SeaPlaque® GTG® or 1.5% NuSieve® GTG® Agarose in the reaction mixture.
6. Mix components by resuspending with a pipette.
7. Ligate at room temperature for 2 - 3 hours.

### Preparing competent cells

If you prepare your own competent cells, set up a small overnight culture of cells. High transformation efficiencies can be obtained with frozen competent cells that are either prepared in the laboratory or purchased. Similar transformation efficiencies of 10⁷ transformants/μg pUC18 DNA with insert have been obtained using the Hanahan Procedure [see citation at end of section]; transformation efficiencies are reduced when using the CaCl₂ method.

**NOTE:** Electroporation can NOT be used with in-gel ligation/transformation procedures.
Section V: In-Gel Reactions

Cloning in the Presence of Agarose — continued

Transformation reaction

The transformation procedure outlined below is a modification of the Invitrogen procedure included with their DH5α® Competent Cells. When using other frozen competent cells follow manufacturer’s directions.

1. Prepare competent cells if working with fresh cells. If using frozen competent cells, remove cells from storage and thaw on ice.
2. Heat the ligation reaction at 68°C for 5 minutes to remelt the agarose.
3. Add competent cells into a prechilled polypropylene tube (17 mm x 100 mm). We use 100 μl of thawed DH5α® cells.
4. Add 1 μl - 2 μl of diluted ligation reaction (70 pg - 140 pg vector) to competent cells.
5. Mix and incubate on ice for 30 minutes.
NOTE: Exceeding 0.02% SeaPlaque® GTG® or NuSieve® GTG® Agarose concentration in the final mixture may reduce transformation efficiencies.
6. Heat shock the cells by placing the tubes in a 42°C water bath for 45 seconds.
7. Place the cells back on ice for 2 minutes.
8. Add 0.9 ml of SOC medium to cells.
9. Incubate at 37°C with shaking for 30 - 60 minutes.
10. Spread 1 μl - 200 μl of the transformation mixture onto an agar plate with the appropriate selective medium.
11. Allow the liquid to soak in.
12. Invert the plates.
13. Incubate overnight at 37°C.

Reagents

- SOC medium
- 2.0 g Bacto-tryptone, 0.5 g Yeast extract, 1.0 ml 1 M NaCl 0.25 ml 1 M KCl
- Dissolve in a final volume of 100 ml distilled water; autoclave
- Add aseptically:
  - 1.0 ml 2 M Mg²⁺
    (1 M MgCl₂, 1 M MgSO₄, filter sterilized)
  - 1.0 ml 2 M glucose (filter sterilized)
- LB Agar Plates Supplemented with Ampicillin and X-gal (1 L)
  - 10.0 g tryptone
  - 5.0 g yeast extract
  - 10.0 g NaCl
  - 15.0 g agar
  - 800 ml distilled water
  - Stir to dissolve components
  - Adjust to pH 7.5 with NaOH
  - Adjust volume to 1 L with distilled water
  - Carefully heat while stirring until agar is dissolved
  - Remove stir bar and sterilize the medium by autoclaving 25 minutes
  - Place the ∆ ask of medium in a water bath set at 50°C and let the medium cool for at least 30 minutes
  - While medium is cooling, prepare ampicillin and X-gal stock solutions.
    - Add 1 ml of ampicillin stock and 2 ml of X-gal stock to the tempered medium and pour into plates
    - Allow plates to sit on bench overnight to dry; then package and store at 4°C
- Ampicillin stock
  - 100.0 mg of ampicillin to 1 ml of sterile distilled water
- X-gal (5-bromo-4-chloro-3-indoyl-ß-D-galactoside) stock
  - 40.0 mg of X-gal to 2 ml of dimethylformamide

SOC medium

- 2.0 g Bacto-tryptone, 0.5 g Yeast extract, 1.0 ml 1 M NaCl 0.25 ml 1 M KCl
- Dissolve in a final volume of 100 ml distilled water; autoclave
- Add aseptically:
  - 1.0 ml 2 M Mg²⁺
    (1 M MgCl₂, 1 M MgSO₄, filter sterilized)
  - 1.0 ml 2 M glucose (filter sterilized)
- LB Agar Plates Supplemented with Ampicillin and X-gal (1 L)
  - 10.0 g tryptone
  - 5.0 g yeast extract
  - 10.0 g NaCl
  - 15.0 g agar
  - 800 ml distilled water
  - Stir to dissolve components
  - Adjust to pH 7.5 with NaOH
  - Adjust volume to 1 L with distilled water
  - Carefully heat while stirring until agar is dissolved
  - Remove stir bar and sterilize the medium by autoclaving 25 minutes
  - Place the ∆ ask of medium in a water bath set at 50°C and let the medium cool for at least 30 minutes
  - While medium is cooling, prepare ampicillin and X-gal stock solutions.
    - Add 1 ml of ampicillin stock and 2 ml of X-gal stock to the tempered medium and pour into plates
    - Allow plates to sit on bench overnight to dry; then package and store at 4°C
  

Ampicillin stock

- 100.0 mg of ampicillin to 1 ml of sterile distilled water

X-gal stock

- 40.0 mg of X-gal to 2 ml of dimethylformamide

---

Reagents

- SOC medium
- 2.0 g Bacto-tryptone, 0.5 g Yeast extract, 1.0 ml 1 M NaCl 0.25 ml 1 M KCl
- Dissolve in a final volume of 100 ml distilled water; autoclave
- Add aseptically:
  - 1.0 ml 2 M Mg²⁺
    (1 M MgCl₂, 1 M MgSO₄, filter sterilized)
  - 1.0 ml 2 M glucose (filter sterilized)
- LB Agar Plates Supplemented with Ampicillin and X-gal (1 L)
  - 10.0 g tryptone
  - 5.0 g yeast extract
  - 10.0 g NaCl
  - 15.0 g agar
  - 800 ml distilled water
  - Stir to dissolve components
  - Adjust to pH 7.5 with NaOH
  - Adjust volume to 1 L with distilled water
  - Carefully heat while stirring until agar is dissolved
  - Remove stir bar and sterilize the medium by autoclaving 25 minutes
  - Place the ∆ ask of medium in a water bath set at 50°C and let the medium cool for at least 30 minutes
  - While medium is cooling, prepare ampicillin and X-gal stock solutions.
    - Add 1 ml of ampicillin stock and 2 ml of X-gal stock to the tempered medium and pour into plates
    - Allow plates to sit on bench overnight to dry; then package and store at 4°C
  

Ampicillin stock

- 100.0 mg of ampicillin to 1 ml of sterile distilled water

X-gal stock

- 40.0 mg of X-gal to 2 ml of dimethylformamide

---
Section V: In-Gel Reactions

Controls

We recommend the following controls be run in parallel with the test plates:

<table>
<thead>
<tr>
<th>Vector</th>
<th>Ligase</th>
<th># of blue colonies</th>
<th># of white colonies</th>
<th>Reason for control</th>
</tr>
</thead>
<tbody>
<tr>
<td>pUC 18-native</td>
<td>-</td>
<td>+++++</td>
<td>-</td>
<td>Cell competency test</td>
</tr>
<tr>
<td>pUC18-singly digested efficiency</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>Background for ligation efficiency</td>
</tr>
<tr>
<td>pUC18-singly digested</td>
<td>+</td>
<td>+++++</td>
<td>-</td>
<td>Determines ligation efficiency</td>
</tr>
<tr>
<td>pUC18-doubly digested background</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>Unrestricted vector background</td>
</tr>
<tr>
<td>pUC18-doubly digested</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Determines the efficiency of double digestion</td>
</tr>
<tr>
<td>pUC18-native</td>
<td>+</td>
<td>+++++</td>
<td>-</td>
<td>Reagent check</td>
</tr>
</tbody>
</table>

Assessing the results

Count colonies and assess the results of ligation-transformation. Colonies containing recombinant plasmids should be white. Blue colonies result from non-recombinant pUC18 plasmid.

References

Section V: In-Gel Reactions

Restriction Digestion in the Presence of Agarose

Procedure

**NOTE:** The urea sample buffer prevents the samples from regelling after digestion. It will also result in good separation of the DNA during electrophoresis.

1. Electrophorese DNA (several μg) in a low melting temperature agarose prepared in 1X TAE Buffer (0.1 mM EDTA).
2. Briefly stain the gel with GelStar® or SYBR® Green I Nucleic Acid Gel Stain or ethidium bromide.
3. Excise the gel slice containing the DNA of interest and place in a preweighed microcentrifuge tube.
4. Estimate the volume of the gel slice based upon the weight and determine the concentration of the DNA in the gel slice (e.g., 100 mg = 100 μl). Assume 100% recovery of the DNA.
5. Store excised band at 4°C until ready for use.
6. Add sterile distilled water to bring volume of the gel slice to 200 μl (200 mg).
7. Remelt the gel slice by heating to 68°C for 10 minutes.
8. Mix the melted gel slice by pipetting.
9. Remove a volume containing the quantity of DNA needed for restriction digestion.
10. Maintain the sample at 37°C to prevent gelling.
11. Dilute the restriction endonuclease with the appropriate reaction buffer.
12. Digest DNA at the appropriate temperature and time for your particular enzyme.
13. Stop the reaction by adding 30 μl of urea sample buffer to 50 μl of the digestion mixture and mixing.
14. Heat the sample to 65°C for 10 minutes if it has regelled.
15. Load the sample onto appropriate agarose gel for analysis.

**Materials**
- Horizontal electrophoresis chamber
- Scalpel or razor blade
- Microcentrifuge tubes (1 ml)
- Water bath or heating block

**Reagents**
- SeaPlaque® GTG® or NuSieve® GTG® Agarose
- 1X TAE (0.1 mM EDTA) Gel and Running Buffer
- Sterile distilled water
- GelStar® or SYBR® Green I Nucleic Acid Gel Stain or ethidium bromide
- Restriction endonuclease(s)
- Urea sample buffer
  - 8% Ficoll® (Type 400) Polymer
  - 27 mM EDTA
  - 0.27% bromophenol blue
  - 5 M urea
  - Adjust to pH 8.0

**Caution:** Materials and methods shown here present hazards to the user and the environment. Refer to the safety information on page 210 before beginning these procedures.

References
Section V: In-Gel Reactions

DNA Amplification in the Presence of Agarose

Procedure 1

1. Electrophorese PCR products in low melting temperature agarose prepared in 1X TAE (0.1 mM EDTA) Buffer.
2. Briefly stain the gel with GelStar® or SYBR® Green I Gel Stain or ethidium bromide.
3. Excise a gel slice containing the template DNA and place into a preweighed microcentrifuge tube.
4. Store the gel slice at 4°C protected from light until ready for use.
5. Melt the agarose gel slice containing template DNA at 65°C for 10 minutes.
6. Dilute with 65°C, sterile distilled water to a final DNA concentration of 0.1 ng/μl.
7. Add a portion of the DNA into the amplification reaction mixture (do not exceed 1 ng DNA/reaction).
8. Combine the components of the reaction mixture.
9. Vortex the reaction mixture to mix the contents.
10. Spin briefly in a microcentrifuge.
11. Overlay the mixture with mineral oil if necessary.
12. Perform amplification reactions with conditions appropriate for the template DNA and primers.
13. Remove mineral oil.
14. Remelt the reaction mixture at 65°C prior to analyzing.

Procedure 2

1. Follow steps 1 - 2 in Procedure 1.
2. Turn the gel upside down while it is on the UV transilluminator.
3. Stab the band of interest with a glass Pasteur pipette. When the pipette is removed from the gel, a small plug of agarose will be contained in the tip.
4. Remove the plug of agarose.
5. Add a portion of the plug of agarose to the amplification reaction. The plug does not require melting; it will melt during the first denaturing step.
6. Follow steps 8 - 14 in Procedure 1.

Amplification Reaction Mixture

If the reaction buffer does not contain magnesium ion, add sufficient amount for your template/primer.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume (μl)</th>
<th>FINAL Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>10X Reaction Buffer</td>
<td>5</td>
<td>1X</td>
</tr>
<tr>
<td>dNTP mix (1.25 mM each)</td>
<td>8</td>
<td>200 μM each</td>
</tr>
<tr>
<td>Primer #1, 10 μM</td>
<td>2.5</td>
<td>0.5 μM or 1.0 μM</td>
</tr>
<tr>
<td>Primer #2, 10 μM</td>
<td>2.5</td>
<td>0.5 μM or 1.0 μM</td>
</tr>
<tr>
<td>Template DNA + Agarose</td>
<td>5 - 10</td>
<td>(0.3 ng - 1.0 ng DNA)</td>
</tr>
<tr>
<td>Polymerase</td>
<td>0.5</td>
<td>(2.5 units)</td>
</tr>
<tr>
<td>Sterile distilled water</td>
<td>to 50 μl</td>
<td></td>
</tr>
</tbody>
</table>

Materials

- Horizontal electrophoresis chamber
- Scalpel or razor blade
- Vortex mixer
- Microcentrifuge
- Thin-walled microcentrifuge tube
- Microcentrifuge tubes (1 ml)
- Thermal cycler

Reagents

- SeaPlaque® GTG® or NuSieve® GTG® Agarose
- 1X TAE (0.1 mM EDTA) Gel and Running Buffer
- DNA amplification kit
- Forward and reverse primers
- Sterile distilled water
- GelStar® or SYBR® Green I Nucleic Acid Gel Stain or ethidium bromide

Caution: Materials and methods shown here present hazards to the user and the environment. Refer to the safety information on page 210 before beginning these procedures.

References

References for other in-gel reactions

**Cycle Sequencing**

**Random Prime Labeling**

**Blunt-end Ligation**

**Attaching Linkers**
Section VI: Recovery of DNA from Agarose Gels

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</thead>
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<tr>
<td>References</td>
<td>124</td>
</tr>
</tbody>
</table>
Recovery of DNA from Agarose Gels

Section VI: Recovery of DNA from Agarose Gels

Introduction

This section discusses various tips which will increase the efficiency of recovery of DNA from agarose gels. These tips and recommendations can be applied to all recovery techniques.

The section is divided into the following topics:
— Choosing the appropriate agarose
— Choosing the appropriate electrophoresis buffer
— Gel casting and DNA loading tips
— Staining and recovery tips

Choosing the appropriate agarose for recovery

When recovering DNA, the choice of agarose is one of the most important factors. To avoid recovery altogether, one can choose to perform in-gel reactions.

Lonza offers Genetic Technology Grade™ (GTG®) Products that are specially prepared for demanding molecular biology applications. Lonza’s GTG® Product quality tests go beyond standard nuclease assays to include enzymatic performance measurements. Our additional testing provides a more realistic index of overall product quality and reliability. You no longer need to screen agarose lots to find those which yield biologically active DNA.

Lonza agaroses and compatible recovery techniques

Buffer types

When recovering DNA from agarose gels, 1X Tris-acetate (TAE) Buffer is recommended for electrophoresis.

Casting and DNA loading tips

— Prepare the gel in 1X TAE Buffer
— Do not cast the gel with ethidium bromide
— Cast a gel 3 mm - 4 mm thick
— Use a combs ≤1 mm thick
— Load no more than 100 ng of DNA per band

Staining and recovery tips

When recovering DNA from agarose gels, we recommend the following:
— Stain the gel for 15 - 20 minutes
— Destain the gel in distilled water for two, 20-minute washes
— Do not expose the DNA to UV light for any longer than 1 minute; long exposure of DNA to UV light can nick the DNA
— The addition of 1 mM guanosine or cytidine to the gel and electrophoresis buffer is effective in protecting DNA against UV-induced damage
— Cut the smallest gel slice possible

It is possible to avoid staining samples which will be used for recovery by running an additional lane containing a small amount of your sample immediately next to the molecular weight marker. However, DNA is damaged by UV light in the absence of ethidium bromide so keep exposure to UV light as brief as possible. Cut the lanes containing the marker and the small amount of the sample from the rest of the gel and stain. To recover the preparative loading, line up the stained portion of the gel with the unstained portion. Check by placing on UV transilluminator and cut out the area that lines up with your sample on the unstained portion of the gel.

The FlashGel® System for Recovery, (page 20) offers a non-UV alternative for DNA recovery.

Reference

Introduction

β-Agarase is an enzyme that digests the polysaccharide backbone of agarose to alcohol-soluble oligosaccharides. DNA in a low melting temperature agarose gel can be recovered after the gel is melted and digested with this enzyme. The remaining oligosaccharides will not gel or interfere with subsequent DNA manipulations such as cloning, labeling, restriction digestion or sequencing. Lonza’s β-Agarase is free of any detectable DNase, RNase and phosphatase activities.

β-Agarase recovery is particularly useful for recovering large DNA (>10 kb) which could be sheared by other methods of recovery.

Compatible agaroses

— SeaPlaque® GTG® Agarose (certified and tested for the recovery of DNA)
— NuSieve® GTG® Agarose (certified and tested for the recovery of DNA)
— SeaPlaque® Agarose

Tips

— Transfer no more than 200 mg of the agarose gel to a microcentrifuge tube for equilibration with digestion buffer
— Completely melt the gel slice prior to the addition of enzyme
— Ethanol precipitations should be incubated at room temperature with ammonium acetate rather than sodium acetate to decrease the likelihood of co-precipitation of agarose-oligosaccharides with the DNA
— Polynucleotide kinase is inhibited by 7 mM ammonium ion; use sodium acetate for your precipitation if you will be phosphorylating your DNA after recovery
— The enzyme preparation retains full activity for several hours at 45°C; however, it will gradually lose activity upon longer incubations; for this reason, incubate at 40°C for overnight digestions
— For efficient recovery of small nucleic acids (<500 bp) and/or very dilute samples (<0.05 μg/ml), we recommend either carrier tRNA or nuclease-free glycogen be added; in addition, overnight precipitation at room temperature can be helpful
— The enzyme preparation has maximum activity between pH 6 - 7 and is relatively unaffected by salt concentrations between 0.1 M and 0.25 M; the equilibration of the gel slice with β-Agarase Buffer is necessary to provide the enzyme with optimal buffer conditions; this equilibration is more important for gels prepared in TBE than in TAE Buffer due to the greater buffering capacity of TBE Buffer
— Ethanol precipitate the DNA at room temperature or on ice. The most effective DNA precipitation can be achieved at 0°C to 22°C, rather than at -20°C to -70°C. At higher temperatures, yields are more consistent and precipitation of oligosaccharides will be avoided

β-Agarase unit definition

— One unit of Lonza β-Agarase will completely digest 200 mg of a molten 1% SeaPlaque® GTG® Agarose gel which has been dialyzed in 1X β-Agarase Buffer in 1 hour at 40°C; similar activities are obtained for other low melting temperature agaroses such as NuSieve® GTG® Agarose.
— Concentration: 1 unit per μl
Recovery of DNA from Agarose Gels

Section VI: Recovery of DNA from Agarose Gels — continued

β-Agarase Recovery of DNA from Agarose Gels — continued

Procedure for β-Agarase digestion

BEFORE YOU BEGIN: Set one water bath or heating block for 70°C. Set another water bath or heating block for 45°C.

1. Electrophorese DNA in a low melting temperature agarose gel (such as SeaPlaque®, SeaPlaque® GTG®, MetaPhor® or NuSieve® GTG® Agarose) which has been prepared in TAE or TBE Buffer.

2. Briefly stain the gel with GelStar® or SYBR® Green I Nucleic Acid Stain or ethidium bromide.

3. Photograph the gel.

4. Excise a gel slice containing the DNA of interest.

5. Place the gel slice containing the DNA sample into a microcentrifuge tube.

6. Equilibrate the gel slice with 10 volumes of 1X β-Agarase Buffer for 60 minutes at room temperature OR follow steps 6a - 6d.

   6a. Melt the gel slice at 70°C for 15 minutes. Make sure that the gel slice is completely melted as the enzyme preparation will not digest agarose in the gelled state.

   6b. Add 2 μl of the 50X β-Agarase buffer to approximately 100 μl of melted gel solution.

   6c. Mix the solution.

   6d. Proceed to steps 9, 10 and 11.

7. Discard the buffer.

8. Melt the gel slice at 70°C (approximately 15 minutes). Make sure the gel slice is completely melted as the enzyme preparation will not digest agarose in the gelled state.

9. Cool the melted agarose solution to 45°C.

10. Add 1 unit of β-Agarase for 200 mg (approximately 200 μl) of 1% agarose gel (add proportionally more or less of the enzyme preparation for larger or smaller gel slices, or with higher or lower agarose concentrations, respectively). Skip to step 12.

11. If you have followed the protocol in steps 6a - 6d, the amount of enzyme will need to be adjusted.

   If the gel was prepared with... Then you will need to add...
   TAE Buffer Twice the amount of enzyme
   TBE Buffer Seven times the amount of enzyme

12. Mix.

13. Incubate at 45°C for 60 minutes.

14. If the DNA is ≤30 kb...

   Place the solution at -20°C for 15 minutes. Centrifuge the solution for 15 minutes at 4°C.

   Continue with Step 15

   OR

   The sample can be concentrated without ethanol precipitation by centrifugation in a molecular weight-cut-off-spin column (e.g., Amicon®’s Microcon™ Microconcentrator).

   If the DNA is >30 kb...

   The DNA/β-Agarase solution may be used directly without precipitation for subsequent enzymatic manipulations.

   OR

   The sample can be concentrated without ethanol precipitation by centrifugation in a molecular weight-cut-off-spin column (e.g., Amicon’s Microcon or Centricon™ Microconcentrators).

15. Transfer the supernatant to a new microcentrifuge tube leaving behind any undigested agarose in the pellet.

16. Add ammonium acetate to the supernatant to a final concentration of 2.5 M.

17. Add 2 to 3 volumes of 100% ethanol.

18. If the DNA is ≤0.05 μg/ml...

   Precipitate at room temperature for 24 hours

   OR

   Add 10 μg of RNA.

   If the DNA is >0.05 μg/ml...

   Precipitate at room temperature for 30 minutes.

19. Collect the precipitate by centrifugation.

Caution: Materials and methods shown here present hazards to the user and the environment. Refer to the safety information on page 210 before beginning these procedures.
Section VI: Recovery of DNA from Agarose Gels

β-Agarase Recovery of DNA from Agarose Gels — continued

Materials

- Microcentrifuge tubes (1 ml)
- Two water baths or heating blocks
- Scalpel or razor blade
- Microcentrifuge

Reagents

- SeaPlaque®, SeaPlaque® GTG®, NuSieve® GTG® or MetaPhor® Agarose
- GelStar® or SYBR® Green I Nucleic Acid Gel Stain or ethidium bromide
- β-Agarase buffer
- Supplied as a 50X concentrate; 1X concentration is: 40 mM Bis Tris/HCl, 40 mM NaCl, 1 mM EDTA (pH 6.0)
- β-Agarase Enzyme
- 10 M ammonium acetate
- 100% ethanol

Caution: Materials and methods shown here present hazards to the user and the environment. Refer to the safety information on page 210 before beginning these procedures.

References

Electroelution of DNA from Agarose Gels

Introduction

Electroelution is a reliable and consistent recovery method. The recovered DNA is suitable for a wide range of applications. For small fragments, a typical yield would be 50 - 85%, but as fragment length increases, yield can drop as low as 20%. The procedure below describes the electroelution of DNA into dialysis bags and has been adapted from Sambrook, et al. Alternatively, if you have a commercially available apparatus, follow the manufacturer’s instructions.

Compatible agaroses

- SeaKem® GTG® Agarose (certified and tested for the recovery of DNA)
- SeaPlaque® GTG® Agarose (certified and tested for the recovery of DNA)
- NuSieve® GTG® Agarose (certified and tested for the recovery of DNA)
- SeaPlaque® Agarose
- NuSieve® 3:1 Agarose
- MetaPhor® Agarose

Tips

- Electrophorese DNA in 1X TAE Buffer
- Have 1 μg of DNA in your band of interest
- Minimize exposure of DNA to UV light for no more than 1 minute
- Cut the smallest gel slice possible

Materials

- Scalpel or razor blade
- Spatula
- Dialysis tubing
- Dialysis tubing clips
- Electrophoresis chamber
- Hand-held, long-wavelength ultraviolet lamp
- Disposable plastic tube
- Pasteur pipette

Reagents

- 1X TAE Buffer
- 2% w/v sodium bicarbonate, 1 mM EDTA pH 8.0, prepare in distilled water
- 1mM EDTA, pH 8.0
- Distilled water
- GelStar® or SYBR® Green I Nucleic Acid Gel Stain or ethidium bromide

Caution: Materials and methods shown here present hazards to the user and the environment. Refer to the safety information on page 210 before beginning these procedures.

Procedure for preparing dialysis tubing

1. Cut the tubing into 10 cm to 20 cm long pieces.
2. Boil for 10 minutes in a large volume of 2% sodium bicarbonate/1 mM EDTA, pH 8.0.
3. Rinse the tubing with distilled water.
4. Boil for 10 minutes in 1 mM EDTA, pH 8.0.
5. Cool the tubing.
6. Store at 4°C submerged in 1 mM EDTA, pH 8.0.
Electroelution of DNA from Agarose Gels — continued

Procedure for electroeluting DNA from agarose gels

1. Electrophorese DNA in an agarose gel.
2. Stain the gel with GelStar® or SYBR® Green I Nucleic Acid Stain or ethidium bromide.
3. Locate the band of interest using a UV light source. Minimize exposure of DNA to UV light to less than 1 minute.
4. Excise the band of interest using a scalpel or razor blade.
5. Wet a spatula with 1X TAE Buffer.
6. Place the agarose slice containing the DNA on a wetted spatula or a scalpel.
7. Photograph the gel for a record of which band was eluted.
8. Seal one end of treated dialysis tubing with a dialysis clip.
9. Fill the bag to the top with 1X TAE Buffer.
10. Transfer the slice of agarose into the bag with the spatula.
11. Allow the slice of agarose to sink to the bottom of the bag.
12. Remove the buffer from the dialysis bag, leaving just enough to keep the gel slice in constant contact with the buffer.
13. Clip the dialysis bag above the gel slice, avoiding air bubbles.
14. Place a shallow layer of 1X TAE Buffer in an electrophoresis chamber.
15. Immerse the bag in the electrophoresis chamber.
16. Pass electric current through the bag (4 - 5 V/cm for 2 - 3 hours). During this time, the DNA is eluted out of the gel onto the inner wall of the dialysis tubing. This process can be monitored with a hand-held, long-wavelength UV lamp. Expose to UV light briefly.
17. Reverse the polarity of the current for 1 minute. This will remove the DNA from the wall of the bag.
18. Recover the bag from the electrophoresis chamber.
19. Gently massage the side of the bag with gloved fingers where the DNA has accumulated. This will remove the DNA from the wall of the bag. This process can be monitored with a hand-held UV lamp. Expose to UV light briefly.
20. Open the dialysis bag.
21. Transfer all of the buffer surrounding the gel slice to a plastic tube.
22. Wash out the bag with a small amount of 1X TAE Buffer.
23. Transfer the solution to the plastic tube.
24. Remove the gel slice from the bag.
25. **OPTIONAL:** Stain the slice with GelStar® or SYBR® Green I Nucleic Acid Stain or ethidium bromide to ensure all of the DNA has been eluted from the slice.
26. Purify the DNA from 1X TAE Solution using phenol/chloroform extractions.*

*After electroelution, it is recommended that the DNA is further purified with a phenol/chloroform extraction followed by ethanol precipitation. Oligosaccharides and other contaminants (found in low-grade agarose) can copurify with the DNA. Phenol extractions will remove any oligosaccharides, avoiding their coprecipitation during ethanol precipitations.

References

Phenol/Chloroform Extraction of DNA from Agarose Gels

Compatible agaroses

- SeaPlaque® GTG® Agarose (certified and tested for the recovery of DNA)
- NuSieve® GTG® Agarose (certified and tested for the recovery of DNA)
- SeaPlaque® Agarose

Tips

Recovery failures when extracting DNA from agarose using phenol/chloroform most often result from either extracting too large a piece of agarose, or precipitating agarose along with the DNA at the ethanol precipitation step. To address these difficulties, we recommend the following:

- No more than 200 mg (200 μl) of agarose should be extracted in a single tube; if your gel slice containing the DNA is larger than this, separate it into smaller pieces, then combine the extracted solutions prior to ethanol precipitation
- Ethanol precipitation of agarose can be avoided by chilling the extracted solution on ice for 15 minutes, then centrifuging the sample(s) in a cold room for 15 minutes at maximum speed in a microcentrifuge prior to adding salts and ethanol. The supernatant is then carefully decanted, and the DNA in the supernatant is precipitated following standard protocols
- Not useful for large DNA (>10kb). Vortexing will shear the DNA

Procedure

This protocol has been adapted from Sambrook, et al.

1. Electrophorese DNA in a low melting temperature agarose gel prepared in 1X TAE Buffer.
2. Set a water bath for 67°C.
3. Prewarm the TE at 67°C.
4. Excise the gel fragment containing the DNA.
5. Place the DNA slice in a microcentrifuge tube.
6. Estimate the volume of the slice. If the slice is significantly greater than 200 mg, break the agarose slice into smaller pieces and place each agarose piece in a separate microcentrifuge tube.
7. Melt the gel slice at 67°C for 10 minutes.
8. Add the appropriate volume of TE Buffer (prewarmed to 67°C) so that the final concentration of agarose is 0.5%.
9. Maintain the samples at 67°C until you are ready to phenol extract.
10. Add an equal volume of buffer-equilibrated phenol.
11. All subsequent steps can be done at room temperature.
12. Vortex for 15 seconds.
13. Centrifuge at maximum speed in a microcentrifuge for 3 minutes.
14. Carefully remove the top aqueous phase. The interface of white debris is the agarose, which can contain some trapped DNA. This can be back-extracted with TE to maximize yield.
15. Place aqueous phase in a clean microcentrifuge tube.
17. Add an equal volume of phenol/chloroform to the aqueous phase.
18. Vortex for 15 seconds.
19. Centrifuge at maximum speed in a microcentrifuge for 3 minutes.
20. Remove the aqueous phase and place in a clean microcentrifuge tube.
21. Repeat the extraction with an equal volume of chloroform.
22. Remove the aqueous phase and place in a clean microcentrifuge tube.
23. Chill the aqueous phase for 15 minutes on ice.
24. Centrifuge in a microcentrifuge at high speed for 15 minutes at 4°C.
25. Carefully decant the supernatant into a clean microcentrifuge tube.
26. Ethanol precipitate the DNA in the supernatant following standard protocols.
Section VI: Recovery of DNA from Agarose Gels

Phenol/Chloroform Extraction of DNA from Agarose Gels — Continued

Materials
- Water bath set to 67°C
- Microcentrifuge tubes (1 ml)
- Ice bucket and ice
- Vortex mixer
- Scapel or razor blade

Reagents
- SeaPlaque® GTG®, NuSieve® GTG® or SeaPlaque® Agarose
- TE Buffer warmed to 67°C
- Buffer-equilibrated phenol
- Chloroform
- Phenol/chloroform (1:1)

Caution: Materials and methods shown here present hazards to the user and the environment. Refer to the safety information on page 210 before beginning these procedures.

Introduction
This protocol is relatively fast and will work with low and standard melting temperature agaroses. However, like many recovery techniques, the DNA yield decreases with increasing DNA size, but should be approximately 50% for DNA 5 kb.

Compatible agaroses
- SeaKem® GTG® Agarose (certified and tested for the recovery of DNA)
- SeaPlaque® GTG® Agarose (certified and tested for the recovery of DNA)
- NuSieve® GTG® Agarose (certified and tested for the recovery of DNA)
- SeaPlaque® Agarose
- MetaPhor® Agarose
- NuSieve® 3:1 Agarose
- SeaKem® Gold Agarose

Procedure
This protocol has been adapted from Benson. For more detailed protocols on phenol chloroform extractions, refer to the previous section.

1. Electrophorese DNA in an agarose gel prepared in 1X TAE Buffer.

2. Excise gel slice containing DNA.
3. Place in a microcentrifuge tube.
4. Estimate volume based on weight.
5. Mash the gel slice with the stir rod.
7. Vortex for 10 seconds.
8. Freeze at -70°C for 5 to 15 minutes.
9. Centrifuge for 15 minutes at room temperature.
10. Remove the supernatant which contains the DNA.
11. Place the supernatant, in a clean microcentrifuge tube.
12. Phenol/chloroform extract the supernatant.
13. Follow with a chloroform extraction.
14. Ethanol precipitate following standard procedures.

Materials
- Microcentrifuge tubes (1 ml)
- Ice bucket and ice
- Vortex mixer
- Scapel or razor blade
- Glass or plastic stir rod

Reagents
- TE Buffer warmed to 67°C
- Buffer equilibrated phenol
- Chloroform
- Phenol/chloroform (1:1)
Introduction

This method is compatible with all the recovery techniques listed in this chapter.

Tips

- Prior to adding salts and ethanol, precipitation of agarose can be avoided by chilling the supernatant on ice for 15 minutes, then centrifuging the sample(s) in a cold room for 15 minutes at maximum speed in a microcentrifuge. The supernatant is then carefully decanted, and the DNA in the supernatant is ethanol precipitated following standard protocols.
- Ethanol precipitations should be incubated at room temperature with ammonium acetate rather than sodium acetate in order to decrease the likelihood of coprecipitation of agarose-oligosaccharides with the DNA or RNA.

Procedure

This protocol has been adapted from Sambrook, et al.

1. Chill the supernatant on ice for 15 minutes.
2. Centrifuge the sample(s) in a cold room for 15 minutes at maximum speed in a microcentrifuge.
3. Carefully decant the supernatant.
4. Place in a clean microcentrifuge tube.
5. Measure the volume of the sample.
6. Add 0.2 volumes of 10 M ammonium acetate to the sample.
7. Add 2 volumes of 100% ice-cold ethanol.
8. Briefly vortex.
9. Store the mixture for 30 minutes to overnight at room temperature.
10. Centrifuge for 30 minutes at 12,000 rpm.
11. Decant the supernatant.
12. Wash the pellet three times with 70% ethanol.
13. Allow to air-dry at room temperature on the bench top.
14. Dry under vacuum for 5 - 10 minutes.
15. Dissolve the DNA in TE Buffer.

References


References for other recovery methods

- References for Electrophoresis onto DEAE-cellulose membrane

- References for Passage through DEAE-sephacel

- References for Recovery using glass beads

- General References
  - Hengen, P.N., Methods and Reagents; Recovering DNA from Agarose Gels. TIBS 19, 1994.
Overview

Polyacrylamide gels can separate DNA that differs by 0.2% in length, well beyond the resolving capabilities of agarose (2% difference in DNA length). Another advantage to using polyacrylamide gels is that they can accommodate large amounts of DNA (up to 10 μg) without any loss in resolution.

Depending upon the application, TBE gels can be prepared as denaturing or nondenaturing gels.

Lonza offers PAGEr® Precast TBE Gels for DNA separation. Refer to page 17 for information.

Applications

Denaturing gels: concentrations range from 8 - 20%
- Oligonucleotide purification
- Separation of single-stranded DNA
- Isolate radiolabeled DNA probes
- S1 nuclease assay
- DNA footprinting
- RNase protection assays

Nondenaturing gels: concentrations range from 3 - 20%
- Separation of di-nucleotide repeats
- Separation of DNA ranging from 20 bp - 2000 bp in length
- Study DNA-Protein interactions (Gel Shift Assays)

Buffers for Electrophoresis

To ensure adequate buffering power during vertical electrophoresis, TBE Buffer is used for polyacrylamide gel electrophoresis at a working strength of 1X. Lower dilutions of the buffer or the use of TAE Buffer may cause gels to overheat and result in band smearing throughout the gel.

TBE is commercially available as 5X or 10X solutions (Lonza AccuGENE® TBE Buffer). Alternatively, it can be prepared as follows:

10X TBE Stock Solution

(890 mM Tris base, 890 mM Boric acid, 20 mM EDTA)

1X = 89 mM Tris base, 89 mM Boric acid, 2 mM EDTA

108.0 g Tris base
55.0 g Boric acid
7.44 g Na₂EDTA • 2H₂O

Adjust volume to 1 liter with distilled water
Filter through a 0.45 μm filter
pH adjustment is not necessary

Suggested Polyacrylamide Concentrations

<table>
<thead>
<tr>
<th>Nondenaturing polyacrylamide gels</th>
<th>Fragment Sizes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gel Percentage</td>
<td>1,000 bp - 2,000 bp</td>
</tr>
<tr>
<td>3.5%</td>
<td></td>
</tr>
<tr>
<td>5.0%</td>
<td>80 bp - 500 bp</td>
</tr>
<tr>
<td>6.0%</td>
<td>75 bp - 2,000 bp</td>
</tr>
<tr>
<td>8.0%</td>
<td>60 bp - 400 bp</td>
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<tr>
<td>10.0%</td>
<td>30 bp - 1,000 bp</td>
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<tr>
<td>12.0%</td>
<td>40 bp - 200 bp</td>
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<tr>
<td>15.0%</td>
<td>25 bp - 150 bp</td>
</tr>
<tr>
<td>20.0%</td>
<td>5 bp - 100 bp</td>
</tr>
<tr>
<td>4 - 20%</td>
<td>10 bp - 2,000 bp</td>
</tr>
</tbody>
</table>
Section VII: Separation of DNA in Polyacrylamide Gels

Detecting DNA in Polyacrylamide Gels

Detecting DNA in Polyacrylamide Gels with GelStar® or SYBR® Green Nucleic Acid Gel Stains

GelStar® and SYBR® Green Nucleic Acid Gel Stains are highly sensitive fluorescent stains for detecting nucleic acids. These stains provide high sensitivity detection of double-stranded or single-stranded DNA. See table below for a comparison of staining sensitivities and limits of detection.

<table>
<thead>
<tr>
<th>Stain</th>
<th>ssDNA</th>
<th>dsDNA</th>
<th>Native RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>GelStar® Stain</td>
<td>25 pg</td>
<td>20 pg</td>
<td>10 ng</td>
</tr>
<tr>
<td>SYBR® Green I Stain</td>
<td>–</td>
<td>20-30 pg</td>
<td>–</td>
</tr>
<tr>
<td>SYBR® Green II Stain</td>
<td>60 pg</td>
<td>–</td>
<td>15 ng</td>
</tr>
<tr>
<td>Ethidium bromide</td>
<td>350 pg</td>
<td>100 pg</td>
<td>30 ng</td>
</tr>
</tbody>
</table>

Tips for Staining Gels with GelStar® and SYBR® Green Nucleic Acid Gel Stains

— The powder used on some laboratory gloves may contribute to background fluorescence. We recommend using powder-free gloves and rinsing gloves prior to handling gels
— Fibers shed from clothing or lab coats may be fluorescent; be cautious when handling gels
— Staining of nucleic acids with these dyes has minimal impact on blotting efficiency. To ensure efficient hybridization, use of prehybridization and hybridization solutions containing 0.1 - 0.3% SDS is important to remove stain retained during transfer
— These stains can be removed from nucleic acids by ethanol precipitation. Isopropyl alcohol precipitation is less effective at removing the dye; butyl alcohol extraction, chloroform extraction and phenol do not remove the dye efficiently
— Allow time for the stock solution to thaw completely. Removal of stain from partially thawed solutions will result in depletion of stain over time. These stains may be diluted in most common electrophoresis buffers with a pH range from 7.0 - 8.5 or in TE Buffer. Staining solutions prepared in water or in buffer with a pH below 7.0 or above 8.5 are less stable and show reduced staining efficiency
— Prepare and store the stain in polypropylene containers such as Rubbermaid® containers or pipette-tip box lids. The stain may adsorb to glass surfaces and some plastic surfaces, particularly if the surfaces carry residues of anionic detergents or reagents
— As an alternative to the protocol presented for staining gels on the cassette plate, smaller gels such as mini gels may be removed from both plates then stained using the protocol for post-staining agarose gels found in Section IV.
— Treatment of one plate with a "release" agent, such as Gel Slick® Solution, increases the ease of separating the glass plates while keeping the gel in place on the other plate for staining
— Handling or compression of gels (particularly polyacrylamide-type gels) can lead to regions of high background after staining. If possible, gels should not be handled directly; use a spatula (or other tool) and a squirt bottle to slide the gel off the plates and into the stain or onto the light box

Procedure for Staining

Incorporating these dyes into the gel or prestaining the nucleic acid in a vertical format is not recommended. The dye binds to glass or plastic plates and DNA may show little to no signal. Gels should be post-stained as described below.

1. Remove the concentrated stock solution from the freezer and allow the solution to thaw at room temperature
2. Spin the solution in a microcentrifuge to collect the dye at the bottom of the tube
3. Dilute the 10,000X concentrate to a 1X working solution for DNA and a 2X working solution for RNA, in a pH 7.5 - 8.5 buffer, in a clear plastic polypropylene container
4. Open the cassette, and leave the gel in place on one plate
5. Place the plate, gel side up, in a staining container
6. Gently pour the stain over the surface of the gel; a disposable pipette may be used to help distribute the stain evenly over the gel surface
7. Stain the gel for 5 - 15 minutes. No destaining is required
8. Remove the gel from the staining solution and view with a 300 nm UV transilluminator, Dark Reader® Transilluminator or CCD imaging system.
For highest sensitivity, the gel should be carefully removed from the plate and placed directly on the transilluminator or scanning stage. Alternatively, if a relatively low fluorescence plate is used, the results may be visualized by placing the gel and plate gel side down on the transilluminator and photographing or by scanning the gel directly on the plate.

**NOTE:** More detailed information on photographing gels and decontaminating staining solutions is described in Detecting DNA with GelStar® or SYBR® Green I Stains (see Section IV). 

### Detecting DNA in Polyacrylamide Gels with Ethidium Bromide

The procedure for post-staining DNA in polyacrylamide gels with ethidium bromide is identical to the procedures used for post-staining agarose gels. Follow the procedures described in Detecting DNA with Ethidium bromide (see Section IV). 

#### Materials
- Clear polypropylene container (e.g., Rubbermaid® Recycling #5 Plastics)
- Microcentrifuge
- UV transilluminator
- Photographic Filter for GelStar® Stain or SYBR® Green Stain (Wratten® #15 or Wratten® #9 Filter respectively)

#### Reagents
- GelStar® Nucleic Acid Gel Stain or SYBR® Green I or II Nucleic Acid Gel Stain
- Buffer between pH 7.5-8.5 (TBE or TE)

**Caution:** Materials and methods shown here present hazards to the user and the environment. Refer to the safety information on page 210 before beginning these procedures.
The two primary methods for recovering DNA from polyacrylamide gels are the “Crush and Soak” method or electroelution. Described below is the “Crush and Soak” procedure. The procedure for electroeluting DNA from polyacrylamide gels is similar to the procedures used for agarose gels with one exception; 0.5X TBE Buffer should be used rather than TAE Buffer. Follow the procedures described in Electroelution of DNA from Agarose Gels (see Section VI).

Tips for increasing DNA recovery from polyacrylamide gels

When recovering DNA from polyacrylamide gels, we recommend the following:
- Stain the gel for no more than 15 minutes
- If ethidium bromide staining, destain the gel in distilled water for two, 20-minute washes
- Do not expose the DNA to UV light for any longer than 1 minute. Longer exposures may result in DNA nicking
- Cut the smallest gel slice possible
- If recovering small amounts of DNA, the addition of 10 μg of carrier RNA prior to ethanol precipitation may improve recovery yields

Crush and soak procedure

1. While the gel is on the transilluminator, cut out the band of interest using a razor blade or scalpel. Cut the smallest size gel slice possible.

NOTE: If the gel has been covered with plastic wrap, do not remove the plastic wrap before cutting.

2. Peel the small piece of gel containing the DNA from the plastic wrap.

3. Transfer the gel slice to a microfuge tube.

4. Crush the gel slice against the wall of the microfuge tube with the disposable pipette tip.

5. Add 1 - 2 volumes of elution buffer to the gel slice (e.g., if the estimated gel slice volume is 200 μl add 200 μl - 400 μl of elution buffer).

6. Incubate the tube at 37°C on a rotating wheel or rotary platform. For fragments less than 500 bp incubate for 3 - 4 hours. For fragments greater than 500 bp incubate for 12 - 16 hours.

7. Centrifuge the samples at 12,000 rpm for 1 minute at 4°C.

8. Transfer the supernatant to a fresh microfuge tube, being careful to avoid transferring fragments of polyacrylamide.

9. Add 0.5 volumes of elution buffer to the pellet of polyacrylamide.

10. Vortex briefly.

11. Centrifuge at 12,000 rpm for 1 minute at 4°C.

12. Combine the two supernatants.

13. Remove any remaining polyacrylamide by passing the supernatant through a disposable plastic column or a syringe barrel containing a Whatman® GF/C Filter or packed siliconized glass wool.

14. Add 2 volumes of cold ethanol.

15. Store the solution on ice for 30 minutes.

16. Recover the DNA by centrifugation at 12,000 rpm for 10 minutes at 4°C.

17. Decant the supernatant.

18. Redissolve the DNA in 200 μl of AccuGENE® 1X TE Buffer, pH 7.6.


20. Repeat steps 14 - 17.

21. Rinse pellet once with 70% ethanol.

22. Add 10 μl of AccuGENE® 1X TE, pH 7.6 to pellet and dissolve.
Section VII: Separation of DNA in Polyacrylamide Gels

Recovery of DNA in Polyacrylamide Gels — continued

Materials
- Scalpel or razor blade
- Microfuge tube
- Disposable pipette tip
- Rotary wheel or platform
- 37°C oven
- Microcentrifuge at 4°C
- Vortex
- Disposable plastic column or a syringe barrel containing a Whatman® GF/C Filter or packed siliconized glass wool
- Ice

Reagents
- Elution buffer
  - (3.85 g ammonium acetate, 0.215 g magnesium acetate, 0.2 ml AccuGENE® 0.5 M EDTA Solution, 1.0 ml AccuGENE® 10% SDS Solution, fill to 100 ml with distilled water)
- 100% and 70% ethanol
- AccuGENE® 1X TE, pH 7.6
- AccuGENE® 3M Sodium Acetate, pH 5.2

References
Section VIII: Separation of RNA in Agarose Gels

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<th>Page</th>
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</tr>
<tr>
<td>References</td>
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</tr>
</tbody>
</table>
Introduction
Separation of RNA in agarose gels is used for a number of different purposes, including Northern blots to monitor RNA expression levels, checking RNA integrity and size-selection of RNA for cloning experiments. Separation of RNA based on fragment length requires conditions that are different from DNA analysis. These include sample preparation, the use of sample and gel denaturants, electrophoresis buffers, and visualization.

Denaturing systems
The purpose of the experiment and the size of the RNA being separated are the primary drivers in determining which denaturing system to use. The most frequently used denaturants for RNA agarose gel electrophoresis are formaldehyde, formaldehyde/formamide, and glyoxal plus DMSO. In each system, the denatured RNA migrates through the agarose gel in a linear relation to the log of its molecular weight (similar to DNA). The most efficient RNA denaturant is methylmercury hydroxide. Because of the hazards associated with this denaturant, it is the least used system for RNA analysis.

The resolving powers of the glyoxal/DMSO and the formaldehyde buffer systems are nearly identical. For detection by Northern analysis, glyoxal/DMSO denaturant is preferable because these gels tend to produce sharper bands than the formaldehyde system. Glyoxal gels require more care to run than formaldehyde gels and because of the lower buffering capacity of glyoxal, these gels must be run at lower voltages than gels containing formaldehyde. Glyoxal gels require a phosphate electrophoresis buffer and the buffer must be recirculated during electrophoresis. If the pH of the buffer rises above 8.0, glyoxal dissociates from RNA, causing the RNA to renature and migrate in an unpredictable manner.

For staining purposes, either denaturant can be used. Ethidium bromide, GelStar® Nucleic Acid Gel Stain and SYBR® Green II Gel Stain bind formaldehyde-denatured RNA more efficiently than glyoxal-denatured RNA. Glyoxal denaturant can interfere with binding of the stain, but gel backgrounds are often lower in these gels than with formaldehyde-denatured gels.

It is important to minimize RNase activity when running agarose gels by following certain precautions. There are several agents on the market that effectively remove RNase’s or consult Sambrook, et al.

Which sample denaturation method to choose depends on the final goal of the experiment and the secondary structure of the RNA. There are several procedures to choose from, the most useful of which are described here. Any sample denaturation method can be used with any of the gel buffering systems. If simply checking the integrity of cellular RNA, no sample denaturation is necessary and TAE or TBE Buffer can be used.

<table>
<thead>
<tr>
<th>Common denaturants</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formamide</td>
<td>Retain biological activity</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>Sample recovery</td>
</tr>
<tr>
<td>Glyoxal</td>
<td>Northern blotting; significant secondary structure</td>
</tr>
</tbody>
</table>

Caution: Materials and methods shown here present hazards to the user and the environment. Refer to the safety information on page 210 before beginning these procedures.

Formamide-only denaturation
Formamide denaturation is suitable for almost all RNA samples and is recommended if you need to retain biological activity. Gels can be cast and run in standard TAE or TBE Buffer Systems or MOPS Buffer. If there is a significant amount of secondary structure, another sample denaturation method should be chosen.

- **Materials**
  - Water bath set to 70°C
  - Ice

- **Reagents**
  - AccuGENE® Molecular Biology Water (RNase-free)
  - Deionized formamide
  - AccuGENE® 10X MOPS Buffer
    - [200 mM MOPS, pH 7.0, 50 mM sodium acetate, 10 mM EDTA, 10 mM EGTA], pH 7.0
Section VIII: Separation of RNA in Agarose Gels

Preparation of RNA Samples — continued

**Formamide denaturation of RNA samples**

1. Bring the RNA volume up to 8 μl with RNase-free water.
2. Add 2 μl of 10X MOPS Buffer.
3. Add 9 μl of deionized formamide.
4. Mix thoroughly.
5. Heat at 70°C for 10 minutes.
6. Chill on ice for at least 1 minute before loading.

**Formaldehyde denaturation**

Formaldehyde denaturation is suitable when samples are to be recovered. It is necessary to ensure that the formaldehyde is fully removed from the recovered RNA prior to subsequent studies. Some enzymatic reactions, such as in vitro transcription, may be problematic even after complete removal of the formaldehyde.

- **Materials**
  - Water bath set to 70°C
  - Ice

- **Reagents**
  - AccuGENE® Molecular Biology Water (RNase-free)
  - AccuGENE® 10X MOPS Buffer (200 mM MOPS, pH 7.0, 50 mM sodium acetate, 10 mM EDTA, 10 mM EGTA), pH 7.0
  - 37% (v/v) formaldehyde
  - Deionized formamide

**Caution:** Materials and methods shown here present hazards to the user and the environment. Refer to the safety information on page 210 before beginning these procedures.

**Formaldehyde denaturation of RNA samples**

1. Bring the RNA volume up to 6 μl with RNase-free water.
2. Add 2 μl of 10X MOPS Buffer.
3. Add 2 μl of 37% formaldehyde.
4. Add 9 μl deionized formamide.
5. Mix thoroughly.
6. Heat at 70°C for 10 minutes. Chill on ice for at least 1 minute before loading.

**Glyoxal denaturation**

Glyoxal is a very efficient denaturant, but should not be used if samples are to be recovered. Glyoxal denatures RNA by introducing an additional ring into the guanosine residues, thus interfering with G-C base pairing. Glyoxal denaturation would be the recommended procedure for Northern blotting. Typically a phosphate electrophoresis buffer is recommended with recirculation to prevent formation of a pH gradient. Alternatively, a 10 mM PIPES, 30 mM bis-Tris buffer, or a 20 mM MOPS, 5 mM sodium acetate, 1 mM EDTA, 1 mM EGTA Buffer systems can also be used without recirculation.

- **Materials**
  - Water bath set to 50°C
  - Ice

- **Reagents**
  - AccuGENE® Molecular Biology Water (RNase-free)
  - DMSO
  - 100 mM Sodium phosphate, pH 7.0
    - Mix 5.77 ml of 1 M Na2HPO4 with 4.23 ml of 1 M NaH2PO4
    - Adjust volume to 100 ml with RNase-free water
  - 6 M Glyoxal, 40% (v/v) solution, deionized immediately before use
    - Pass solution through a small column of mixed-bed ion exchange resin until the pH is >5.0; large volumes can be deionized then stored frozen in aliquots at −20°C

**Caution:** Materials and methods shown here present hazards to the user and the environment. Refer to the safety information on page 210 before beginning these procedures.

**Glyoxal denaturation of RNA samples**

1. Bring the RNA volume up to 11 μl with RNase-free water.
2. Add 4.5 μl of 100 mM sodium phosphate.
3. Add 22.5 μl of DMSO
4. Add 6.6 μl of deionized glyoxal.
5. Mix thoroughly
6. Heat at 50°C for 1 hour. Chill on ice for at least 1 minute before loading.
### Buffers for RNA Electrophoresis

The two commonly used buffer systems for RNA electrophoresis are a phosphate buffer for glyoxal/DMSO denatured RNA and a MOPS Buffer for formaldehyde or formamide denatured RNA. These buffers are very low in ionic strength. During electrophoresis, a pH gradient may be generated along the length of the gel, resulting in the hydrolysis (melting) of the agarose gel. This problem can be avoided by recirculating the buffer. For glyoxal-denatured gels, if the pH of the buffer rises above pH 8.0, the glyoxal will dissociate from the RNA, causing the RNA to renature and migrate in an unpredictable manner.

### Buffer preparation

**NOTE:** Use RNase-free chemicals, water and containers

<table>
<thead>
<tr>
<th>Buffer preparation</th>
<th>g/l</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>100 mM Sodium Phosphate Buffer, pH 7.0</strong></td>
<td></td>
</tr>
<tr>
<td>(Glyoxal/DMSO denatured RNA)</td>
<td></td>
</tr>
<tr>
<td>1 M Na₃HPO₄</td>
<td>57.7 ml</td>
</tr>
<tr>
<td>1 M NaH₂PO₄</td>
<td>42.3 ml</td>
</tr>
</tbody>
</table>

— Adjust volume to 1 liter with RNase-free water
— Adjust volumes accordingly to prepare more buffer

<table>
<thead>
<tr>
<th>Buffer preparation</th>
<th>g/l</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>10X MOPS Buffer</strong></td>
<td></td>
</tr>
<tr>
<td>(Formaldehyde or Formamide denatured RNA)</td>
<td></td>
</tr>
<tr>
<td>200 mM MOPS (free acid)</td>
<td>41.86 g</td>
</tr>
<tr>
<td>50 mM sodium acetate</td>
<td>6.80 g</td>
</tr>
<tr>
<td>10 mM EDTA·2H₂O</td>
<td>3.72 g</td>
</tr>
<tr>
<td>10 mM EGTA (free acid)</td>
<td>3.80 g</td>
</tr>
</tbody>
</table>

— Mix with 850 ml of distilled water
— Adjust pH to 7.0 with 10 M NaOH
— Adjust volume to 1 liter with RNase-free water
— Filter through a 0.2 μm nitrocellulose filter and store in the dark

**Caution:** NaOH is a corrosive material. Use safety glasses and gloves to protect from burns.

### Section VIII: Separation of RNA in Agarose Gels

#### Electrophoresis of RNA

The choice of an agarose free of RNase contamination is of major importance. Lonza offers a variety of agarose products for RNA electrophoresis including The FlashGel® System for RNA, Reliant® and Latitude® Precast RNA Gels, Lonza agarose, AccuGENE® 10X MOPS Buffer, RNA Marker, GelStar® and SYBR® Green Nucleic Acid Gel Stains.

#### General guidelines

— Northern blotting requires a standard melting temperature agarose such as SeaKem® LE or NuSieve® 3:1 Agarose or Reliant® or Latitude® Precast RNA Gels
— If samples are to be recovered, a low melting temperature agarose can be used such as NuSieve® GTG® or SeaPlaque® GTG® Agarose
— A 1.5 - 2.0% gel made with SeaKem® GTG® or SeaKem® Gold Agarose or FlashGel® System, Reliant® or Latitude® Precast RNA Gels will work for RNA molecules of 500 - 10,000 nucleotides
Section VIII: Separation of RNA in Agarose Gels

Electrophoresis of RNA — continued

— For RNA smaller than 500 nucleotides, use a 3 or 4% NuSieve® 3:1 or MetaPhor® Agarose Gel
— For RNA larger than 10,000 nucleotides, SeaKem® Gold Agarose and FlashGel® System, Reliant® or Latitude® Precast RNA Gels will be a better choice for tighter bands and better resolution
— If a low melting temperature agarose is required, a 1.5 or 2.0% SeaPlaque® GTG® Gel can be used for separation of RNA from 500 - 10,000 nucleotides, while a 3.0% or 4.0% NuSieve® GTG® Gel should be used for fine resolution of RNA smaller than 500 nucleotides; NuSieve® GTG® Agarose is not recommended for formaldehyde/MOPS gels

Dye mobilities for RNA gels

The following table is a migration table of single-stranded RNA in relation to bromophenol blue (BPB) and xylene cyanol (XC) in formaldehyde or glyoxal agarose gels.

<table>
<thead>
<tr>
<th>Agarose (%)</th>
<th>XC (bp)</th>
<th>BPB (bp)</th>
<th>XC (bp)</th>
<th>BPB (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SeaKem® Gold Agarose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>6,300</td>
<td>660</td>
<td>9,500</td>
<td>940</td>
</tr>
<tr>
<td>1.5</td>
<td>2,700</td>
<td>310</td>
<td>4,300</td>
<td>520</td>
</tr>
<tr>
<td>2.0</td>
<td>1,500</td>
<td>200</td>
<td>2,300</td>
<td>300</td>
</tr>
<tr>
<td>SeaKem® GTG® and SeaKem® LE Agarose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>4,200</td>
<td>320</td>
<td>7,200</td>
<td>740</td>
</tr>
<tr>
<td>1.5</td>
<td>1,700</td>
<td>140</td>
<td>2,800</td>
<td>370</td>
</tr>
<tr>
<td>2.0</td>
<td>820</td>
<td>60</td>
<td>1,600</td>
<td>220</td>
</tr>
<tr>
<td>SeaPlaque® and SeaPlaque® GTG® Agarose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>2,400</td>
<td>240</td>
<td>4,400</td>
<td>400</td>
</tr>
<tr>
<td>1.5</td>
<td>800</td>
<td>80</td>
<td>1,900</td>
<td>180</td>
</tr>
<tr>
<td>2.0</td>
<td>490</td>
<td>30</td>
<td>1,050</td>
<td>120</td>
</tr>
<tr>
<td>NuSieve® 3:1 Agarose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>950</td>
<td>70</td>
<td>1,600</td>
<td>155</td>
</tr>
<tr>
<td>3.0</td>
<td>370</td>
<td>20</td>
<td>740</td>
<td>75</td>
</tr>
<tr>
<td>4.0</td>
<td>190</td>
<td>5</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>NuSieve® GTG® and MetaPhor® Agarose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>Not applicable</td>
<td>1,300</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>3.0</td>
<td>Not applicable</td>
<td>480</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>4.0</td>
<td>Not applicable</td>
<td>260</td>
<td>40</td>
<td></td>
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</tbody>
</table>

Agarose selection guide for RNA electrophoresis

<table>
<thead>
<tr>
<th>Separation</th>
<th>Northern Blotting</th>
<th>Preparative</th>
</tr>
</thead>
<tbody>
<tr>
<td>NuSieve® 3:1 Agarose</td>
<td>NuSieve® 3:1 Agarose</td>
<td>NuSieve® GTG® Agarose</td>
</tr>
<tr>
<td>MetaPhor® Agarose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SeaKem® LE Agarose</td>
<td>SeaKem® LE Agarose</td>
<td>SeaPlaque® GTG® Agarose</td>
</tr>
<tr>
<td>SeaKem® GTG® Agarose</td>
<td>SeaKem® GTG® Agarose</td>
<td></td>
</tr>
<tr>
<td>SeaKem® Gold Agarose</td>
<td>SeaKem® Gold Agarose</td>
<td>SeaPlaque® GTG® Agarose</td>
</tr>
</tbody>
</table>

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Electrophoresis of gels containing formaldehyde

A formaldehyde denaturant with a MOPS buffer is the most commonly used system for RNA electrophoresis. Care should be taken when handling gels containing formaldehyde. These gels are less rigid than other agarose gels. NuSieve® GTG® Agarose is not compatible with the MOPS buffering system.

Formaldehyde electrophoresis of RNA

NOTE: Do not exceed 20 μg of RNA per lane as larger amounts can result in loss of resolution.

1. For a 1% gel, dissolve 1.0 g of agarose in 72 ml of water. Adjust the amounts for different percent gels
2. Cool agarose to 60°C in hot water bath
3. Place in fume hood
4. Immediately add 10 ml of prewarmed 10X MOPS Buffer
5. Add 5.5 ml of prewarmed 37% formaldehyde
6. Cast gel in a fume hood
7. Denature the RNA sample following one of the methods previously described
8. Add 2 μl of formaldehyde loading buffer per 20 μl of sample
9. Mix thoroughly
10. Remove the gel comb
11. Place the gel in the electrophoresis chamber
12. Cover surface of the gel to a depth of 1 mm with 1X MOPS Buffer
13. Load the samples
14. Electrophorese at a maximum of 5 V/cm (interelectrode distance) until the bromophenol blue has traveled at least 80% of the way through the gel (see mobility table on page 135)

Caution: Materials and methods shown here present hazards to the user and the environment. Refer to the safety information on page 210 before beginning these procedures.
Electrophoresis of gels containing glyoxal/DMSO

These gels should be run slower than formaldehyde gels with buffer recirculation to avoid the formation of a pH gradient. Glyoxylated RNA will give sharper bands than formaldehyde-treated RNA.

Glyoxal/DMSO electrophoresis of RNA

1. For a 1% gel, dissolve 1.0 g of agarose in 100 ml of 10 mM sodium phosphate, pH 7.0. Adjust amounts for different percent gels.
2. Cool agarose to 60°C in hot water bath.
3. Cast the gels to a thickness that will accommodate a loading volume of 60 μl.
4. Remove the comb.
5. Place the gel in the electrophoresis chamber.
6. Cover gel with 10 mM sodium phosphate buffer to a depth of 1 mm.
7. Add 12 μl of glyoxal loading buffer per 45 μl of sample.
8. Mix thoroughly.
9. Load 0.5 μg - 1.0 μg of RNA per lane.
10. Electrophorese at 4 V/cm (interelectrode distance) while the buffer is recirculated. If no recirculation apparatus is available, pause electrophoresis every 30 minutes and remix the buffer.
11. Electrophorese until the bromophenol blue has traveled at least 80% of the way through the gel (see mobility table on page 135).

Materials
- Water bath set to 60°C
- Fume hood
- Accessories to cast an agarose gel
- Electrophoresis chamber and power supply
- Flask or beaker
- Recirculating unit

Reagents
- 100 mM sodium phosphate pH 7.0
- Glyoxal loading buffer
  (10 mM sodium phosphate, pH 7.0, 0.25% bromophenol blue and xylene cyanol, 50% glycerol)

Caution: Materials and methods shown here present hazards to the user and the environment. Refer to the safety information on page 210 before beginning these procedures.
**Introduction**

Detection of RNA in agarose gels varies depending on the denaturant, stain and photographic conditions used. Lonza offers two highly sensitive stains, GelStar® and SYBR® Green II Gel Stains, for the detection of RNA in agarose gels. These stains exhibit higher RNA detection sensitivity than ethidium bromide, allowing you to load less RNA sample on your gel. Unlike ethidium bromide, GelStar® and SYBR® Green II Stain only fluoresce upon binding to the nucleic acid resulting in lower background fluorescence which is particularly useful when including the stain in the gel for glyoxal denatured samples or when concentrations of formaldehyde in the gel exceed 2 M. The chart below shows the detection sensitivity of various dyes for RNA using in-gel or post-staining techniques.

<table>
<thead>
<tr>
<th>Stain</th>
<th>Method</th>
<th>Native RNA</th>
<th>Glyoxal RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>GelStar® Stain</td>
<td>in-gel</td>
<td>10 ng</td>
<td>10 ng</td>
</tr>
<tr>
<td>Ethidium bromide, no destain</td>
<td>in-gel</td>
<td>30 ng</td>
<td>150 ng</td>
</tr>
<tr>
<td>Ethidium bromide, with destain</td>
<td>in-gel</td>
<td>30 ng</td>
<td>50 ng</td>
</tr>
<tr>
<td>SYBR® Green II Stain</td>
<td>post stain</td>
<td>15 ng</td>
<td>50 ng</td>
</tr>
</tbody>
</table>

Limits are based on optimal detection methods for each stain. Samples detected with SYBR® Green Stain were post-stained, samples detected with ethidium bromide or GelStar® Stain were detected by in-gel staining (post-stained gels showed similar results).

FlashGel® System (see page 25), uses a similar stain system and is capable of RNA detection <10ng.

### Tips for staining gels with GelStar® or SYBR® Green II Gel Stains

Follow the guidelines below to increase the detection sensitivity of GelStar® or SYBR® Green II Gel Stains.

- New clear polypropylene containers [e.g., Rubbermaid® Recycling #5 Plastics] should be obtained for use with GelStar® and SYBR® Green Stains. When stored in the dark in polypropylene containers, the diluted stain can be used for up to 24 hours and will stain 2 to 3 gels with little decrease in sensitivity. The containers should be rinsed with distilled water [do not use detergents] after each use and dedicated to GelStar® or SYBR® Green Stain use only.

- These stains bind to glass and some non-polypropylene (polystyrene) plastics, resulting in reduced or no signal from the nucleic acid.

- A 2X working solution of GelStar® or SYBR® Green Stain should be prepared just prior to use from the 10,000X stock solution by diluting in 1X MOPS Buffer or a pH 7.5 to 8.5 buffer [e.g., TAE, TBE or TE].

- For non-denaturing or denaturing polyacrylamide gels, dilute GelStar® or SYBR® Gel Stain 1:10,000X in 1X electrophoresis buffer.

- Agarose gels should be cast no thicker than 4 mm. As gel thickness increases, diffusion of the stain into the gel is decreased, lowering the efficiency of RNA detection.

- Optimal sensitivity for GelStar® and SYBR® Green Stains is obtained by using the appropriate photographic filters for each stain.
  
  - GelStar® Stain: Wratten® or Tiffen® #9 Filter
  
  - SYBR® Green Stains: Wratten or Tiffen #15 Filter

- We do not recommend photographing gels with a 254 nm transilluminator. An outline of the UV light source may appear in photographs. A filter that will allow a 525 nm transmission and exclude infrared light is required.
Procedure for staining RNA with GelStar<sup>®</sup> or SYBR<sup>®</sup> Green II Gel Stains

For optimal resolution, sharpest bands and lowest background, stain the gel with GelStar<sup>®</sup> or SYBR<sup>®</sup> Green II Gel Stain following electrophoresis.

The photographs below demonstrate the detection sensitivity of various stains with different sample denaturants.

Follow the steps below to stain RNA after electrophoresis:

1. Remove the concentrated stock solution of the stain from the freezer and allow the solution to thaw at room temperature.
2. Spin the solution in a microcentrifuge to collect the dye at the bottom of the tube.
3. Dilute the 10,000X concentrate to a 2X working solution (2 μl/10 ml) in 1X MOPS Buffer, in a clear plastic polypropylene container. Prepare enough staining solution to just cover the top of the gel.
4. Remove the gel from the electrophoresis chamber.
5. Place the gel in staining solution.
6. Gently agitate the gel at room temperature.
7. Stain the gel for 60 minutes.
8. Remove the gel from the staining solution and view with a transilluminator, CCD camera or Dark Reader<sup>®</sup> Transilluminator.

NOTE: Gels stained with GelStar<sup>®</sup> or SYBR<sup>®</sup> Green II Gel Stains do not require destaining. The dyes’ fluorescence yield is much greater when bound to RNA than when in solution.

Caution: Materials and methods shown here present hazards to the user and the environment. Refer to the safety information on page 210 before beginning these procedures.

Materials
- Clear polypropylene container (e.g., Rubbermaid® Recycling #5 Plastics)
- GelStar<sup>®</sup> Photographic Filter (Wratten® #9 equivalent) or SYBR<sup>®</sup> Green Photographic Filter (Wratten® #15 equivalent)
- Microcentrifuge
- UV transilluminator Dark Reader<sup>®</sup> Transilluminator (Clare Chemical Research, Inc.) or CCD imaging system

Reagents
- 1X MOPS Buffer
- GelStar<sup>®</sup> or SYBR<sup>®</sup> Green II Gel Stain stock solution

Section VIII: Separation of RNA in Agarose Gels
Detection of RNA with GelStar<sup>®</sup> or SYBR<sup>®</sup> Green II Gel Stains — Continued
Follow this procedure when including GelStar® Nucleic Acid Gel Stain in the agarose gel

**NOTE:** Unlike ethidium bromide, GelStar® Nucleic Acid Gel Stain does not interact with glyoxal.

1. Remove the concentrated stock solution of GelStar® Stain from the freezer and allow the solution to thaw at room temperature.
2. Spin the solution in a microcentrifuge tube.
3. Prepare the agarose solution.
4. Once the agarose solution has cooled to 70°C, add the stain by diluting the stock 1:5,000 into the gel solution prior to pouring the gel (2 μl per 10 ml).
5. Slowly swirl the solution.
6. Pour the gel into the casting tray.
7. Load samples onto the gel.
8. Run the gel.
9. Remove the gel from the electrophoresis chamber.
10. View with a 300 nm UV Transilluminator, CCD camera or Clare Chemical’s Dark Reader® Transilluminator.

**NOTE:** GelStar® Stained Gels do not require destaining. The dye’s fluorescence yield is much greater when bound to RNA than when in solution.
Visualization by photography

Gels stained with GelStar® and SYBR® Green II Gel Stains exhibit negligible background fluorescence, allowing long film exposures when detecting small amounts of RNA. Use the appropriate photographic filter for the stain you are using (see page 138). The table below provides suggested film types and photographic conditions.

<table>
<thead>
<tr>
<th>Polaroid® Film</th>
<th>f-stop</th>
<th>Exposure time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 5? or 667</td>
<td>4.5</td>
<td>0.5-2 seconds</td>
</tr>
<tr>
<td>Type 55</td>
<td>4.5</td>
<td>15-45 seconds</td>
</tr>
</tbody>
</table>

Visualization by image capture system

GelStar® and SYBR® Green II Nucleic Acid Gel Stains are compatible with most CCD and video imaging systems. Due to variations in the filters for these systems, you may need to purchase a new filter. Lonza does not sell filters for this type of camera. Contact your systems manufacturer and using the excitation and emission information listed, they can guide you to an appropriate filter.

<table>
<thead>
<tr>
<th>Stain</th>
<th>Emission (nm)</th>
<th>Excitation (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GelStar® Stain</td>
<td>527</td>
<td>493</td>
</tr>
<tr>
<td>SYBR® Green I Stain</td>
<td>521</td>
<td>494</td>
</tr>
<tr>
<td>SYBR® Green II Stain</td>
<td>513</td>
<td>497</td>
</tr>
</tbody>
</table>

Application notes

— The fluorescent characteristics of GelStar® and SYBR® Green II Gel Stains make them compatible with argon ion lasers
— Gels previously stained with ethidium bromide can subsequently be stained with GelStar® or SYBR® Green II Gel Stain following the standard protocol for post-staining. There will be some decrease in sensitivity when compared to a gel stained with only GelStar® or SYBR® Green II Gel Stain
— We recommend the addition of 0.1% to 0.3% SDS in the prehybridization and hybridization solutions when performing Northern blots on gels stained with these dyes

Materials

— Staining vessel larger than the gel
— UV transilluminator, Dark Reader® Transilluminator (Clare Chemical Research, Inc.) or CCD imaging system
— Magnetic stir plate
— Magnetic stir bar

Reagents

— Ethidium bromide stock solution (10 mg/ml) (1.0 g Ethidium bromide, 100 ml distilled water, stir on magnetic stirrer for several hours, transfer the solution to a dark bottle, store at room temperature)
— Distilled water
— 0.1 M ammonium acetate

Caution: Materials and methods shown here present hazards to the user and the environment. Refer to the safety information on page 210 before beginning these procedures.
Section VIII: Separation of RNA in Agarose Gels

Detection of RNA with Ethidium Bromide

Introduction

Ethidium bromide does not stain RNA as efficiently as it does DNA, so be certain that sufficient RNA is loaded to see the band of interest. Although as little as 10 ng - 20 ng of DNA can usually be visualized by ethidium bromide fluorescence, as much as 10-fold more RNA may be needed for good visualization. Visualization of poorly staining RNA is made even more difficult by the higher background fluorescence of RNA gels. In formaldehyde gels, background fluorescence can be minimized by dropping the formaldehyde concentration in the gel from 2.2 M to 0.66 M. Staining with ethidium bromide is not recommended when performing Northern blot analysis onto nylon membranes.

Follow the steps below to stain RNA after electrophoresis

1. Prepare enough working solution of ethidium bromide (0.5 mg/ml of ethidium bromide in 0.1 M ammonium acetate) to cover the surface of the agarose gel.
2. Remove the gel from the electrophoresis chamber.
3. Submerge the gel for 30 - 45 minutes in the ethidium bromide solution.
4. Remove the gel from the solution.
5. Submerge the gel for 60 minutes in a new container filled with distilled water.

NOTE: For gel concentrations of 4% or greater, these times may need to be doubled. If after destaining the background is still too high, continue to destain.
6. Repeat in fresh distilled water.
7. Gels can be viewed with a UV light transilluminator, Dark Reader® Transilluminator (Clare Chemical Research, Inc.) or CCD imaging system.

Follow the steps below when including ethidium bromide in the agarose gel

1. Prepare agarose solution.
2. While the agarose solution is cooling, add ethidium bromide to a final concentration of 0.5 μg/ml to the solution.
3. Slowly swirl the solution.
4. Pour the gel into the casting tray.
5. Add ethidium bromide to the running buffer to a final concentration of 0.5 μg/ml.
6. Load and run the gel.
7. Destain the gel by submerging the gel in distilled water for 60 minutes.
8. Repeat in fresh distilled water.
9. Gels can be viewed with a UV light transilluminator, Dark Reader® Transilluminator (Clare Chemical Research, Inc.) or CCD imaging system.

Decontamination of GelStar® and SYBR® Green Gel Stains and ethidium bromide solutions

Staining solutions should be disposed of by passing through activated charcoal followed by incineration of the charcoal. For absorption on activated charcoal, consult Sambrook, et al., pp. 6.16 - 6.19, [1989]. Follow state and local guidelines for decontamination and disposal of Nucleic Acid Staining Solutions.
Recommended agaroses for Northern blotting

When transferring RNA from an agarose gel to a membrane, a standard melting temperature agarose should be used. Standard melting temperature agaroses have higher gel strength than low melting temperature agaroses and do not fracture during transfer. The table below is a list of Lonza agaroses and precast gels that are recommended for blotting.

<table>
<thead>
<tr>
<th>Agarose</th>
<th>Size Range (bases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NuSieve® 3:1 Agarose</td>
<td>&lt;500</td>
</tr>
<tr>
<td>SeaKem® LE Agarose</td>
<td>500 - 10,000</td>
</tr>
<tr>
<td>SeaKem® GTG® Agarose</td>
<td>500 - 10,000</td>
</tr>
<tr>
<td>SeaKem® Gold Agarose</td>
<td>500 - 10,000</td>
</tr>
<tr>
<td>Reliant® &amp; Latitude® RNA Gels</td>
<td>25 - 10,000</td>
</tr>
</tbody>
</table>

Tips for agarose gel preparation

— Use the lowest agarose concentration required to resolve your fragments
— If staining with ethidium bromide prior to transfer, thoroughly destain the gel with distilled water
— Avoid casting thick gels (>4 mm) unless absolutely necessary. Thick gels not only require longer electrophoretic times, but may interfere with the free transfer of nucleic acids to the hybridization membrane
— Do not use more than a 500.0 gm weight on top of the stack. Excess weight can compress the gel. This will increase the agarose concentration and decrease the pore size, which can inhibit movement of the buffer and RNA

Choosing the appropriate membrane

When transferring RNA, nylon membranes have several advantages over nitrocellulose:

<table>
<thead>
<tr>
<th>Nylon</th>
<th>Nitrocellulose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncharged/charged</td>
<td>Supported/unsupported</td>
</tr>
<tr>
<td>High strength</td>
<td>Good strength*</td>
</tr>
<tr>
<td>Good for reprobing</td>
<td>Poor for reprobing</td>
</tr>
<tr>
<td>UV crosslinking</td>
<td>Baking in vacuum oven at 80ºC</td>
</tr>
<tr>
<td>50 nucleotides**</td>
<td>500 nucleotides**</td>
</tr>
</tbody>
</table>

*Good for supported nitrocellulose, poor for unsupported nitrocellulose. **Lower size limit for efficient nucleic acid retention.

Choosing a blotting method

Consider the following when choosing a blotting method:

— Gel concentration
— Fragment size
— Speed and transfer efficiency

Capillary transfer

Traditional passive capillary transfer uses paper towels to draw the transfer buffer from a reservoir through the gel. Passive capillary transfer takes 16 - 24 hours to complete. The most common complaints are poor transfer efficiency for larger RNA molecules and long transfer time.

Vacuum transfer

Vacuum transfer systems use negative pressure to pull the transfer buffer through the gel along with the nucleic acid. These systems take approximately 30 - 60 minutes to complete. Vacuum blotting can be useful for high concentration gels where compression of the gel is a concern.

Electroblotting

Electroblotting is most often used for polyacrylamide gels. A voltage gradient pulls the sample out of the gel and onto a membrane. Semi-dry electroblotting requires minimal buffer with a low voltage and current. Care should be taken so smaller fragments are not pulled completely through the membrane.
General guidelines for Northern blotting

It is important to minimize RNase activity by following certain precautions. There are several agents on the market that effectively remove RNase's or consult Sambrook, et al

Wear gloves throughout the procedure. RNAs are not safe from nuclease degradation until they have been immobilized on the membrane

Cut or mark membrane for orientation. Do this before wetting the membrane

The blotting membrane should be in contact with the underside of the gel. Since nucleic acids will concentrate near the bottom of the gel, there is less distance for them to travel during the transfer

Cut all papers and membranes to the correct size, such that, the only thing pulling the transfer buffer is the buffer solution

Avoid bubbles, ensure that there is even contact between all the layers of the blotting system

Incorrect denaturation of probe can cause poor transfer results

Glyoxal gels can be transferred immediately after electrophoresis. The glyoxal will be removed in the post transfer, prehybridization wash

Destain gels that contain formaldehyde or ethidium bromide to avoid sample loss and inefficient transfer efficiency

NOTE: Soak gel 3 times for 5 - 10 minutes each in either 1X MOPS, transfer buffer, or sterile water. This will remove most of the formaldehyde and excess background fluorescence seen with ethidium bromide. Complete destaining usually takes 2 hours or longer.

Lonza's Reliant® and Latitude® Precast RNA Gels do not contain any denaturants or stains and do not require destain prior to transfer.

Materials
- Nitrocellulose or nylon membrane
- 2-3 glass dishes larger than gel
- Paper towels
- Whatman® 3MM Chromatography Paper
- Glass or plastic pipette
- Flat ended forceps
- Glass plate to serve as a platform for the gel
- Plastic wrap
- <500.0 g weight
- Orbital or rocking platform shaker

Reagents
- AccuGENE® Molecular Biology Water
- AccuGENE® 20X SSPE or AccuGENE® MOPS Buffer

Procedure for RNA transfer by passive capillary electrophoresis transfer buffer

The concentration of the transfer buffer will vary depending on the method and type of membrane used. The capillary method outlined here uses a nylon membrane and 5X SSPE Buffer.

20X SSPE

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>175.3 g</td>
</tr>
<tr>
<td>NaH₂PO₄</td>
<td>27.6 g</td>
</tr>
<tr>
<td>EDTA</td>
<td>7.4 g</td>
</tr>
</tbody>
</table>

Adjust to pH 7.4 with 10 N NaOH
To 1 liter with distilled water
Follow the steps below for gel preparation and setting up the transfer

1. Cast a standard melting temperature agarose gel no thicker than 4 mm.
2. Electrophorese RNA following standard protocols.
3. For formaldehyde gels, follow the steps below prior to transfer. For glyoxal gels, proceed to step 4.
4. Soak the gel in an excess of 1X MOPS Buffer, distilled water or 5X SSPE for 10 minutes.
5. Repeat three times with new wash solution each time.
6. Float membrane in RNase-free water for 5 minutes.
7. Equilibrate membrane in 5X SSPE for 5 minutes. The membrane may remain in the transfer buffer until it is used.
8. Set up transfer using either an upward capillary transfer set up or downward capillary transfer set up.
9. Allow the transfer to proceed as follows:

<table>
<thead>
<tr>
<th>Transfer set-up</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upward</td>
<td>16 - 24 hours</td>
</tr>
<tr>
<td>Downward</td>
<td>1 - 4 hours depending on gel thickness and concentration</td>
</tr>
<tr>
<td>Turboblotter™ System</td>
<td>1 - 3 hours</td>
</tr>
</tbody>
</table>

10. Remove the paper towel or chromatography paper.
11. Remove the gel and membrane together.
12. Mark the positions of the wells on the membrane with a pencil for orientation then remove the gel from the transfer setup.
13. Rinse the membrane for 30 - 60 seconds in transfer buffer.
14. Place the membrane on a sheet of Whatman® 3MM Chromatography Paper.
15. Treat membrane as described in Immobilizing RNA on a membrane, next section.

**Materials**
- Membrane
- Plastic wrap
- UV light source
- Vacuum dryer set to 80°C or hot oven at 65°C.

**Reagents**
- AccuGENE® 1 M Tris pH 8.0
- RNase-free water
- AccuGENE® Molecular Biology Water or AccuGENE® 10X Mops Buffer
- AccuGENE® 20X SSPE

**Immobilizing RNA on a membrane**

The methods and procedures for immobilizing RNA on the membrane are essentially the same as they are for DNA.

If samples have been denatured under the formaldehyde system, immobilization can take place immediately after transfer without any pretreatment steps. The formaldehyde has been removed from the system prior to transfer during the gel destaining steps. If the glyoxal denaturing system was used, the glyoxal must now be removed from the filter. Follow one of the procedures below to remove glyoxal from the membrane.

**Option 1**

1. Air dry the membrane.
2. Bake the membrane as follows:

<table>
<thead>
<tr>
<th>Membrane Type</th>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrocellulose</td>
<td>80°C</td>
<td>2 hours under vacuum</td>
</tr>
<tr>
<td>Nylon</td>
<td>65°C</td>
<td>1 - 1.5 hours</td>
</tr>
</tbody>
</table>

3. Immerse the filter in 200 ml of preheated 20 mM Tris, pH 8.0.
4. Cool to room temperature.

**Option 2**

1. Immobilize RNA on membrane either by UV irradiation or baking.
2. Wash membrane 65°C for 15 minutes in 20 mM Tris, pH 8.0.

**References**

Section VIII: Separation of RNA in Agarose Gels

The FlashGel® System completes RNA analysis in less than 30 minutes and requires one-fifth the amount of total RNA for detection. The FlashGel® System is recommended for verification and analysis of total RNA (Figure 1), quick checks of native RNA (Figure 3) and checks for RNA degradation (Figure 2).

Rapid RNA analysis

The FlashGel® System for RNA separates up to 34 samples of RNA in 8 minutes or less. RNA samples are visible on the FlashGel® Dock for up to 4 minutes, after which they fade and then reappear with increasing intensity following a 10-20 minute post-run hold. Full analysis is complete in less than 30 minutes, compared to the 1-3 hours required for typical agarose gels. Figure 1 demonstrates analysis time of the FlashGel® System compared to other agarose gel methods for RNA.

Sensitive detection

RNA quantities <10 ng per band are clearly detected on the FlashGel® System for RNA. Some RNA preparations deliver such low yields that there may not be the surplus RNA needed to assess integrity. The FlashGel® System for RNA uses a stain that reduces the amount of required RNA by a factor of five or greater compared to ethidium bromide. Figure 1 illustrates detection sensitivity of the FlashGel® System for RNA compared to other agarose gel and staining methods.

Clean, enclosed system

The FlashGel® System for RNA eliminates the hazards and tedium associated with RNA gel preparation. The cassettes fully enclose the gel and running buffer, eliminating operator exposure to hazardous reagents, and protecting samples from contaminating RNases. The cassettes contain a 1.2% agarose and buffer blend which are selected for purity, manufactured in a dedicated clean room, and guaranteed RNase-free. The FlashGel® Dock does not come in contact with samples or gels, so it is not necessary to dedicate a unit for RNA work.

Figure 1. Sample of RNA Marker (Cat. # 50575) lanes 1 & 2, and E. coli Total RNA (Ambion) Lanes 3 & 4 contain 50 ng (lanes 1 & 3 or 250 ng (lanes 2 & 4) of RNA per 5μl load.

Figure 2. Checking Sample Quality with the FlashGel® System for RNA Sample degradation is clearly visible on the FlashGel® System for RNA. 8 min run at 225 V, followed by 20 min hold prior to imaging. Lane 1: RNA Marker (Lonza); Lane 2: 250 ng E.coli Total RNA (Ambion); Lanes 3-7: 250 ng E.coli Total RNA incubated with increasing levels of RNase A. Intact, denatured RNA shows sharp, clear bands on the FlashGel® System. Partially degraded RNA has a smeared appearance, and completely degraded RNA appears as a low molecular weight smear.
Section VIII: Separations of RNA in Agarose Gels

FlashGel® System for RNA Analysis — continued

Simple Protocol

Procedure

1. Insert cassette into FlashGel® Dock.
2. Pre-load wells with RNase-free Water (Cat. #51200).
3. Load samples.\footnote{Formaldehyde Loading Buffer and RNA Markers are recommended for best performance. Denatured RNA samples will migrate according to their true size on FlashGel® RNA Cassettes.}
4. Plug in and turn on light and power (225 V).
5. Run for 8 minutes.\footnote{The FlashGel® DNA Marker may be used to monitor separation since it will immediately be visible on the dock in real time and be visible for the full length of the run - stop the run when the 100 bp band reaches the end of the gel. The visible blue xylene cyanol band may also be used to track migration - stop the run when the blue band has migrated approximately 1/3 the distance of the gel.}
6. Turn off power and hold for 10-20 minutes or until desired RNA bands are visible on the gel.
7. Photograph as usual.\footnote{RNA bands are visible for up to 4 minutes in real-time runs (see Figure 3). If desired bands are seen within that time, then a 10-20 minute hold time prior to imaging is not required. The longer the post-run incubation period (up to 20 minutes), the brighter the RNA bands become. Unlike typical agarose gels where samples diffuse immediately, RNA samples on FlashGel® Cassettes remain intact for >2 hours. Caution: Materials and methods shown here present hazards to the user and the environment. Refer to the safety information on page 210 before beginning these procedures.}

<table>
<thead>
<tr>
<th>Lane</th>
<th>Sample Description</th>
<th>Load Volume</th>
<th>Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RNA Marker (Lonza)</td>
<td>1.25 l</td>
<td>Native samples prepared with FlashGel® Loading Dye</td>
</tr>
<tr>
<td>2</td>
<td>E. coli Total RNA (Ambion)</td>
<td>1.25 l</td>
<td>Formaldehyde denaturation at 65°C for 5 minutes</td>
</tr>
<tr>
<td>3</td>
<td>RNA Marker</td>
<td>5 l</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>E. coli Total RNA</td>
<td>5 l</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>RNA Marker</td>
<td>1.25 l</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>E. coli Total RNA</td>
<td>1.25 l</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>RNA Marker</td>
<td>5 l</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>E. coli Total RNA</td>
<td>5 l</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3. Quick checks of Native RNA on the FlashGel® System for RNA Analysis. Native and formaldehyde denatured RNA samples were run at 225 V for 4 min. Native, non-denatured RNA samples do not require additional incubation time compared to formaldehyde denatured samples, and may be suitable for very fast checks of RNA integrity. After 20 additional minutes, the denatured samples would absorb stain and increase in visibility.

Some components and technology of the FlashGel® System are sold under licensing agreements. The nucleic acid stain in this product is manufactured and sold under license from Molecular Probes, Inc., and the FlashGel® Cassette is sold under license from Invitrogen IP Holdings, Inc, and is for use only in research applications or quality control, and is covered by pending and issued patents. The FlashGel® Dock technology contains Clare Chemical Research, Inc. Dark Reader® Transilluminator technology and is covered under US Patents 6,198,107; 6,512,236; and 6,914,250. The electrophoresis technology is licensed from Temple University and is covered under US Patent 6,905,385.
## In This Section

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplification of Plasmid cDNA Libraries with SeaPrep® Agarose</td>
<td>150</td>
</tr>
<tr>
<td>Preparing Agarose for use in Cell Culture Applications</td>
<td>152</td>
</tr>
<tr>
<td>References</td>
<td>154</td>
</tr>
</tbody>
</table>
Introduction

This section describes an amplification technique developed to address the common problem of disproportionate amplification of plasmids seen when expression cloning mRNAs of very low abundance encoding cytokines, receptors, and cell surface molecules from plasmid cDNA libraries derived from highly complex tissue sources. This amplification technique allows amplification of a plasmid cDNA library in a representative fashion, decreasing the possibility that less abundant clones would vanish during the amplification due to differential rates of replication.

The technique below describes a method where bacterial transformants are suspended, not plated, in low gelling temperature agarose. With this method, it has been found that, from generation to generation of amplification, the relative abundance of bacterial cells containing plasmids encoding selected hematopoietic factors varies less than two fold.

Preparing the bacterial growth media plus agarose

NOTE: Alternatively, prepared solutions of L Broth, LB Broth or Super Broth can also be used.

1. In a 2 liter Erlenmeyer flask, add the following reagents to 1 liter of double distilled water:
   1a. 20.0 g bacto-tryptone
   1b. 10.0 g bacto-yeast extract
   1c. 10.0 g NaCl
2. Measure pH of solution.
3. If necessary, adjust to pH 7.0 using 1 N NaOH or 1 N HCl.
4. Add 3.0 g of SeaPrep® Agarose to the solution.
5. Autoclave the solution on a liquid cycle for 15 - 20 minutes.
6. Cool the solution to 40°C.
7. Store in a 37°C water bath until ready-for-use.
8. Prior to use add 2 ml of 50 mg/ml ampicillin.

Materials

- Autoclave
- 2 L Erlenmeyer flasks
- Water bath set to 37°C
- pH meter
- Sterile 50 ml polypropylene centrifuge tubes
- Ice water bath
- Sterile 500 ml centrifuge bottle
- Centrifuge
- Sterile Nunc® Tubes
- 0.2 μm filter

Reagents

- SeaPrep® Agarose
- 2X L Broth (LB)
- 50 mg/ml ampicillin
- Double distilled water
- Bacto-tryptone
- Bacto-yeast extract
- NaCl
- 1 N NaOH or 1 N HCl
- pH standards
- LB-amp plates
- Filter sterilized glycerol

1. Prepare vector using a tailing method.
2. Prepare and purify cDNA following standard procedures.
3. Optimize the tailing of cDNA inserts following standard procedures.
4. Scale up the appropriate cDNA/vector-annealing reaction to generate up to 2.5 x 10^6 cfu/2000 ml.
5. Perform the appropriate number of standard bacterial transformations following standard procedures.
6. Pool all the transformations after the 37°C incubation.

NOTE: General procedures for vector and cDNA preparation, ligation and transformation can be found in Sambrook, J., et al. and Kriegler, M.
Plasmid cDNA library amplification

1. Add ampicillin to a final concentration of 100 μg/ml (2.0 ml of 50 mg/ml stock solution) to the 2X LB-agarose solution.
2. Add transformants (1.25 x 10^6 cfu) to the LB-agarose solution.
3. Gently swirl to avoid foaming.
4. Aliquot the transformation mix into 25 ml aliquots in 50 ml polypropylene centrifuge tubes.
5. Place the tubes in an ice-water bath for 20 - 60 minutes to allow the agarose gel to set.
6. Incubate overnight at 37°C undisturbed.
7. Plate 100 μl of the cell-agarose suspension directly on LB-amp plates for titer determination or prepare the suspension for library storage.

Library Storage

1. Prepare 12.5% glycerol by diluting in double distilled water.
2. Filter solution through a 0.2 μm filter into a sterile container.
3. Pour the colony-containing gel into a sterile 500 ml centrifuge bottle.
4. Pellet the cells: at 8,000 rpm for 20 minutes at room temperature. It is not necessary to melt the gel, the cells will pellet through the soft agarose.
5. Decant the media from the cell pellets.
6. Resuspend the cell pellets in 100 ml of 12.5% glycerol in 2X L broth.
7. Aliquot into sterile Nunc® tubes.
8. Stores library at —70°C.

Materials
- Scalpel or razor blade
- GelBond® Film
- Glass plate or press board
- Clamps or elastic bands
- A forced hot-air oven

Reagents
- SeaKem® LE or GTG® Agarose
- SeaKem® Gold Agarose
- NuSieve® 3:1 or NuSieve® GTG® Agarose
- MetaPhor® Agarose

References
The concentration and type of agarose to use largely depends on your cell system, the application, and the lot specific gel strength of the agarose. There are three types of agarose which can be used for cell culture media, each having unique properties making one more suitable for a given application than another.

— SeaKem® LE Agarose – A standard melting and gelling temperature agarose which can be used as a solid medium to support cell growth by supplementation with growth factors and nutrients, top agar enriched with magnesium for baculovirus screening, substrate for bacterial growth and for colony lifts.

— SeaPlaque® Agarose – A low gelling temperature agarose (28°C) that remains a liquid at 37°C allowing the manipulation of cells within the solution; SeaPlaque® Agarose can be used as a semi-solid media for anchorage independent assays, plaque assays or overlays; SeaPlaque® Agarose has also been found to be very effective as a medium for protoplast culture.

— SeaPrep® Agarose – Unique ultra-low gelling temperature (15°C) and gel strength agarose (>75 g/cm²); SeaPrep® Agarose is ideal for hybridoma cloning; Cells can be recovered from the gel by increasing the temperature slightly, allowing transfer to a viable cell suspension for subsequent growth in liquid medium.
Section IX: Special Applications in Agarose Gels

Preparing Agarose for use in Cell Culture Applications — continued

Suggested Agarose Products and Concentration Guidelines

The table below provides general guidelines on agaroses and gel concentrations for given applications. General guidelines and specific information pertaining to a given cell type and application can be obtained from the current literature and *Cell Biology: A Laboratory Manual*.

<table>
<thead>
<tr>
<th>Application</th>
<th>SeaKem® LE Agarose</th>
<th>SeaPlaque® Agarose</th>
<th>SeaPrep® Agarose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammalian cell culture</td>
<td>0.3% - 1%</td>
<td>0.25% - 1.5%</td>
<td></td>
</tr>
<tr>
<td>Baculovirus screening</td>
<td></td>
<td>0.3% - 0.6%</td>
<td></td>
</tr>
<tr>
<td>Bacterial culture</td>
<td>0.7% - 1.0%</td>
<td>1.0%</td>
<td></td>
</tr>
<tr>
<td>Colony lifts</td>
<td>0.7% - 1.0%</td>
<td>1.0%</td>
<td></td>
</tr>
<tr>
<td>Anchorage independent assays</td>
<td>0.25% - 0.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plaque assays</td>
<td>0.3% - 1%</td>
<td>0.50% - 1.5%</td>
<td></td>
</tr>
<tr>
<td>Overlays</td>
<td>0.3% - 0.6%</td>
<td>0.3% - 1%</td>
<td></td>
</tr>
<tr>
<td>Protoplast culture</td>
<td></td>
<td>0.35% - 0.7%</td>
<td>0.8% - 1.5%</td>
</tr>
<tr>
<td>Hybridoma cloning</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virus enumeration</td>
<td></td>
<td></td>
<td>1%</td>
</tr>
<tr>
<td>Cell matrix interactions</td>
<td></td>
<td>1.0% - 3.0%</td>
<td></td>
</tr>
<tr>
<td>Attachment dependent cell culture</td>
<td></td>
<td></td>
<td>0.6%</td>
</tr>
<tr>
<td><em>In vitro</em> cloning – mammalian</td>
<td>0.5% - 2%</td>
<td></td>
<td>1% - 4%</td>
</tr>
</tbody>
</table>

**NOTES:** Higher agarose concentration gels can affect and possibly restrict cell proliferation. Optimal gel concentrations should be determined for each culture system on a case-by-case basis. In some cases, such as hybridoma cloning using SeaPrep® Agarose, it is advisable to sample different lots of agarose for the desirable gel strength qualities.

Agarose Preparation

When preparing agarose for cell culture work, it is always best to prepare the agarose in water suitable for cell culture and separate from any growth media or nutrients. Agarose solutions and media solutions should be prepared at 2X concentrations (i.e., if desired final agarose concentration is 0.6%, prepare a 1.2% agarose solution), autoclaved separately, and aliquoted into useable aliquots.
Procedure for Autoclaving Agarose

1. Choose a flask that is 2 - 4 times the volume of the solution.
2. Add water to the flask.
3. Sprinkle in the pre-measured agarose powder at a 2X final agarose concentration.
4. Cover the flask with aluminum foil.
5. Place the flask in the autoclave.
6. Sterilize the agarose by autoclaving for 10 minutes at 15 lb/in². If using SeaPrep® Agarose, autoclave for no longer than 5 minutes.

**NOTE:** Agarose may lose gel strength when exposed to longer periods of time in the autoclave.

7. Once the agarose solution has cooled, aliquot into useable aliquots and store at 4°C prior to use.

<table>
<thead>
<tr>
<th>Materials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flask that is 2-4 times the volume of the solution</td>
</tr>
<tr>
<td>Autoclave</td>
</tr>
<tr>
<td>Aluminum foil</td>
</tr>
<tr>
<td>Sterile flasks or culture tubes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reagents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agarose powder</td>
</tr>
<tr>
<td>Water suitable for cell culture</td>
</tr>
</tbody>
</table>

General Procedure for Using Agarose in Culture Medium

1. Remelt the agarose by placing in a hot water bath or microwave.
2. Allow the agarose solution to cool to 37°C.
3. Prewarm the 2X media solution to 37°C.
4. Mix equal volumes of the sterile 2X agarose solution with sterile 2X media containing growth factors and nutrients.
5. Cast the agarose/media solution into plates or sterile culture tubes.
6. Allow the agarose solution to gel for 20 minutes if using as a feeder or overlay or maintain the solution at 37°C if using as a liquid culture.

**NOTE:** A solution containing 1% SeaPlaque® Agarose will stay liquid for approximately 18 hours. The amount of time an agarose solution will stay in a liquid state at 37°C, largely depends on the agarose concentration (increased agarose concentrations will decrease the time the solution stays in a liquid state), the age of the agarose and the particular lot of agarose you are using. When purchasing a new lot of agarose, we recommend you test this prior to culturing cells.

<table>
<thead>
<tr>
<th>Materials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microwave or hot water bath</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reagents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelled agarose solution</td>
</tr>
<tr>
<td>2X media solution</td>
</tr>
</tbody>
</table>

References

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Buffers for Protein Electrophoresis 156
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**Overview**

Polyacrylamide gel electrophoresis (PAGE) is a powerful tool for separating and identifying mixtures of proteins and peptides. Several systems exist for performing PAGE and consideration should be given to which system best suits a given sample prior to running the samples.

**Gradient vs. homogeneous (straight percentage) gels**

Gradient gels are suitable for a wide range of size resolutions, and will result in tighter band separation than single concentration gels. A homogeneous, or single concentration gel is appropriate where the proteins of interest are known to be within a narrow size range.

**Discontinuous and continuous buffer systems**

A discontinuous buffer system utilizes a large-pore gel (the stacking gel) layered on top of a small pore gel (the resolving gel). In this system, the buffer used to prepare the gels is different from the buffer used in the tank. The different buffers create two ion fronts concentrating the proteins into a very tight zone.

A continuous buffer system uses the same buffer in the gel and the tank. This system uses a single separating gel (no stacking gel) and has a single ion front. This system is less used than discontinuous systems, however, the separation of specific proteins and protein complexes that precipitate or aggregate may require the use of a continuous system.

---

**Buffers for Protein Electrophoresis**

The Laemmli Buffer System (Tris-Glycine) is a discontinuous buffer system, widely used for fine resolution of a broad molecular weight range of proteins. In this system, the gel is prepared with Tris-HCl Buffer and the Tris-glycine is used as the running buffer.

In the Tris-Tricine Buffer System, tricine replaces glycine in the running buffer. The result is more efficient stacking and destacking, and higher resolution of proteins and peptides with lower molecular weights (under 10 kDa - 15 kDa).

**Buffer Preparation**

**Tris-Glycine SDS Buffer, pH 8.3**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Amount (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>.25 M Tris base</td>
<td>30.3 g Tris Base</td>
</tr>
<tr>
<td>1.92 M Glycine</td>
<td>144.0 g Glycine</td>
</tr>
<tr>
<td>1.0% SDS*</td>
<td>10.0 g SDS</td>
</tr>
</tbody>
</table>

Adjust volume to 1 liter with distilled water

(1X = 25 mM Tris base, 192 mM Glycine, 0.1% SDS*)

*Omit SDS if running native proteins.

**Tris-Tricine SDS Buffer, pH 8.3**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Amount (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M Tris base</td>
<td>121.1 g Tris base</td>
</tr>
<tr>
<td>1 M Tricine</td>
<td>179.0 g Tricine</td>
</tr>
<tr>
<td>1.0% SDS*</td>
<td>10.0 g SDS</td>
</tr>
</tbody>
</table>

Adjust volume to 1 liter with distilled water

(1X = 100 mM Tris base, 100 mM Tricine, 0.1% SDS*)

*Omit SDS if running native proteins.
Casting Polyacrylamide Gels

Follow the steps below to cast a straight percentage polyacrylamide gel with a stacking gel. Alternatively, Lonza offers ready-to-use ProSieve® 50 Gel Solution, or PAGEr® Gold Precast Gels in a wide variety of concentrations and well formats.

Casting the resolving gel

1. Assemble plates according to the manufacturer’s instructions.
2. Place the specified quantity of the first four components from the table on page 175 into a side arm flask.
3. Mix gently by swirling.

NOTE: Omit SDS if running a native gel.

4. Degas the solution for 15 minutes. A high concentration of APS (0.3%) can be used to speed polymerization and skip the degassing step.
5. Add the specified amounts of TEMED and 10% APS.
6. Mix gently by swirling.
7. Pour the resolving gel, leaving space for the stacking gel.
8. Gently overlay the acrylamide with water-saturated isobutanol. The overlay blocks oxygen from inhibiting polymerization of the resolving gel.
9. Allow the gel to polymerize for 30 - 60 minutes. A very sharp liquid-gel interface will be visible when the gel has polymerized.
10. Pour off the overlay.
11. Rinse the top of the gel several times with water.
12. Blot any remaining water with a paper towel.

Materials
- Vertical electrophoresis plate assembly with comb
- Sidearm flask assembly or filter unit for degassing solutions
- Paper towels

Reagents
- 30% Acrylamide mix, 29% (w/v) Acrylamide, 1% (w/v) bisacrylamide, prepared in distilled water
- 1.5 M Tris Buffer pH 8.8
- 10% SDS
- 10% Ammonium Persulfate (APS)
- TEMED
- Distilled water
- Water saturated isobutanol

Caution: Wear gloves, safety glasses and lab coats when handling acrylamide solutions.
## Preparation of a 5% stacking gel

1. Place the specified quantity of the first four components from the table below into a side arm flask.

### Component volumes (ml)

<table>
<thead>
<tr>
<th>Solution components</th>
<th>1 ml</th>
<th>5 ml</th>
<th>10 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>0.68</td>
<td>3.4</td>
<td>6.8</td>
</tr>
<tr>
<td>30% Acrylamide mix</td>
<td>0.17</td>
<td>0.83</td>
<td>1.7</td>
</tr>
<tr>
<td>1.5 M Tris [pH 8.8]</td>
<td>0.13</td>
<td>0.63</td>
<td>1.25</td>
</tr>
<tr>
<td>10% SDS</td>
<td>0.01</td>
<td>0.05</td>
<td>0.1</td>
</tr>
<tr>
<td>10% Ammonium persulfate</td>
<td>0.01</td>
<td>0.05</td>
<td>0.1</td>
</tr>
<tr>
<td>TEMED</td>
<td>0.001</td>
<td>0.005</td>
<td>0.001</td>
</tr>
</tbody>
</table>

2. Mix gently by swirling.

### NOTE: Omit SDS if running a native gel.

3. Degas the solution for 15 minutes.

4. Add the specified amounts of TEMED and 10% APS.

5. Mix gently by swirling.

6. Pour the stacking gel directly onto the resolving gel.

7. Insert the comb immediately.

8. Allow the gel to polymerize for at least one hour.

9. Prepare samples while the gel is polymerizing.

10. Carefully remove the comb.

### NOTE: Gels may be stored overnight at 4°C with the comb in place and wrapped in plastic wrap.

## Casting gradient polyacrylamide gels

Hand casting gradient gels is not covered in this resource. Due to the complexity involved in hand casting gradient gels, precast gels have become a popular alternative. We recommend PAGEr® Precast Gels [see page 48], or ProSieve® Gel Solution [see page 60], which provides gradient quality separation from a linear gel format. Detailed discussions and protocols for preparing gradient gels can be found in *Electrophoresis in Practice, 2nd Edition*.
Introduction

Protein load levels will vary depending upon sample purity and staining method used. For highly purified proteins, 0.5 μg to 5 μg protein per lane on a minigel is generally sufficient. Complete mixtures such as cell lysates may require as much as 50 μg protein per lane. The table below provides lower detection limits for protein detection.

Protein stain detection limits

<table>
<thead>
<tr>
<th>Protein Stain</th>
<th>Lower Detection Limit (protein/band)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coomassie® Blue Stain</td>
<td>100 ng</td>
</tr>
<tr>
<td>Silver Stain</td>
<td>1 ng</td>
</tr>
<tr>
<td>SYPRO® Orange Protein Gel Stain</td>
<td>1 ng–2 ng</td>
</tr>
<tr>
<td>SYPRO® Red Protein Gel Stain</td>
<td>1 ng–2 ng</td>
</tr>
<tr>
<td>SYPRO® Tangerine Protein Gel Stain</td>
<td>4 ng–8 ng</td>
</tr>
</tbody>
</table>

**NOTE:** Limits are based on optimal detection methods for each stain.

The photographs below demonstrate the detection sensitivities of commonly used stains:

- Coomassie® Blue Stain (CCD camera)
- SYPRO® Red Protein Gel Stain diluted in 7.5% acetic acid (Polaroid® Photo UV light)
- SYPRO® Tangerine Protein Gel Stain diluted in PBS (Polaroid® Photo UV light)
- Silver Stain (Amersham Plus™ One Kit)
- SYPRO® Ruby Stain

Serial dilutions of ProSieve® Protein Markers were separated on 12% PAGE® Gold Precast Gels, stained and photographed as noted. The figure shows the staining sensitivity seen for the 50 kDa band of the marker. The level of protein present is indicated in nanograms. Staining was performed following the manufacturer’s instructions provided with the stains. Exposure times for photography were adjusted to obtain highest possible detection levels. Note that in both silver stain examples shown, development was allowed to proceed for an extended time period relative to the range given in the instructions to maximize detection. In the case of the Amersham kit; this results in overexposure of higher protein loading levels. Images of the silver stained gels were captured using a CCD camera system.
Section X: Protein Separation in Polyacrylamide Gels

Loading Buffers

Introduction

In general, loading buffers for protein electrophoresis contain Tris-HCl, pH 6.8; 2% SDS; a reducing agent such as dithiothreitol (DTT), β-mercaptoethanol (βME), or Tris[2-carboxyethylphosphine]hydrochloride (TCEP); glycerol (a sinking agent) and a marker dye. An alternative reducing agent to βME, Bond-Breaker™ TCEP Solution from Thermo Scientific is an odor-free, ready-to-use solution added to the sample buffer prior to denaturation.

Gel loading buffers serve four purposes in protein electrophoresis:

— Reduction of protein complexes if performing denaturing PAGE
— Dissociation of proteins to allow them to run through the gel
— Increase the density of the sample ensuring samples drop evenly into the well
— Addition of a dye to the sample to simplify loading and monitor the electrophoretic process

Sample preparation tips for sample preparation

— Keep samples on ice prior to adding the sample buffer
— Add room temperature sample buffer to the cold samples
— If preparing samples for future use, aliquot treated samples into usable aliquots to avoid freeze thawing
— Do not leave the samples in SDS sample buffer at room temperature without first heating to 95°C to inactivate proteases. A loss of high-molecular weight bands and general smearing of the bands are indications of protease activity

Procedure for sample preparation

Follow the guidelines below for preparing protein samples for electrophoresis.

1. Add 0.5 ml βME or 1 ml TCEP to 10 ml of 2X Tris-Glycine SDS sample buffer.
2. Add 1 part 2X sample buffer to 1 part sample on ice.
3. Mix well.
4. Heat sample at 95°C - 100°C in a boiling water bath for 4 minutes.
5. Place on ice until ready-to-use or store at –20°C for up to 6 months.

Loading the samples tips for loading samples

— Load the same sample volume in each well
— If the well is not needed for a sample, load with 1X sample buffer

NOTE: If a well is left empty, adjacent samples may spread

Materials

— Boiling water bath
— Ice

Reagents

— βME or TCEP
— 10 ml 2X Tris-Glycine SDS sample buffer

2X Tris-Glycine SDS sample buffer

<table>
<thead>
<tr>
<th>2X concentrate</th>
<th>126 mM Tris-HCl, pH 6.8</th>
<th>20% Glycerol</th>
<th>4% SDS</th>
<th>0.005% Bromophenol blue</th>
</tr>
</thead>
<tbody>
<tr>
<td>amount to add for 2X concentrate</td>
<td>2.5 ml of 0.5 M Tris-HCl, pH 6.8</td>
<td>2 ml Glycerol</td>
<td>4 ml of 10% SDS</td>
<td>0.5 ml of 0.1% Bromophenol blue</td>
</tr>
</tbody>
</table>

Adjust volume to 10 ml with distilled water

(1X = 63 mM Tris-HCl, 10% glycerol, 2% SDS, 0.0025% Bromophenol blue, 2.5% βME)
**Section X: Protein Separation in Polyacrylamide Gels**

**Loading Buffers — continued**

**Procedure for sample loading**

1. Slowly and gently lift the comb straight up from the gel. Allow air to enter the well area to release the vacuum which forms between the gel and the comb.
2. Rinse each well with 1X electrophoresis buffer.
3. Place the gel into the electrophoresis chamber.
4. Add 1X electrophoresis buffer to cover the wells.
5. Gently load the desired volume of sample beneath the buffer in each well.

**NOTE:** Loading the sample too fast will lead to diffusion of the sample in the well.

**Section X: Protein Separation in Polyacrylamide Gels**

**Optimal Voltage, Running Times and Power Settings**

**Optimal voltage**

Tris-Glycine polyacrylamide minigels are typically run at constant voltage between 125 - 200 volts. During electrophoresis, the current drops and heat decreases. Voltage set too high, or not limited causes excessive heating, resulting in band distortion and potential damage to the gel and apparatus. Constant voltage allows the same voltage to be used with multiple gels in an apparatus. Gel thickness is not a factor when using constant voltage. For large format gels, a constant current setting with a voltage limit set slightly higher (5 volts) than the expected voltage for the run may also be used to maintain sample velocity.

**Optimal electrophoretic time**

The gel should be run until the bromophenol blue dye has migrated to the bottom of the gel. Gel running times are dependent upon the buffer system used, the length of the gel and the polyacrylamide concentration. Typically minigels will take approximately 30 - 90 minutes to run. Whereas large format gels may take as long as 5 hours to run.

**Section X: Protein Separation in Polyacrylamide Gels**

**ProSieve® 50 Gel Solution**

ProSieve® 50 Gel Solution is a modified acrylamide formulation for electrophoresis of large proteins.

**Advantages**

- **Gradient separation** – From easy-to-cast single concentration gels
- **Easy-to-handle** – Gels are more durable than standard acrylamide
- **Sharp resolution** – Resolves large proteins (>200 kDa)
- **Fast** – Shorter destaining times and faster protein mobility times
- **Low Background** – Even when used with Silver Stain

See page 60 for ordering information.
Introduction

PAGEr® Gold Precast Gels are Tris-Glycine (Tris-HCl) gels with a 4% stacking gel. The gels contain no SDS, so can be used for native gel electrophoresis or SDS may be added to the sample and running buffer for denaturing conditions.

Advantages

- Eliminates gel preparation time
- Printed well markings for ease of sample loading
- Easy open cassette
- Compatible with most commonly used vertical mini-gel apparatuses

The photograph below demonstrates the resolution performance of PAGEr® Gold Precast Gels

Separations were run using a 10 well 4-20% Tris-Glycine PAGEr® Gold Precast Gel. The gel was run at 120 volts until the tracking dye reached the base of the gel (approximately 90 minutes). ProSieve® Protein Markers were loaded in lanes 1, 5 and 10; aqueous extracts of three different strains of E. coli cells were run in lanes 2 - 4 and 7 - 9. Lanes 2 - 4 were loaded at one half the load level of lanes 7 - 9. Lane 6 contains a mixture of purified proteins. Proteins were detected by Coomassie® Blue Stain.

Separation ranges for proteins in PAGEr® Gold Tris-Glycine Gels

<table>
<thead>
<tr>
<th>Polyacrylamide Concentration</th>
<th>Size Separation Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5%</td>
<td>50 kDa - 200 kDa</td>
</tr>
<tr>
<td>10%</td>
<td>25 kDa - 200 kDa</td>
</tr>
<tr>
<td>12%</td>
<td>20 kDa - 100 kDa</td>
</tr>
<tr>
<td>15%</td>
<td>10 kDa - 50 kDa</td>
</tr>
<tr>
<td>4 - 20%</td>
<td>5 kDa - 200 kDa</td>
</tr>
<tr>
<td>10 - 20%</td>
<td>5 kDa - 150 kDa</td>
</tr>
<tr>
<td>4 - 12%</td>
<td>25 kDa - 250 kDa</td>
</tr>
<tr>
<td>8 - 16%</td>
<td>15 kDa - 200 kDa</td>
</tr>
</tbody>
</table>

See page 48 for ordering information.
The figure below demonstrates the separation patterns of ProSieve® Protein Markers on PAGE® Gold Precast Gels at various concentrations. Smaller molecular weight bands do not separate on some lower concentration gels.

### Percentage PAGE® Precast Gel

<table>
<thead>
<tr>
<th>Gel Concentration</th>
<th>15%</th>
<th>12%</th>
<th>10%</th>
<th>7.5%</th>
<th>10-20%</th>
<th>4-20%</th>
<th>8-16%</th>
<th>4-12%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Separation Pattern</td>
<td><img src="1" alt="Image" /></td>
<td><img src="1" alt="Image" /></td>
<td><img src="1" alt="Image" /></td>
<td><img src="1" alt="Image" /></td>
<td><img src="1" alt="Image" /></td>
<td><img src="1" alt="Image" /></td>
<td><img src="1" alt="Image" /></td>
<td><img src="1" alt="Image" /></td>
</tr>
<tr>
<td>Size Range</td>
<td>10 – 50 kDa</td>
<td>20 – 200 kDa</td>
<td>25 – 200 kDa</td>
<td>50 – 200 kDa</td>
<td>5 – 150 kDa</td>
<td>5 – 200 kDa</td>
<td>15 – 200 kDa</td>
<td>25 – 250 kDa</td>
</tr>
</tbody>
</table>

ProSieve® Protein Markers contain 10 proteins with exact masses of 225, 150, 100, 75, 50, 35, 25, 15, 10 and 5 kDa. Gels were run at 175 volts until the dye front reached the bottom of the gel (approximately 60 minutes). 8 ml - 10 ml of ProSieve® Protein Marker was loaded per lane (0.8 μg - 1 μg per band). Gels were stained with Coomassie® Blue Stain.

### Chamber Compatibility

PAGE® Gels are available in 9 cm x 10 cm and 10 cm x 10 cm sizes and fit most standard mini-vertical systems.

Contact Scientific Support for information about your specific chamber.

<table>
<thead>
<tr>
<th>Standard Vertical Systems</th>
<th>PAGE® Gels</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAGE® Minigel Chamber</td>
<td>9 cm x 10 cm</td>
</tr>
<tr>
<td>10 cm x 10 cm gels</td>
<td></td>
</tr>
<tr>
<td>Bio-Rad® MiniPROTEAN® II, MiniPROTEAN® 3 or ReadyGel® Cell Systems</td>
<td>9 cm x 10 cm gels</td>
</tr>
<tr>
<td>Reverse the inner core gasket so the flat side faces outward</td>
<td></td>
</tr>
<tr>
<td>Novex® XCell SureLock® Mini-Cell</td>
<td>9 cm x 10 cm</td>
</tr>
<tr>
<td>10 cm x 10 cm gels</td>
<td></td>
</tr>
<tr>
<td>FisherBiotech® Vertical Minigel FBVE121, Owl Separations Systems Wolverine® PB2</td>
<td>10 cm x 10 cm gels</td>
</tr>
<tr>
<td>Chamber comes with 2 sets of wedges. Use the thinner wedges for the PAGE® Gold Gels.</td>
<td></td>
</tr>
<tr>
<td>FisherBiotech® Vertical Minigel FB VE101, Owl Separations Systems Penguin® Model P905</td>
<td>10 cm x 10 cm gels</td>
</tr>
<tr>
<td>Request adaptor for these chambers from Scientific Support, (Cat. No. 59902).</td>
<td></td>
</tr>
<tr>
<td>Hoefer® Mighty Small (SE250)</td>
<td>9 cm x 10 cm</td>
</tr>
<tr>
<td>10 cm x 10 cm gels</td>
<td></td>
</tr>
<tr>
<td>Gaichi 2, ISS chambers</td>
<td>10 cm x 10 cm gels</td>
</tr>
<tr>
<td>See page 164 for modification instructions.</td>
<td></td>
</tr>
</tbody>
</table>

Some chambers may require adjustment for optimal fit, see page 164.
The following guidelines will ensure optimal performance of PAGEr® Gold Precast Gels in these systems.

Bio-Rad® Mini-PROTEAN® II, Mini-PROTEAN® III or Ready Gel® Cell Systems
Remove the rubber gasket from the inner core. Replace the gasket in the reverse orientation into the unit so the flat side faces outward.

Daiichi 2
To run one gel: Place one cassette on wedge side of chamber. Use the taller half of an Owl glass cassette or an equivalent as a buffer dam on the other side. Use Daiichi wedges. The PAGEr® Gold Cassettes cannot be used as the dam in this system.
To run two gels: Widen the hole on the yellow port of the inner core. Replace the long arm wedges with modified wedges, which are thicker and shorter. This chamber modification and the new wedges are available from Lonza free of charge. Contact Lonza Scientific Support for details.

FisherBioTech® Vertical Minigel Protein System: FB-VE10-1 Mini Chamber
Lonza offers an adapter for this chamber, contact Lonza Scientific Support for details. The adapter only works if the inner gasket is white.
Replace black-plastic side spacer with Lonza adapter. Use one on each side of the inner core.

FisherBioTech® Vertical Minigel Protein System: FB-VE12-1
Chamber comes with 2 sets of wedges. Use the thinner wedges with PAGEr® Gold Precast Gels.

Hoefer® Mighty Small (SE250)
Replace the lower buffer chamber with a Deep Lower Buffer Chamber for the SE260. Available from GE Healthcare (Part No. 80-6148-78). The extra depth of the SE260 buffer chamber allows the lid to lock into place.

Novex® XCell SureLock® Mini-Cell
Lonza offers a spacer for this chamber, contact Lonza Scientific Support for details.
To run one gel: Put the gel in the front of the chamber. Put the buffer dam on the back. Place the Lonza spacer between the buffer dam and the buffer core, on the side of the chamber with the gel tension wedge. Lock the gel tension wedge.
To run two gels: Put a gel on each side of the buffer core. Place the Lonza spacer between the gel and the buffer core, on the side of the chamber with the gel tension wedge. Lock the gel tension wedge.

Owl Separation Systems Penguin™ Model P8DS-1
Lonza offers an adaptor for this chamber, contact Lonza Scientific Support for details. The Lonza adaptor for the Penguin™ model only works if the inner gasket is white. Replace black-plastic side spacer with Lonza adaptor. Use one on each side of the inner core.
Procedure for electrophoresis using PAGE® Gold Precast Gels

1. Cut open pouch and remove gel.
2. Rinse the gel with distilled or deionized water.
3. Slowly and gently lift the comb straight up.
   **NOTE:** Put the comb aside so it can be used to separate the cassette plates at the end of the run.
4. Remove white tape from bottom of the cassette.
   **NOTE:** The sharp end of the comb can be used to peel off the tape.
5. Mount the cassette[s] into the electrophoresis chamber so the printed side faces the outer (anode) buffer chamber. If running only one gel, mount the appropriate buffer dam.
6. Fill the buffer chambers with 1X running buffer.
7. Wash the wells with 1X running buffer, displacing any air bubbles in the wells.
8. Load samples.
   **NOTE:** For best results, load 1X sample buffer in the wells without samples.
9. Run the gels at a constant voltage of 125 - 200 volts until the dye front is near the bottom of the gel (approximately 30 - 90 minutes).
10. Remove the gel[s] from electrophoresis chamber.
11. Place the cassette on a flat surface with the short side of the cassette facing up.
12. Using the end of the comb and starting at the top of the cassette separate the two plates by using a twisting motion to crack the cassette.
13. Carefully remove the short plate.
14. Hold the plate with the gel over an open container.

<table>
<thead>
<tr>
<th>If the gel is adhered to</th>
<th>Then</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large Plate</td>
<td>Run end of comb or a gloved finger behind slot on cassette to push out the lip at the bottom of the gel.</td>
</tr>
<tr>
<td>Small Plate</td>
<td>Use the comb or a spatula to loosen one lower corner of the gel.</td>
</tr>
</tbody>
</table>
15. Allow gel to peel away and gently drop into the container.
16. Fix, stain and destain or blot the gel as desired.

**Materials**
- Scissors
- Pipette
- Electrophoresis apparatus
- Power Supply
- Container
- Spatula

**Reagents**
- Distilled water
- Running Buffer AccuGENE® 10X Tris-Glycine or Tris-Glycine SDS Buffer

PAGE® Gold Precast Gels can be run at higher voltages to achieve faster run times. If using Tris-glycine Running Buffer gels can be run at 200 - 250 volts for approximately 40 minutes. If using Tris-Tricine running buffer gels can be run at 150 - 200 volts for approximately 30 minutes.

The photographs below show the separation of protein samples on PAGE® Gold Precast Gels under increased voltage.

Samples were prepared using Tris-Glycine SDS Sample Buffer and β-Mercaptoethanol as a reducing agent. Lane 1: ProSieve® Protein Markers; Lanes 2 & 3: E. coli extracts; Lane 4: Bovine Serum Albumin (BSA). Gel 1: 4-20% gel in 1X Tris-Glycine SDS Buffer run at 230 volts for 40 minutes. Gel 2: 8-16% gel in 1X Tris-Tricine SDS Buffer run at 185 volts for 30 minutes. Samples were run until the tracking dye reached the base of the gels. Proteins were detected using Coomassie® Blue Stain.
Detecting proteins with SYPRO® Protein Gel Stains

SYPRO® Protein Gel Stains are highly sensitive fluorescent stains for the rapid detection of proteins in polyacrylamide gels. These stains can detect as little as 4 ng - 8 ng of protein per band in 40 - 60 minutes without destaining. Gels stained with SYPRO® Protein Gel Stains exhibit low background and minimal protein-to-protein staining variability. Gels can be documented using standard imaging systems.

Tips for staining gels with SYPRO® Protein Gel Stains

— The SDS front at the bottom of the gel stains heavily with SYPRO® Stains. Unless the protein of interest comigrates with the SDS front, we recommend running the SDS front off the end of the gel

— Colored stains such as Coomassie® Blue Stain and colored protein markers may interfere with SYPRO® staining and quench fluorescence. To stain gels previously stained with Coomassie® Blue Stain, soak the gel in several changes of 7.5% acetic acid to remove the Coomassie® Stain. Then incubate the gel in 0.05% SDS for 30 minutes and stain with SYPRO® Stain as usual

— Glove powder can leave background markings on gels. Rinse or wash gloves prior to handling gels

— Clean the surface of the transilluminator after each use with deionized water and a soft cloth. Fluorescent stains (such as SYPRO® Stains or ethidium bromide) can accumulate on the transilluminator and may cause high background

— Handle gels carefully to avoid non-specific staining of areas of the gel that have been squeezed

— SYPRO® Stains may be photobleached after several minutes of exposure to UV light. If a gel becomes photobleached, restain by incubating in the staining solution

— SYPRO® stained gels can be restained with Coomassie® Blue or Silver Stain procedures

— Plastic wraps and GelBond® Film will autofluoresce when exposed to UV light resulting in very high background. Gels backed with GelBond® Film can be photographed by inverting the gel on the transilluminator

Select the Best Stain for your Application

<table>
<thead>
<tr>
<th>Application</th>
<th>SYPRO® Ruby</th>
<th>SYPRO® Tangerine</th>
<th>SYPRO® Red</th>
</tr>
</thead>
<tbody>
<tr>
<td>High performance staining</td>
<td>![ ]</td>
<td>![ ]</td>
<td>![ ]</td>
</tr>
<tr>
<td>Staining prior to Western blotting</td>
<td>![ ]</td>
<td>![ ]</td>
<td>![ ]</td>
</tr>
<tr>
<td>2D electrophoresis</td>
<td>![ ]</td>
<td>![ ]</td>
<td>![ ]</td>
</tr>
<tr>
<td>Edman microsequencing</td>
<td>![ ]</td>
<td>![ ]</td>
<td>![ ]</td>
</tr>
<tr>
<td>Mass spectrometry</td>
<td>![ ]</td>
<td>![ ]</td>
<td>![ ]</td>
</tr>
<tr>
<td>Quantitation</td>
<td>![ ]</td>
<td>![ ]</td>
<td>![ ]</td>
</tr>
<tr>
<td>Zymography</td>
<td>![ ]</td>
<td>![ ]</td>
<td>![ ]</td>
</tr>
<tr>
<td>Electroelution</td>
<td>![ ]</td>
<td>![ ]</td>
<td>![ ]</td>
</tr>
<tr>
<td>Membrane staining</td>
<td>![ ]</td>
<td>![ ]</td>
<td>![ ]</td>
</tr>
<tr>
<td>Protein expression</td>
<td>![ ]</td>
<td>![ ]</td>
<td>![ ]</td>
</tr>
<tr>
<td>Detection prior to Immunostaining</td>
<td>![ ]</td>
<td>![ ]</td>
<td>![ ]</td>
</tr>
<tr>
<td>Difficult to stain proteins</td>
<td>![ ]</td>
<td>![ ]</td>
<td>![ ]</td>
</tr>
<tr>
<td>IEF Gels</td>
<td>![ ]</td>
<td>![ ]</td>
<td>![ ]</td>
</tr>
</tbody>
</table>

Materials

— Clear polypropylene container (e.g., Rubbermaid® Recycling #5 Plastics)

Reagents

— 7.5% (v/v) acetic acid
— SYPRO® Protein Gel Stain stock solution

Caution: Materials and methods shown here present hazards to the user and the environment. Refer to the safety information on page 210 before beginning these procedures.

Caution: Acetic acid causes burns and respiratory irritation. Take precautions to prevent exposure.
**General Procedure for staining proteins with SYPRO® Protein Gel Stains**

1. Run SDS-polyacrylamide gels according to standard protocols.

**NOTE:** To reduce background staining with SYPRO® Red and Tangerine Stains, use 0.05% SDS in the running buffer. Gels run in 0.05% SDS show no change in the migration pattern of proteins.

2. Dilute the 5,000X concentrate to a 1X solution, in 7.5% (v/v) acetic acid, in a clear plastic polypropylene container. For example, for every 10 ml acetic acid add 2 μl stock SYPRO Stain solution.

3. Mix well.

4. Place the gel into the staining container and cover with a lid to protect from light.

5. Gently agitate the gel at room temperature.

6. Stain the gel for 40 - 60 minutes.

**NOTE:** The optimal staining time depends on the thickness of the gel and the gel concentration. Longer staining times may be required as gel thickness increases.

7. Briefly rinse the gel in 7.5% acetic acid.

8. Remove the gel from the staining container and photograph the gel following the procedure on page 168.

**Procedure for staining non-denaturing gels with SYPRO® Red Protein Gel Stain**

Options for staining proteins with SYPRO® Red Stain after native gel electrophoresis:

1. Dissolve the stain 1:5,000 in distilled water and follow the general protocol for staining proteins with SYPRO® Protein Gel Stains. **This will be highly protein-selective, and will be less sensitive than staining proteins in SDS gels. To increase the signal, a long film exposure can be used since the background fluorescence is essentially zero.**

2. Soak the gel after electrophoresis in 0.05% SDS for 30 minutes, then stain with a 1:5,000 solution of SYPRO® Stain diluted in 7.5% acetic acid. **Proteins will be denatured and fixed after electrophoresis using this treatment.**

**Procedure for staining proteins with SYPRO® Tangerine Protein Gel Stain: Non-fixing protocol**

SYPRO® Tangerine Protein Gel Stain is an extremely versatile fluorescent stain that does not alter protein structure, interfere with the transfer of proteins to blotting membranes, or use organic solvents. The stain can be diluted into a wide range of buffers with a pH range from 4 - 10 and if fixing is necessary, it can be diluted into 7.5% acetic acid following the General Protocol for Staining Proteins with SYPRO® Gel Stains.

If proteins are to be used for subsequent analysis, dilute SYPRO® Tangerine stock solution into 50 mM phosphate, 150 mM NaCl, pH 7.0 or use one of the buffers listed below prepared as 50 mM - 100 mM solutions containing 150 mM NaCl.

**Compatible Buffers**

<table>
<thead>
<tr>
<th>Buffer</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formate</td>
<td>4.0</td>
</tr>
<tr>
<td>Citrate</td>
<td>4.5</td>
</tr>
<tr>
<td>Acetate</td>
<td>5.0</td>
</tr>
<tr>
<td>MES</td>
<td>6.0</td>
</tr>
<tr>
<td>Imidazole</td>
<td>7.0</td>
</tr>
<tr>
<td>HEPES</td>
<td>7.5</td>
</tr>
<tr>
<td>Tris acetate</td>
<td>8.0</td>
</tr>
<tr>
<td>Tris-HCl</td>
<td>8.5</td>
</tr>
<tr>
<td>Tris borate</td>
<td></td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>10.0</td>
</tr>
</tbody>
</table>

**Materials**

- Clear polypropylene container (e.g., Rubbermaid® Recycling #5 Plastics)

**Reagents**

- 50 mM Phosphate, 150 mM NaCl, pH 7.0 or suitable buffer

**Caution:** Materials and methods shown here present hazards to the user and the environment. Refer to the safety information on page 210 before beginning these procedures.
Follow this procedure if the gel will be used for subsequent analysis such as zymography or Western blotting. Stained proteins can also be eluted from gels and used for further analysis such as mass spectrometry.

1. Run SDS-polyacrylamide gels according to standard protocols. It is not necessary to decrease the amount of SDS present in the running buffer when using SYPRO® Tangerine Gel Stain.

2. Dilute the 5,000X concentrate to a 1X solution, in 7.5% (v/v) acetic acid solution, in a clear plastic polypropylene container. For example, for every 10 ml acetic acid add 2 μl stock SYPRO® Stain solution.

3. Mix well.

4. Place the gel into the staining container and cover to protect from light.

5. Gently agitate the gel at room temperature.

6. Stain the gel for 40 - 60 minutes. The optimal staining time depends on the thickness of the gel and the gel concentration. Longer staining times may be required as gel thickness increases.

7. Remove the gel from the staining container and immediately photograph the gel following the procedure on page 168.

**NOTE:** If the gel will be blotted after staining with SYPRO® Tangerine Stain, stain the gel according to the procedure above, using 50 mM phosphate, 150 mM NaCl as the stain diluant. Add 0.1% SDS to the transfer buffer. This will help in the transfer of some proteins to the membrane.

**Viewing gels stained with SYPRO® Protein Gel Stains**

All SYPRO® Protein Gel Stains have two excitation wavelengths; in the UV region at approximately 300 nm and in the blue range of visible spectrum, between 470 and 610 nm. The stains can be visualized using a UV transilluminator or the Dark Reader® Transilluminator.

**Photographing gels stained with SYPRO® Protein Gel Stains**

— Protein bands stained with SYPRO® Protein Gel Stains are best seen by photographing the gel. The integrating effect of a camera or imaging system can detect bands that are not visible to the eye.

— Gels stained with SYPRO® Protein Gel Stains can be photographed using Polaroid® Cameras, CCD camera systems or laser scanners.

— The highest sensitivity using a Polaroid® Camera can be obtained using Polaroid® 667 black-and-white print film and the SYPRO® Protein Gel Stain Photographic Filter. Exposure time will vary with the intensity of the illumination source. Begin with an f-stop of 4.5 and an exposure of 2 - 8 seconds. Use of an ethidium bromide filter is not recommended as it blocks much of the light and leads to lower detection sensitivity.

— The SYPRO® Photographic Filter does not work with CCD Camera systems. For CCD cameras, use the emission and excitation data below and check with the camera manufacturer for the appropriate filter.

<table>
<thead>
<tr>
<th>Stain</th>
<th>Emission (nm)</th>
<th>Excitation (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SYPRO® Red</td>
<td>630</td>
<td>300 and 550</td>
</tr>
<tr>
<td>SYPRO® Orange</td>
<td>570</td>
<td>300 and 470</td>
</tr>
<tr>
<td>SYPRO® Tangerine</td>
<td>640</td>
<td>300 and 490</td>
</tr>
</tbody>
</table>

**Detecting proteins with Coomassie® Blue Stain**

Coomassie® Blue Stain binds nonspecifically to most proteins. Proteins are fixed and stained in a Coomassie® Blue staining solution and subsequently destained to eliminate the blue background from the gel. Gels can be dried, photographed or stored wet.

**Coomassie® Blue Stain solution**

<table>
<thead>
<tr>
<th>Solution</th>
<th>Amount for 1X working Solution*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1X Working Solution</td>
<td></td>
</tr>
<tr>
<td>40% Ethanol</td>
<td>400 ml Ethanol</td>
</tr>
<tr>
<td>0.125% Coomassie® Blue</td>
<td>1.25 g Coomassie® Blue R-250</td>
</tr>
<tr>
<td>Distilled water</td>
<td>500 ml Distilled water</td>
</tr>
<tr>
<td>10% Acetic acid</td>
<td>100 ml Acetic acid</td>
</tr>
</tbody>
</table>

*Add reagents in the order provided

**Coomassie® Blue destain solution**

<table>
<thead>
<tr>
<th>Solution</th>
<th>Amount for 1X working Solution*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1X Working Solution</td>
<td></td>
</tr>
<tr>
<td>5% Ethanol</td>
<td>50 ml Ethanol</td>
</tr>
<tr>
<td>7.5% Acetic acid</td>
<td>75 ml Acetic acid</td>
</tr>
<tr>
<td>Adjust volume to 1 liter</td>
<td></td>
</tr>
</tbody>
</table>

**Caution:** These solutions should be prepared under the fume hood.
Detecting proteins with silver stain

Silver staining is based on differential reduction of silver ions bound to sulphydryl and carboxyl side chains on proteins. After electrophoresis, proteins are fixed, exposed to silver nitrite and developed to form a black precipitate of silver. All silver staining procedures are time consuming. Many good kits are commercially available which make the procedure faster and easier. The BioRad® Silver Stain Plus™ Kit is recommended for its versatility and ease of use.

The procedure described below is a modification of Morrisey. This procedure uses dithiothreitol (DTT) to improve reproducibility. An advantage of this version is that development occurs more slowly than many silver staining protocols, giving more control over the final image.

Procedure

1. Prepare enough Coomassie® Blue Staining Solution to cover the surface of the polyacrylamide gel.
2. Prewarm the Coomassie® Blue Stain Solution to 55°C.
3. Remove the gel from the glass plates.
4. Submerge the gel in the Coomassie® Blue Stain Solution.
5. Gently agitate the gel for 20 minutes at 55°C until the gel becomes blue. Alternatively, solutions may be heated to 45°C and incubated at room temperature.
6. Decant stain solution from container.

NOTE: Coomassie® Blue Staining Solutions can be saved and stored to stain multiple gels. As the stain solution reaches its “use limit”, gels will appear grainy and will not destain completely and new staining solutions should be prepared.

7. Briefly rinse excess stain from gel in water
8. Transfer the gel into the destain solution.
9. Gently agitate the gel at 55°C until the gel has destained and bands are visible. (Approximately 1 hour).

NOTE: The destain solution may need to be changed occasionally until the background is clear. Do not over destain, which can lead to loss of band intensity. Pieces of paper towel or a Kimwipe® can be placed in the corner of the container to speed up this process. Change the tissues when they are saturated with Coomassie® Blue Stain.

Materials

– Kimwipes or paper towels
– 3 plastic containers with covers
– Shaking incubator set to 55°C

Reagents

– Stain solution
  – 0.125% Coomassie® Blue, 40% Ethanol, 10% Acetic acid
– Destain solution
  – 5% Ethanol, 7.5% Acetic acid

Detecting proteins with silver stain

Silver staining is based on differential reduction of silver ions bound to sulphydryl and carboxyl side chains on proteins. After electrophoresis, proteins are fixed, exposed to silver nitrite and developed to form a black precipitate of silver. All silver staining procedures are time consuming. Many good kits are commercially available which make the procedure faster and easier. The BioRad® Silver Stain Plus™ Kit is recommended for its versatility and ease of use.

The procedure described below is a modification of Morrisey. This procedure uses dithiothreitol (DTT) to improve reproducibility. An advantage of this version is that development occurs more slowly than many silver staining protocols, giving more control over the final image.

Procedure

Wear gloves and use only glass-distilled water and glass staining containers. All steps can be done at room temperature.

1. Gently agitate the gel in 100 ml Destain I for 30 minutes to overnight.
2. Remove Destain solution I.
3. Gently agitate the gel in 100 ml Destain II for 30 minutes.
4. Remove Destain II.
5. Gently agitate the gel in 100 ml cross-linking solution for 30 minutes.
6. Remove cross-linking solution.
7. Wash gel with several changes of water over a 2 hour period.

NOTE: For small peptides, incubate with glutaraldehyde overnight to insure retention of the peptides in the gel.
8. Gently agitate the gel in DTT solution for 30 minutes.
9. Remove DTT solution and drain well, but do not rinse the gel.
10. Gently agitate the gel in 100 ml silver nitrate solution for 30 minutes.

Continued on next page.
Section X: Protein Separation in Polyacrylamide Gels

Detection of Proteins in Polyacrylamide Gels — continued

11. Place the staining tray under running deionized water and swirl for a few seconds.
12. Remove the water.
13. Add 50 ml of Developing solution, swirl briefly, then discard the solution.

**NOTE:** This reacts with excess silver and prevents non-specific staining of the gel.
15. Add 100 ml of Developing solution and shake slowly.

**NOTE:** Staining occurs slowly at first but then progresses rapidly. Development takes approximately 5 to 10 minutes.
16. When the bands are slightly lighter than the desired staining level, remove developing solution, rinse quickly with water, add Destain II to cover the gel, as the stop solution. Alternatively, add 5 ml of Stop Solution to the developer to stop development. In either case, development will not stop immediately but continues for approximately 5 minutes after adding Stop Solution.
17. Wash the gel several times in Destain II.
18. Rinse the gel with water.
19. Store in water or dry the gel.

### Reagents

- Glass distilled water
- Destain I \( (40\% \text{ Methanol} \ 7\% \text{ Acetic acid}) \)
  - 400 ml Methanol
  - 70 ml Acetic acid
  - Adjust volume to 1 liter with distilled water
  - Store at room temperature indefinitely
- Destain II \( (5\% \text{ Methanol}, 7\% \text{ Acetic acid}) \)
  - 700 ml Acetic acid
  - 500 ml Methanol
  - Adjust volume to 10 liters with distilled water
  - Store at room temperature indefinitely
- Cross-linking solution \( (10\% \text{ glutaraldehyde}) \)
  - 20 ml of 50% glutaraldehyde stock in 100 ml of distilled water
- Dithiothreitol (DTT) Solution \( (5 \text{ mg/ml}) \)
  - 5.0 mg DTT in 1 liter of distilled water
- Silver Nitrate Solution \( (0.1\% \text{ w/v Silver nitrate}) \)
  - 1.0 g Silver nitrate (AgNO\(_3\)) in 1 liter of distilled water
- 3% Sodium Carbonate \( (3\% \text{ w/v}) \)
  - 60.0 g Sodium carbonate in 2 liters of distilled water
  - Store in glass container
- Developing Solution \( (3\% \text{ Sodium carbonate}, 0.019\% \text{ Formaldehyde}) \)
  - 200 ml of 3% Sodium carbonate
  - 100 ml of 37% Formaldehyde
  - Prepare just prior to use
- Stop Solution \( (2.3 \text{ M Sodium citrate}) \)
  - 67.64 g Sodium citrate, dihydrate \( \text{FW 294.1} \)
  - Adjust volume to 100 ml in distilled water

### Caution: Glutaraldehyde is toxic and must be handled in a fume hood

- 1.0 g Silver nitrate (AgNO\(_3\)) in 1 liter of distilled water
- 3% Sodium Carbonate \( (3\% \text{ w/v}) \)
- 60.0 g Sodium carbonate in 2 liters of distilled water
- Store in glass container
- Developing Solution \( (3\% \text{ Sodium carbonate}, 0.019\% \text{ Formaldehyde}) \)
- 200 ml of 3% Sodium carbonate
- 100 ml of 37% Formaldehyde
- Prepare just prior to use
- Stop Solution \( (2.3 \text{ M Sodium citrate}) \)
- 67.64 g Sodium citrate, dihydrate \( \text{FW 294.1} \)
- Adjust volume to 100 ml in distilled water

### Caution: Materials and methods shown here present hazards to the user and the environment. Refer to the safety information on page 210 before beginning these procedures.

### Materials

- Glass containers
- Orbital shaker

### References

Section XI: Blotting Proteins from Polyacrylamide Gels

Introduction

Protein transfer efficiency in blotting applications is dependent upon multiple factors, including gel percentage, gel thickness, protein size, transfer conditions (e.g., buffer and voltage), and type and quality of membrane.

Choosing the appropriate membrane

<table>
<thead>
<tr>
<th>Nitrocellulose</th>
<th>PVDF</th>
<th>Nylon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrophobic binding</td>
<td>Hydrophobic binding</td>
<td>Hydrophobic &amp; electrostatic binding</td>
</tr>
<tr>
<td>General purpose membrane</td>
<td>SDS tolerant</td>
<td>Stable if baked</td>
</tr>
<tr>
<td>Low background</td>
<td>High background</td>
<td>High background</td>
</tr>
<tr>
<td>Low strength</td>
<td>High strength</td>
<td>High strength</td>
</tr>
<tr>
<td>Becomes brittle if baked</td>
<td>Suitable for protein sequencing</td>
<td>Least suitable for Western transfer</td>
</tr>
</tbody>
</table>

Transfer solutions

Formula for Towbin transfer solution:

<table>
<thead>
<tr>
<th>Towbin Transfer Solution</th>
<th>Amount for 1X Working Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1X Working Solution</td>
<td>1X Working Solution</td>
</tr>
<tr>
<td>25 mM Tris base</td>
<td>30.3 g Tris base</td>
</tr>
<tr>
<td>192 mM Glycine</td>
<td>144.1 g Glycine</td>
</tr>
<tr>
<td>0.1% SDS</td>
<td>10.0 g</td>
</tr>
<tr>
<td>20% Methanol</td>
<td>2 L Methanol</td>
</tr>
<tr>
<td>Adjust volume to 8 liters with distilled water</td>
<td>Adjust volume to 10 liters with distilled water</td>
</tr>
<tr>
<td>Measure, but do not adjust pH; it should be approximately 8.2 to 8.4</td>
<td></td>
</tr>
</tbody>
</table>

It may be necessary to lower the concentrations of methanol, SDS or both to obtain the optimal balance of transfer and binding efficiency. The table below outlines the effects that SDS and methanol have on protein transfer.

<table>
<thead>
<tr>
<th>SDS</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improves transfer of proteins &gt;60 kDa</td>
<td>Improves binding efficiency</td>
</tr>
<tr>
<td>Decreases binding efficiency</td>
<td>Decreases transfer efficiency</td>
</tr>
<tr>
<td>Not compatible with nylon membranes</td>
<td>Do not soak gel in transfer buffer prior to blotting</td>
</tr>
<tr>
<td>Include 0.1% - 0.2% in transfer buffer</td>
<td>Include 20% in transfer buffer</td>
</tr>
</tbody>
</table>
Section XI: Blotting Proteins from Polyacrylamide Gels

Tips for increasing transfer efficiency

— Use the lowest concentration gel that will resolve the protein(s) of interest.
— Avoid using gels 0.1 mm thick. Thick gels may require longer blotting times.
— Decrease the concentration of methanol to optimize transfer efficiency of proteins >150 kDa.
— Small proteins tend to transfer more easily than large proteins. Longer transfer times may be used to ensure complete transfer of large proteins (>60 kDa), proteins from native gels, and thicker gels.
— Use two membranes if transferring for an extended period of time (>1 hour). The second membrane will bind any protein that may transfer through the first. This can be verified by membrane staining.
— Use ProSieve® Color Protein Markers (see page 51) to confirm that transfer has occurred to the membrane and not the filter paper.
— Use a chopping motion when removing the well and foot area. Slicing the gel may cause tearing.
— Gently roll out air bubbles between transfer stack layers using a wet glass rod or pipette.
— If the gel sticks to the filter paper or membrane after transfer, soak for 5 - 15 minutes in water then gently peel the filter paper away.
— If proteins are left in the gel after following recommended transfer conditions, increasing the voltage by no more than 5 volts may be helpful.

Section XI: Blotting Proteins from Polyacrylamide Gels

Semi-dry Blotting PAGEr® Gold Precast Gels

This protocol is for use with the BioRad® Trans-Blot® Semi-Dry Cell. If using another manufacturer’s blotting apparatus, follow manufacturer’s instructions for use.

Tips for Semi-Dry Blotting

— To prevent the stack from drying out, add extra transfer solution (2 ml - 5 ml) to the top layer of filter paper before closing the lid on the blotting apparatus.
— Center the gel on the membrane. Occasionally, the gel will overlap the membrane and stick to the filter paper below. If this occurs, gently break the seal with a scalpel.

Procedure for semi-dry blotting

NOTE: Nitrocellulose or PVDF membranes are recommended for this method and should be wetted in methanol prior to wetting in transfer solution.

1. Electrophorese gel following standard procedure.
2. Carefully trim off stacking gel and bottom foot from the gel.
3. Soak gel for 20 minutes in chilled 1X transfer solution.
4. Cut the filter paper, blot paper and membrane to the size of the gel.
5. Soak membrane, blotting paper and filter paper for 5 - 10 minutes in 1X transfer solution.
6. To make a semi-dry blotting sandwich, stack in the following order:
   6a. Mask
   6b. Prewet extra thick blot paper
   6c. Prewet nitrocellulose membrane
   6d. Polyacrylamide gel.
   6e. Prewet Whatman® Grade 114 or 54 Filter Paper
   6f. Prewet extra thick blot paper
   6g. Top platen (stainless steel cathode), and safety cover
    
7. Turn on the power and transfer at 25 volts (constant) at 400 mA for:
   60 minutes for 10 kDa - 100 kDa proteins
   90 minutes for 100 kDa - 300 kDa proteins

NOTE: Optimized conditions will be required for different proteins or different membranes.

Continued on next page.
Section XI: Blotting Proteins from Polyacrylamide Gels

Semi-dry Blotting PAGEr® Gold Precast Gels — continued

---

**Materials and Reagents**

- **Mask**
- A piece of GelBond® Film (see page 61), or similar polyester film, the size of the bottom anode, with a rectangular hole the size of the gel cut out of the center. The purpose of the mask is to focus current through the gel stack.
- Whatman® Grade 114 or 54 Filter Paper
- Extra thick blot paper
- Transfer membrane
- Scalpel or razor blade
- Scissors
- Glass rod or pipette
- 1XTowbin transfer solution at 4°C
- Shallow tray for soaking membranes and filter paper

---

For hydrophobic proteins or proteins >100 kDa, tank blotting is preferable to semi-dry blotting because prolonged transfers are possible without gel drying. For more detailed information and protocols concerning tank blotting, consult the blotting apparatus manufacturer’s instructions.

**Procedure for tank blotting**

**NOTE:** Transfer is performed at 4°C.

**NOTE:** Nitrocellulose or PVDF membranes are recommended for this method and should be wetted in methanol prior to wetting in transfer solution.

1. Electrophorese gel following standard procedures.
2. Carefully trim off stacking gel and bottom foot from the gel.
3. Soak gel for 20 minutes in chilled 1X Transfer solution.
4. Cut the filter paper, blotting paper and membrane to the size of the gel.

**NOTE:** Transfer membranes should be handled at the edges with gloves worn.

5. Soak fiber pads, nitrocellulose membrane and blotting paper for 2 minutes in 1X Transfer solution.
6. To make a tank blotting sandwich, stack in the following order:

   6a. Cathode unit
   6b. 1 prewet fiber pad
   6c. 1 sheet prewet extra thick blot paper
   6d. 1 sheet prewet Whatman® 114 or 54 Blotting Paper
   6e. Polyacrylamide gel
   6f. Prewet nitrocellulose membrane
   6g. 1 sheet prewet extra thick blot paper
   6h. 1 prewet fiber pad
   6i. Anode unit

7. Place in tank with nitrocellulose membrane closest to anode (+).
8. Cover with chilled 1X Transfer solution.
9. Turn on the power and transfer by running at 100 volts (constant) at 400 mA for:
   - 90 minutes for 10 kDa–100 kDa proteins
   - 120 minutes for 100 kDa–300 kDa proteins

**NOTE:** Optimized conditions will be required for different proteins or different membranes.

---

Continued on next page
Monitoring protein transfer

Protein transfer can be monitored by staining the gel following transfer (see page 166), and/or staining the membrane. Membrane staining should only be performed when duplicate samples have been run on a gel and the membrane can be cut in half, or when a second membrane has been used. Stains commonly used for this purpose include SYPRO® Ruby Protein Blot Stain, (see page 55), India ink stain or colloidal gold stain. Described below is the use of the GE Healthcare AuroDye® Forte Kit which is a colloidal gold stain.

Staining membranes with the GE Healthcare AuroDye® Forte Kit

This method is compatible with PVDF and nitrocellulose membranes. For more detailed information and protocols, consult the instructions provided with the kit.

1. Place the membrane in a clean glass dish.
2. Add 1X PBS, 0.3% Tween 20.
3. Shake slowly on a shaker for 30 minutes at 37°C.
4. Remove solution and replace with fresh 1X PBS, 0.3% Tween® 20.
5. Shake slowly on a shaker for 5 minutes at room temperature.
6. Repeat steps 4 – 5 two more times.
7. Briefly rinse the membrane in distilled water.
8. Place the membrane in a thermal seal pouch with 15 ml - 20 ml AuroDye® Forte Kit for 2 - 4 hours at room temperature.
9. After fully developed, rinse briefly with distilled water and air dry.

Materials and Reagents

- Previously blotted membrane
- Orbital shaker set to 37°C
- Orbital or rocking platform shaker
- Glass dish
- Thermal seal pouch and sealing unit
- Distilled water
- 1X PBS, 0.3% Tween® 20

Materials

- Whatman® Grade 114 or 54 Filter Paper
- Extra thick blot paper
- Transfer membrane
- Fiber or foam pad
- Scissors
- Glass rod or pipette
- Scalpel or razor blade
- 1X Towbin transfer solution at 4°C
- Shallow tray for soaking membranes and filter paper

References

Section XII: Isoelectric Focusing of Proteins on Agarose Gels

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<td>References</td>
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</table>
Section XII: Isoelectric Focusing of Proteins on Agarose Gels

Introduction

Separation of proteins in complex mixtures for analytical resolution can be achieved by isoelectric focusing (IEF), in which proteins are separated based on their net charge (isoelectric point, or pI) in the presence of a pH gradient. Analytical focusing is carried out either in polyacrylamide gels—most recently prepared with immobilized pH gradients—or in agarose gels prepared with mobile carrier ampholytes, which form a pH gradient when subjected to electrophoresis. Separation in agarose gels is more rapid, since the pores of the agarose gel are larger than those of polyacrylamide gels.

■ Advantages
  — No toxic monomer solutions are required
  — Separation of proteins larger than 2,000 kDa
  — Shorter staining times
  — Nontacky and compressible (blottable)
  — No catalysts to interfere with separation

■ Applications
  — Immunofixation directly in the gel
  — Crossed immunoelectrofocusing
  — Direct tissue isoelectric focusing
  — Preparative isoelectric focusing

Compatible agaroses

Isoelectric focusing on agarose gels requires the use of an agarose that has no measurable electroendosmosis (EEO). Lonza has developed two products that can be used for this application, specifically IsoGel® Agarose and IsoGel® Agarose IEF Plates.

— IsoGel® Agarose is a highly purified agarose that is easy to prepare and produces a less restrictive gel than polyacrylamide, allowing rapid focusing of high molecular weight proteins (>2,000 kDa).
— IsoGel® Agarose IEF Plates are ready-to-use 125 mm x 100 mm precast gels that eliminate gel preparation time and provide easy handling throughout IEF processing.

The photograph below demonstrates the separation performance of proteins focused on an IsoGel® Agarose IEF Plate.

Alternatively, Lonza offers IsoGel® Agarose IEF Precast Plates.

Separation of proteins in a IsoGel® Agarose IEF Plate, pH 3.1-10. Lanes 1 & 4: in-house pl Marker. Lanes 2 & 3: Broad Range pl 4.45-9.6 marker (BioRad). Lane 5: Hemoglobin, Hb Type AFSC (PE Wallace). 2.5 μl of each sample were loaded on the gel and prefocused at 1 watt for 10 minutes and focused at 2000 volts (max), 25 mA (max), 25 W (max) for 60 minutes on a GE Healthcare Multiphor II chamber at 30°C. The gel was stained with Crowle’s Stain.
Section XII: Isoelectric Focusing of Proteins on Agarose Gels

Preparation of Agarose Isoelectric Focusing Gels

Suggested anolytes and catholytes

When selecting anolytes and catholytes for any pH gradient, it is important to closely bracket the ends of the pH range of the ampholytes. Avoid creating pH discontinuities between the ends of the ampholyte range and the bracketing electrolytes.

**NOTE:** pH is dependent on temperature. 25°C pH values are provided for selection of electrolytes. Under running conditions, the pH will be slightly higher.

### Anolytes

<table>
<thead>
<tr>
<th>Anolyte</th>
<th>Concentration</th>
<th>pH (25°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphoric acid</td>
<td>1 M</td>
<td>1.0</td>
</tr>
<tr>
<td>Sulfuric acid</td>
<td>0.2 M</td>
<td>1.6</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>0.5 M</td>
<td>2.6</td>
</tr>
<tr>
<td>L-glutamic acid</td>
<td>40 mM</td>
<td>3.2</td>
</tr>
<tr>
<td>Indole acetic acid</td>
<td>3 mM</td>
<td>3.8</td>
</tr>
<tr>
<td>L-tyrosine</td>
<td>4 mM</td>
<td>4.5</td>
</tr>
</tbody>
</table>

### Catholytes

<table>
<thead>
<tr>
<th>Catholyte</th>
<th>Concentration</th>
<th>pH (25°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threonine</td>
<td>50 mM</td>
<td>5.8</td>
</tr>
<tr>
<td>Glycine</td>
<td>50 mM</td>
<td>6.15</td>
</tr>
<tr>
<td>Hepes</td>
<td>0.4 M</td>
<td>7.3</td>
</tr>
<tr>
<td>L-Histidine (free-base)*</td>
<td>40 mM</td>
<td>7.35</td>
</tr>
<tr>
<td>Bicine</td>
<td>0.1 M</td>
<td>8.0</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>1 M</td>
<td>13.0</td>
</tr>
</tbody>
</table>

*Do not substitute Histidine HCl for free-base.

Preparation of the gel casting assembly

1. Spread a few drops of distilled water or 0.1% nonionic detergent on one glass plate.
2. Lay a sheet of GelBond® Film, cut slightly smaller than the glass plate, onto the plate with the hydrophilic side up.

**NOTE:** Water droplets spread on the hydrophilic side but bead up on the hydrophobic side of the film.

3. Cover the GelBond® Film with a sheet of blotting paper or the interleaving paper supplied with the film.
4. Firmly roll with a rubber roller or wipe with tissues to squeeze out any air bubbles and excess fluid from behind the GelBond® Film.
5. Carefully wipe off any excess liquid at the edges.
6. Place the U-frame spacer on top of the GelBond® Film. If a U-frame spacer is unavailable, place two spacers on the GelBond® Film along either side and one spacer across the bottom edge.
7. Place the second glass plate over the spacer(s).
8. Clamp the assembly with the stationery clamps, using 2 clamps per side and bottom.
9. Warm the cassette in a 55°C forced hot air oven for 15 minutes.

**NOTE:** GelBond® Film may warp if the cassette is heated too long or above 75°C.

### Materials

- GelBond® Film (110 mm x 125 mm, Cat. No. 53745)
- Blotting paper or interleaving paper supplied with Gelbond® Film
- Two thick glass plates (110 mm x 125 mm)
- A plastic 0.8 mm-thick U-frame spacer or 3 single spacers, 0.8 mm-thick
- Six stationery binder clamps
- Blotting paper, rubber roller or tissues
- Forced hot air oven set to 55°C

### Reagents

- Distilled water or 0.1% nonionic detergent
The following procedure is to prepare a 10 ml IsoGel® Agarose gel.

**Solution preparation**

1. Choose a 50 ml beaker or Erlenmeyer flask.
2. Add 8 ml distilled water and a stir bar to the flask or beaker.
3. Premeasure 0.1 g IsoGel® Agarose.
4. Premeasure 1.0 g d-sorbitol.
5. Sprinkle in the premeasured agarose powder, while the solution is rapidly stirring.
6. Using a spatula, break up and disperse any agarose clumps and scrape down any powder adhered to the walls of the flask.
7. Add the d-sorbitol while the solution is rapidly stirring.
8. Remove the stir bar.
9. Follow the procedures on page 83 for dissolving agarose.
10. Cool the solution to approximately 60°C.
11. Add 0.63 ml of ampholyte solution with a 1-cc syringe.
12. Stir the solution well to mix.
13. Maintain the agarose solution at 60°C - 65°C until casting.
14. Correct for evaporation by adding warm distilled water immediately before gel casting.

**Materials**
- Erlenmeyer flask or beaker (50 ml)
- Microwave
- Boiling water bath or hot plate
- Magnetic stir plate
- Magnetic stir bar
- 1-cc tuberculin syringe
- Water bath set to 60°C
- 20-cc syringe
- Prewarmed cassette assembly
- Parafilm® or tape
- Spatula

**Reagents**
- IsoGel® Agarose (see page 56)
- Distilled water
- Ampholytes
- D-sorbitol
- Boiling distilled water
- 60°C distilled water
1. Flush a 20 cc syringe with boiling water to thoroughly heat it.
2. Expel all water from the barrel and needle.
3. Immediately fill the syringe with the warmed agarose solution.
4. Slowly inject the agarose solution into the pre-warmed cassette.

**NOTE:** Try to avoid introducing air bubbles into the cassette by injecting the solution in a slow-steady stream.

5. Fill the cassette assembly to the top with agarose solution.
6. Seal the top of the cassette with Parafilm® or tape to prevent evaporation.
7. Allow the casting assembly to cool at room temperature.
8. Place the gel at 4°C for one hour.

**Disassembly of casting cassette**

1. Remove the tape and clamps.
2. With the cassette lying flat, insert a flat spatula between the glass plates.
3. Twist gently to break the seal.
4. Carefully remove the top plate, leaving the gel and the GelBond® Film attached to the back plate.
5. Remove the spacers.
6. Lift the GelBond® Film from the back plate by inserting a flat spatula under the film.
7. Gently lift film and gel away from glass plate.

**Sample Preparation**

Successful isoelectric focusing, in part, depends upon the condition of the sample. Situations such as insolubility or high salt content, particularly in the case of high sample loading, should be addressed before the sample is loaded onto the gel. Listed below are general guidelines for sample treatment.

**High Salt Content:** Dialyze the sample against distilled water, 1% glycine, or 0.05 M - 0.1 M ammonium bicarbonate solution.

**Dissociation of protein aggregates, subunit assemblies or to unfold peptide chains:** Add urea to a final concentration of 4 M - 9 M to both the sample and the gel.

**Samples that are hydrophobic or poorly soluble at or near their pI point:**
Add either nonionic or zwitterionic detergents to the sample and the gel at a final concentration of 0.05% - 1.0%. Detergents should be added to the agarose solution once the agarose has been dissolved and cooled to 60°C.

**Nonionic detergents**

- Triton® X-100
- Nonidet® (NP-40)
- Tween® 80

**Zwitterionic detergents**

- CHAPS (available from Sigma Chemical Co., St. Louis, MO)
- Zwittergent® 3-14 (available from Calbiochem/Behring, LaJolla, CA)
Section XII: Isoelectric Focusing of Proteins on Agarose Gels

Running Agarose IEF Gels

Procedure for gel placement

1. Set the refrigerated circulator bath to 10°C - 15°C.
   
   **NOTE:** To prevent condensation on the gel and platen, do not circulate the coolant to the IEF chamber just prior to focusing.

2. Spread 0.2 ml - 0.3 ml of distilled water on the cooling platen of the IEF chamber.

3. Lower the gel (film-side down) onto the wetted area. Avoid trapping air under the GelBond® Film.

4. Wipe excess fluid from the edges of the film.

5. Blot the surface of the gel briefly with a sheet of fine-grained blotting paper.

6. If necessary, trim the edges of the gel parallel to the direction of focusing with a razor blade to ensure the edges are even and free of cracks or small tears.

Procedure for electrode wick application

1. Cut two electrode wicks to the exact width of the gel or slightly shorter.

2. Completely immerse one wick in catholyte solution.

3. Place the wick on blotting paper to remove excess fluid.

4. Place on the negative electrode contact of the gel.

5. Completely immerse a second wick in anolyte solution.

6. Place the wick on blotting paper to remove excess fluid.

7. Place on the positive electrode contact of the gel.

   **NOTE:** The wicks must lie parallel to each other on the ends of the gel, evenly touching the surface.

8. Place a glass plate on top of the gel and wicks for approximately one minute.

   **NOTE:** This ensures uniformity of contact between wicks and gel and serves to smooth the wick surface in preparation for electrode contact.

Procedure for sample application

1. Place the sample applicator mask across the gel at least 1 cm from either wick (e.g., 3 cm from cathode).

2. Load sample and pl markers into the slots (2 μl - 5 μl maximum; 2 - 10 mg/ml concentration).

   **NOTE:** In direct tissue isofocusing, tissue samples may be placed directly onto the applicator slots.

3. Ensure electrodes and electrical contacts are clean and there are no breaks in the wire or ribbon.

4. Place the electrodes on the wicks (not the gel surface), aligning them so they lie in parallel upon the wicks.

5. Set the power supply at 1 W constant power.

6. Apply power for 10 minutes.

7. Turn power off.

8. Remove the sample applicator mask.

9. Gently remove any precipitated sample from the gel surface with blotting paper.

**Materials**
- Horizontal IEF chamber
- Fine-grained blotting paper
- Razor blade
- Refrigerated circulator bath set to 10°C - 15°C
- Kimwipe® Tissues or equivalent
- Electrode wicks
- Scissors
- Forceps
- Glass plate slightly larger than the gel
- Sample applicator mask
- Power supply

**Reagents**
- Distilled water
- Catholyte solution
- Anolyte solution
Running Agarose IEF Gels — continued

IEF power settings and focusing time

1. Start circulation of the coolant to the IEF chamber.
2. Set the voltage, current and power according to the appropriate running conditions listed in the table below.
3. Once focusing is complete, turn off the power.
4. Discard the wicks.
5. Place the gel in fixative solution.

NOTE: The separation progress can be monitored by observing the visible proteins in the pI markers coming into focus and noting the decreasing rate of current flow on the power supply’s milliampere indicator. Focusing is attained when the visible pI markers are sharply resolved and the current has stopped decreasing significantly (less than 1 mA in 10 minutes).

Running Conditions

<table>
<thead>
<tr>
<th>Ampholyte pH Range</th>
<th>Voltage (upper limiting)</th>
<th>Current (limiting)</th>
<th>Power</th>
<th>Anolyte</th>
<th>Catholyte</th>
<th>Focusing Time (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 - 4.5</td>
<td>250 V</td>
<td>MAX</td>
<td>10 W</td>
<td>0.05 M H₃PO₄</td>
<td>0.05 M Threonine</td>
<td>90</td>
</tr>
<tr>
<td>3.5 - 9.5</td>
<td>500 V</td>
<td>MAX</td>
<td>10 W</td>
<td>0.5 M HOAc</td>
<td>1 M NaOH</td>
<td>90</td>
</tr>
<tr>
<td>3.5 - 9.5</td>
<td>1000 V</td>
<td>MAX</td>
<td>25 W</td>
<td>0.5 M HOAc</td>
<td>1 M NaOH</td>
<td>40</td>
</tr>
<tr>
<td>3.5 - 9.5</td>
<td>1500 V</td>
<td>MAX</td>
<td>25 W</td>
<td>0.5 M HOAc</td>
<td>1 M NaOH</td>
<td>30</td>
</tr>
<tr>
<td>4 - 6.5</td>
<td>500 V</td>
<td>MAX</td>
<td>10 W</td>
<td>2% solution of pH 2.5 - 4.5 Ampholyte</td>
<td>0.04 M L-Histidine (free-base)</td>
<td>90</td>
</tr>
<tr>
<td>5 - 8</td>
<td>500 V</td>
<td>MAX</td>
<td>10 W</td>
<td>2% solution of pH 2.5 - 4.5 Ampholyte</td>
<td>0.1 M NaOH or 0.1 M Bicine</td>
<td>90</td>
</tr>
</tbody>
</table>

*1 M H₃PO₄ can be replaced by 0.5 M acetic acid
Section XII: Isoelectric Focusing of Proteins on Agarose Gels

Staining Proteins in Agarose IEF Gels

Introduction

Either Coomassie® Blue or Crowle’s Double Stain can be used to stain IEF gels. Coomassie® stain is used when increased sensitivity is desired, and Crowle’s stain produces gels with clear background and sharp resolution.

Staining proteins with Coomassie® Blue Stain or Crowle’s Double Stain

Preparation of staining solutions

<table>
<thead>
<tr>
<th>Fixative solution</th>
<th>Methanol</th>
<th>Trichloroacetic acid</th>
<th>Sulfosalicylic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>180 ml</td>
<td>30.0 g</td>
<td>18.0 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adjust volume to 500 ml with distilled water</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Coomassie® Stain</th>
<th>Coomassie® Brilliant Blue R-250</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 g</td>
<td></td>
</tr>
<tr>
<td>250 ml</td>
<td>Ethanol</td>
</tr>
<tr>
<td>90 ml</td>
<td>Glacial acetic acid</td>
</tr>
<tr>
<td></td>
<td>Adjust volume to 1 liter with distilled water</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Coomassie® Destaining Solution</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>250 ml</td>
<td></td>
</tr>
<tr>
<td>90 ml</td>
<td>Glacial acetic acid</td>
</tr>
<tr>
<td>Adjust volume to 1 liter with distilled water</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Crowle’s Double Stain</th>
<th>Crocein scarlet (C.I. 26905)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 g</td>
<td></td>
</tr>
<tr>
<td>150.0 mg</td>
<td>Coomassie® Brilliant Blue R-250</td>
</tr>
<tr>
<td>50 ml</td>
<td>Glacial acetic acid</td>
</tr>
<tr>
<td>30.0 g</td>
<td>Trichloroacetic acid</td>
</tr>
<tr>
<td>Adjust volume to 1 liter with distilled water</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Crowle’s Destaining Solution</th>
<th>Glacial acetic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 ml</td>
<td></td>
</tr>
<tr>
<td>Adjust volume to 1 liter with distilled water</td>
<td></td>
</tr>
</tbody>
</table>

Materials

- Flask
- Stir bar
- Magnetic stir plate
- Forceps
- Paper towel
- Whatman® 3MM chromatography paper
- 1 kg-2 kg weight
- Glass plate
- Forced hot air oven set to 50°C-55°C
- Staining vessel
- Clamps

Reagents

- Methanol
- Trichloroacetic acid
- Sulfosalicylic acid
- Distilled water
- Coomassie® Brilliant Blue R-250
- Ethanol
- Glacial acetic acid
- Crocein scarlet (C.I. 26905)
Follow the steps below to stain the gel after electrophoresis using either Coomassie® Blue or Crowle’s Double Dtain.

1. Place the gel in Fixative solution for 10 minutes.
2. Remove the gel and rinse the surface with distilled water.
3. Drain excess water.
4. Place on a paper towel, gel side up.
5. Pre-wet a piece of Whatman® 3MM chromatography paper with distilled water.
6. Place on gel surface.
7. Overlay the blotting paper with four to six layers of absorbent paper toweling.
8. Place a glass plate on top of the paper toweling.
9. Place a 1 kg - 2 kg weight on top of the towel ing for 10 minutes.
10. Remove the weight, the glass plate, and the paper toweling.
11. Rewet the blotting paper thoroughly with distilled water.
12. Gently lift the blotting paper off gel surface.
13. Wash the gel in distilled water for 5 minutes to remove residual fixative and ampholytes.
14. Dry the gel completely in a forced hot air oven (50°C - 55°C).

**NOTE:** Drying usually takes less than 30 minutes.

15. Stain with Coomassie® or Crowle’s double stain solution for 15 · 30 minutes without agitation.

**NOTE:** Float gel-face down into the stain, so precipitated stain will not settle on the gel surface.

16. Remove the gel and rinse with distilled water.
17. Place the gel in Destaining solution for 3 minutes.
18. Briefly rinse again in distilled water.
19. Clamp (gel side out), onto a glass plate to prevent curling during drying.
20. Dry the gel in a forced hot air oven (50°C - 55°C) for approximately 15 minutes or dry at room temperature overnight.

**NOTE:** Gel may crack if over dried by heating.
Staining Proteins in Agarose IEF Gels — continued

**Staining Proteins in Agarose IEF Gels with Silver Stain**

A modified silver stain procedure has been developed for use with agarose gels cast on GelBond® Film. After electrophoresis, the gels are fixed, press blotted, and completely dried before staining. Perform all fixing and staining steps in acid-cleaned (50% HNO₃) glassware. All washes are done with constant agitation in a volume of at least 250 ml (gel volume:reagent volume = 1:22). Coomassie® Brilliant Blue stained gels may be silver stained after drying. In this case, proceed directly to step 14.

**Preparation of staining solutions**

<table>
<thead>
<tr>
<th>Materials</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixative solution</td>
<td></td>
</tr>
<tr>
<td>180 ml Methanol</td>
<td></td>
</tr>
<tr>
<td>30.0 g Trichloroacetic acid</td>
<td></td>
</tr>
<tr>
<td>18.0 g Sulfosalicylic acid</td>
<td></td>
</tr>
<tr>
<td>Adjust volume to 500 ml with distilled water</td>
<td></td>
</tr>
<tr>
<td>Silver Stain Solution A</td>
<td></td>
</tr>
<tr>
<td>50.0 g Sodium carbonate, anhydrous in 1 liter distilled water [Stable for 2 - 3 weeks at room temperature]</td>
<td></td>
</tr>
<tr>
<td>Silver Stain Solution B</td>
<td></td>
</tr>
<tr>
<td>In the order given dissolve the following reagents in 1 liter of distilled water, while mixing rapidly. 2.0 g Ammonium nitrate 2.0 g Silver nitrate 10.0 g Dodeca-tungstosilicic acid 6.7 ml 37% formaldehyde [Stable 1 week at room temperature stored in the dark]</td>
<td></td>
</tr>
<tr>
<td>Stop solution</td>
<td></td>
</tr>
<tr>
<td>1% Acetic acid</td>
<td></td>
</tr>
</tbody>
</table>

**Caution:** Materials and methods shown here present hazards to the user and the environment. Refer to the safety information on page 210 before beginning these procedures.

**Caution:** Acetic acid causes burns and respiratory irritation. Take precautions to prevent exposure.

**Caution:** Glutaraldehyde is toxic and must be handled in a fume hood.
Follow the steps below to stain the gel after electrophoresis using silver stain:

1. Place the gel in Fixative solution for 10 minutes. If gel is prestained with Coomassie® Blue and dried, proceed to step 14.
2. Place the gel on a paper towel, gel side up.
3. Pre-wet a sheet of Whatman® 3MM chromatography paper with distilled water.
4. Place on gel surface.
5. Overlay the blotting paper with four to six layers of absorbent paper toweling.
6. Place a glass plate on top of the paper toweling.
7. Place a 1 kg - 2 kg weight on top of the toweling for 10 minutes.
8. Remove the weight, the glass plate, and the paper toweling.
9. Rewet the blotting paper thoroughly with distilled water.
10. Gently lift the blotting paper off the gel surface.
11. Wash the gel in distilled water for 5 minutes to remove residual fixative and ampholytes.
12. Clamp (gel side out), onto a glass plate to prevent curling during drying.
13. Dry the gel in a forced hot air oven (50°C - 55°C) for approximately 15 minutes or until dry.

**NOTE:** Gel may crack if over dried by heating.

14. Soak the dried gel in 2% glutaraldehyde for 10 minutes.
15. Wash in distilled water for 10 minutes using mild agitation.
16. Soak the gel for 10 minutes in 0.01% (DTT) dithiothreitol.
17. Wash in distilled water for 10 minutes using mild agitation.
18. Pour an equal volume of Silver stain solution B into vigorously stirring Silver stain solution A (75 ml B and 75 ml A for each gel to be stained).
19. Transfer the solution to an acid-cleaned glass dish containing one gel.
20. Stain the gel for 10 minutes with gentle agitation.

**NOTE:** There will be some background.

21. Transfer the gel to a Stop solution and gently agitate for 5 minutes.
22. Rinse the gel in distilled water.
23. Wipe any silver deposits from the back of the film.
Press blot transfer is a quick method of removing proteins from agarose gels. The procedure involves overlaying the gel with a buffer-soaked nitrocellulose membrane covered by a thick filter pad and several layers of dry paper toweling. The assembly is then covered by a glass plate. After just 1 1/2 minutes of press blot, approximately 20% of the proteins are transferred from the gel to the membrane. Up to 85% transfer can be achieved with a 35 - 40 minute blotting time. Transferred proteins can be detected on the membrane and on the gel by standard methods.

**Procedure**

1. Prepare Tris-saline buffer, pH 7.5.
2. Cut one piece of nitrocellulose membrane and thick filter paper to the same dimension as the gel.

**NOTE:** Wear gloves to prevent contamination by extraneous proteins.

3. Evenly wet the nitrocellulose membrane in the Tris-saline buffer by holding one end of the membrane with smooth-tipped forceps and lowering the other end into the buffer container, dropping the membrane flat on the buffer surface.

**NOTE:** The membrane must be completely saturated with buffer.

4. Remove the gel from the focusing chamber.
5. Place on a flat surface, gel side up.
6. Place the buffer-soaked nitrocellulose membrane onto the gel surface.

**NOTE:** Avoid trapping air bubbles between the gel and the membrane.

7. Place one piece of buffer soaked filter paper on top of the membrane.
8. Place three layers of dry paper toweling on top of the filter paper.
9. Cover with a glass plate slightly larger than the gel surface. No other weight is required.
10. Press blot for 1 1/2 minutes or longer, as desired.
11. Remove glass plate and discard the paper toweling and filter paper.

**Materials**
- Nitrocellulose membrane
- Thick filter paper
- Scissors
- Smooth-tipped forceps
- Container
- Paper towels
- Glass plate

**Reagents**
- Tris-saline buffer, pH 7.5
  - \(50.0 \text{ g Tris-HCl, 0.94 g Tris-Base, 58.48 g NaCl adjust to 2 liters with distilled water}\)
Section XII: Isoelectric Focusing of Proteins on Agarose Gels

Preparative Isoelectric Focusing

Separation of relatively large amounts of biologically active macromolecules is possible by isoelectric focusing in agarose. Typical high-yield recoveries of applied proteins are obtainable with retention of biological activity. This preparative isoelectric focusing procedure is based on the work of Cantarow, et al. As much as 120.0 mg of protein can be focused in 9.5 ml (0.75 x 10 x 11.5 cm) of 1% IsoGel® Agarose containing 2.5% ampholytes.

Procedure

1. Follow steps 1 - 14 for Gel Preparation on pages 179-181. Also see Sample Preparation on page 181.
2. Add the protein sample to the agarose solution once cooled to 60°C.
3. Stir the solution well to mix.
4. Cast the gel and disassemble the casting cassette following the steps previously described on page 179.
5. Place the gel on the 10°C cooling platen for 10 minutes (if gel has not already been chilled after casting).
6. Focus following the steps previously described on pages 182-183.

Procedure for recovering proteins from preparative gel

1. Excise the gel slice containing the protein of interest by using a spatula to strip the agarose from the GelBond® Film in 5 mm-wide slices.
2. Place the agarose strip in a 5-cc plastic syringe fitted with an 18-gauge needle.
3. Macerate the gel slice by expelling it into a clean tube.
4. Add 4 ml of phosphate-buffered saline (PBS) to the macerated gel.
5. Cover the tube securely with Parafilm®
6. Place on a test tube rocker for 16 hours at 4°C.
7. Centrifuge the tube for one minute at 100 rpm.
8. Separate the supernatant from the gel using a serum separator.

Materials
- Spatula
- 5-cc plastic syringe with an 18-gauge needle
- Clean centrifuge tube
- Test tube rocker at 4°C
- Centrifuge
- Serum separator
- Parafilm®

Reagent
- Phosphate-buffered saline (PBS)
Detection of separate species can be accomplished by protein stains or by overlaying gels with specific antibody solutions coupled to enzymes that will eventually produce a visible end product. The antibody-peroxidase conjugate system or autoradiography with $^{125}$I-labeled antibody are frequently used for this purpose. Immunoperoxidase labeling of focused proteins is performed according to Saravis, et al. After focusing, fixing, and drying the gel, treat the gel as described below.

**Procedure for Immunoperoxidase Staining/Avidin-Biotin Modification**

1. Soak the gel in 3% hydrogen peroxide for 10 minutes.
2. Rinse with distilled water.
3. Soak the gel in 2.28% periodic acid for 5 minutes.
4. Rinse with distilled water.
5. Soak the gel in 0.02% sodium borohydride for 2 minutes.
6. Rinse with distilled water.
7. Place the gel in 0.05 M Tris-saline, pH 7.6 for 10 minutes.
8. Incubate the gel with 1:5 normal serum for 10 minutes (same species as secondary antibody).
9. Incubate the gel for 2 - 4 hours at room temperature with Anti-serum (primary antibody).
10. Rinse the gel with 0.15 M PBS, pH 7.4 for 1 hour at room temperature.
11. Incubate the gel with Secondary* antibody (e.g., goat anti-mouse IgG) for 2 - 4 hours at room temperature.

**NOTE:** If the avidin/avidin modification is used, proceed with steps 12 and 13 using biotinylated reagents (marked with * in steps 11 and 15).

12. Rinse the gel with 0.15 M PBS for 1 hour at room temperature.
13. Incubate the gel with avidin solution [40 mg/gel] in PBS for 1 hour at room temperature (stock solution of avidin is usually 20 mg/ml).
14. Rinse the gel with 0.15 M PBS for 1 hour at room temperature.
15. Incubate the gel with horseradish peroxidase* (33 mg/gel) for 2 hours at room temperature.
16. Rinse the gel with 0.15 M PBS for 1 hour at room temperature.
17. Incubate the gel with diaminobenzidine [0.15 mg/ml] and hydrogen peroxide [0.03%] in 0.01 M Tris 0.15 M NaCl for 1 - 1.7 hours at room temperature.
18. Rinse the gel in PBS and dry.

**Materials**
- Staining containers

**Reagents**
- Hydrogen peroxide
- Distilled water
- Periodic acid
- Sodium borohydride
- 0.05 M Tris-saline (pH 7.6), normal serum (same species as secondary antibody)
- Anti-serum (primary antibody)
- 0.15 M PBS (pH 7.4)
- Secondary antibody
- 20 mg/ml avidin solution
- Horseradish-peroxidase
- 0.15 mg/ml diaminobenzidine
- 0.01 M Tris/0.15 M NaCl
This method employs the application of tissue or cell pellets directly onto the surface of the IEF gel without concentration of samples, dialysis to remove salts, or the salt extraction of soluble proteins from tissue. DTIF allows more soluble protein per milligram of tissue to enter the gel than is recoverable by extraction procedures and minimizes denaturation of biologically active proteins that can be damaged by extraction.

1. Prepare solutions and cast gels as previously described on pages 179-181.
2. Prepare for focusing as previously described on page 182.
3. Place the sample applicator mask across the gel at least 1 cm from either wick (e.g., 3 cm from cathode).
4. Drape a tissue slice over the open slot of the applicator mask.
5. Ensure the electrodes and the electrical contacts are clean and there are no breaks in the wire or ribbon.
6. Place the electrodes on the wicks (not the gel surface), aligning them so they are in parallel upon the wicks.
7. Set power supply at 1 W (constant power).
8. Prefocus for 10 - 15 minutes to allow sample uptake.
9. Turn power supply off.
10. Remove the tissue slice and the applicator mask.
11. Focusing is continued using standard conditions.
12. After focusing is complete, the gel is fixed, stained and dried following standard procedures.
# Section XII: Isoelectric Focusing of Proteins on Agarose Gels

## Resolution Reference Guide

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<th>Phenomenon</th>
<th>IEF Band Appearance</th>
<th>Possible Causes</th>
<th>Remedy</th>
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<tbody>
<tr>
<td>Streaks or gaps perpendicular to bands</td>
<td><img src="image" alt="Streaks or gaps perpendicular to bands" /></td>
<td>• Particulates in sample&lt;br&gt;• Old or denatured sample&lt;br&gt;• Isoelectric precipitation of sample applied too close to pl point&lt;br&gt;• Poor soluble sample, which precipitates</td>
<td>• Filter or centrifuge sample before applying&lt;br&gt;• Replace sample&lt;br&gt;• Do not prefocus gel before applying sample&lt;br&gt;• Apply sample in different location&lt;br&gt;• Use solubilizing additive, i.e., non-ionic, Zwitterionic&lt;br&gt;• Try different application location</td>
</tr>
<tr>
<td>Fuzzy bands</td>
<td><img src="image" alt="Fuzzy bands" /></td>
<td>• Run incomplete&lt;br&gt;• Focusing time too long&lt;br&gt;• Insufficient fixation</td>
<td>• Use pl marker to monitor run or measure pH gradient before removing gel from platen&lt;br&gt;• Decrease focusing time or power/voltage&lt;br&gt;• Increase fixation time. Be sure to place gel in fixative immediately after IEF run</td>
</tr>
<tr>
<td>Skewed bands</td>
<td><img src="image" alt="Skewed bands" /></td>
<td>• Electrode not clean&lt;br&gt;• Uneven electrode contact&lt;br&gt;• Sample placed too close to edge of gel&lt;br&gt;• Electrode wicks too short&lt;br&gt;• Old or incorrect electrolyte solution&lt;br&gt;• Overloading of sample&lt;br&gt;• Excessive salt in sample</td>
<td>• Rinse electrode with distilled water, then dry&lt;br&gt;• Wicks should be evenly wetted and placed on gel parallel to each other&lt;br&gt;• Place electrodes firmly on wicks but not so fluid is squeezed from wicks&lt;br&gt;• Apply ( \geq 5 \text{ mm} ) from edge of gel&lt;br&gt;• Wicks should extend to edge of gel&lt;br&gt;• Make fresh solution&lt;br&gt;• Decrease protein load&lt;br&gt;• Reduce salt concentration by gel filtration or dialyze against 1% glycine</td>
</tr>
<tr>
<td>Missing or faint bands</td>
<td><img src="image" alt="Missing or faint bands" /></td>
<td>• Protein not denatured by fixative&lt;br&gt;• Sample unstable at pH of site of application</td>
<td>• Establish appropriate denaturing conditions&lt;br&gt;• Use alternative application site</td>
</tr>
<tr>
<td>Wavy bands</td>
<td><img src="image" alt="Wavy bands" /></td>
<td>• Excessive salt in sample&lt;br&gt;• Overloading with sample, distorting pH gradient&lt;br&gt;• Improper analyte used&lt;br&gt;• Old or incorrect electrolyte solutions&lt;br&gt;• Dirty electrodes</td>
<td>• Reduce salt concentration by gel filtration or dialyze against 1% glycine&lt;br&gt;• Applying smaller sample volume&lt;br&gt;• Replace analyte&lt;br&gt;• Make fresh solutions&lt;br&gt;• Rinse electrode with distilled water</td>
</tr>
<tr>
<td>Arc-shaped bands</td>
<td><img src="image" alt="Arc-shaped bands" /></td>
<td>• Thin spot in gel</td>
<td>• Reduce evaporation by keeping gel sealed until ready for use&lt;br&gt;• Keep chamber lid closed except when handling gel</td>
</tr>
<tr>
<td>Phenomenon</td>
<td>IEF Band Appearance</td>
<td>Possible Causes</td>
<td>Remedy</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>--------------------</td>
<td>--------------------------------------------------------------------------------</td>
<td>---------------------------------------</td>
</tr>
<tr>
<td>Burning or sparking</td>
<td></td>
<td>• Electrode wicks too dry</td>
<td>• Anodal wick should be wet not dripping wet, cathodal wick should be damp.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Focusing too long</td>
<td>• Reduce focusing time to minimum required to obtain linear pH gradient.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Thin spot in gel</td>
<td>• Keep gel sealed until ready for use.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Using incorrect electrolyte solutions</td>
<td>• Always use recommended solutions.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Excessive power input</td>
<td>• Check power supply settings.</td>
</tr>
<tr>
<td>Sparking along edge of gel onto cooling plate</td>
<td></td>
<td>• Excess moisture on the gel, or under gel</td>
<td>• Remove excess moisture by gently blotting gel.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Electrode strips overhanging ends of gel</td>
<td>• Cut electrode strips to size of gel.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Liquid expelled at sides of electrode due to EEO flow of water to cathode</td>
<td>• Occasional blotting may be necessary.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Excess electrode pressure when placing electrodes on gel</td>
<td>• Use firm but light pressure.</td>
</tr>
<tr>
<td>Formation of ‘ditch’ ultimately causing gel to burn out</td>
<td></td>
<td>• Gradient drift</td>
<td>• Check purity or reagents.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• High current causing excessive heating early in run</td>
<td>• Use a low power for first 10-15 minutes of run during sample uptake.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Uneven gel thickness due to gel drying out</td>
<td>• Open foil package containing gel just before use.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Overblotting of gel, causing gel to dry out</td>
<td>• Initially blot only until blotting paper picks up moisture.</td>
</tr>
<tr>
<td>Sample smearing or precipitation</td>
<td></td>
<td>• Sample overload</td>
<td>• Decrease sample concentration.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Sample aggregation</td>
<td>• Solubilize sample with neutral surfactant or 1% glycine.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Sample applied too near pl or at a pH where it is insoluble</td>
<td>• Change application site. Solubilizing additives or detergents may be necessary.</td>
</tr>
<tr>
<td>Incomplete Sample Uptake</td>
<td></td>
<td>• Sample application mask not left on long enough</td>
<td>• Allow at least 10 minutes.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Insolubles or aggregates in sample</td>
<td>• Filter or centrifuge sample.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Get too wet when sample applied</td>
<td>• Blot gel before sample application.</td>
</tr>
<tr>
<td>Sample retained in sample application well</td>
<td></td>
<td>• Sample applied too near pl</td>
<td>• Change application site.</td>
</tr>
<tr>
<td>Fluid expression</td>
<td></td>
<td>• Unblotted wick</td>
<td>• Blot cathode wick with filter paper until damp before application.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Overfocusing</td>
<td>• Monitor run with pl markers.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Salt in sample</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>• Sample overload</td>
<td>• Decrease sample concentration.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Uneven electrode contact to wicks</td>
<td>• Check location of electrodes.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Electrode wicks too wet</td>
<td>• Remove excess moisture. Wicks must be blotted before use.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Local hot spot from spillage of electrode solution onto gel</td>
<td>• Avoid this area for sample application.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• General (over entire gel)</td>
<td>• Check coolant flow and temperature.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Insufficient/excessive cooling</td>
<td>• Check power supply settings.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Excessive power applied to gel</td>
<td>• Check power supply settings.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Gel not adequately blotted</td>
<td>• Use blotting paper.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• High ambient humidity</td>
<td>• Use tray with desiccant in chamber.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Excessive amount along electrode wicks. Persisting for duration of run</td>
<td>• Check pH of wicks and polarity of plugs into power supply. Reverse polarity if incorrect.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Cathodal drift (pooling of fluid near cathode—over saturating wick)</td>
<td>• Blot pooled liquid of necessary.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Reversed polarity of electrode wicks (lower pH at cathode, higher at anode)</td>
<td>• Check pH of wicks and polarity of plugs into power supply. Reverse polarity if incorrect.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Spotted over gel</td>
<td>• Check for air bubbles—remove if present.</td>
</tr>
</tbody>
</table>
## Isoelectric Focusing of Proteins on Agarose Gels

### Phenomenon | IEF Band Appearance | Possible Causes | Remedy
--- | --- | --- | ---
Condensation inside chamber | • Inefficient cooling | • Adjust coolant temp. to 10°C. |  
 | | • Voltage/power setting too high | • Adjust to 1W for 1st 10 min. 25W limiting thereafter (pH 3-10 e.g.). |  
 | | • Salt in sample | • Desalt sample. |  
 | | • Humid ambient conditions | • Place humectant cartridge or desiccant tray in chamber. |  

Get thinning  
- at electrode  
- near point of application  
- generalized | • Improper electrolyte solution | • Check electrolyte solution. |  

Uneven pH gradient/pH gradient different than stated for ampholyte used | • Incorrect calibration of pH electrode | • Check pH electrode. |  
 | | • Get overfocused—excessive gradient drift | • Do not exceed time required to form a linear pH gradient. |  
 | | • Impure distilled water in sample or electrolyte solutions | • Check water for heavy metals or ionic contaminants. |  
 | | • Incorrect electrolyte solution | • Check solution. |  

pH gradient is not linear | • Insufficient run time | • Use Isofug pl markers to check progress. Measure pH gradient before removing gel. |  

pH gradient not attaining upper limits | • Overfocusing | • Decrease runtime. |  
 | | • pH measurements may be wrong | • Use surface pH electrode. |  

pH range shifted toward cathode | • Cathodal drift (entire gradient shifts towards cathode) | • Reduce run time (as much as possible) to attain desired gradient profile. |  

Current increases with time | • Electrodes applied at the wrong ends of the gel | • Use recommended anolyte/catholyte solution. |  
 | | | • Apply (+) electrode to anode. (-) electrode to cathode. |  
 | | | • Reverse polarity |  

Diagonal band migration | • Uneven electrode contact | • Apply electrodes evenly on wicks. |  
 | | • Too much pressure on electrodes | • Adjust for light but firm contact. |  
 | | • Uneven electrode placement | • Ensure electrodes are parallel and perpendicular to the sample migration. |  

Lateral band migration | • Air pocket(s) trapped beneath mask | • Press gently to expel before sample applied. |  
 | | • Too much sample applied to applicator mask | • Reduce sample volume, increase sample concentration. |  
 | | • Sample mask left on too long/ not long enough | • Remove mask after 10 minutes at 1 watt. |  

Sample “focuses” at wrong position | • Run incomplete | • Allow longer focusing time. |  
 | | • Sample unstable at pH of application | • Use alternative application site. |  
 | | • Removal of ligands during focusing | • Check protein for this possible characteristic. |  
 | | • Formation of complex aggregate | • Use urea to treat sample. |  

A supposedly “pure” sample focuses as multiple bands | • Sample exists in various states of oxidation | • Pretreat sample accordingly. |  
 | | • Sample dissociates into subunits | • Natural phenomenon. |  
 | | • Sample has become denatured | • Check procedures used to prepare sample. |  

High background stain | • Residual ampholytes in gel | • Follow recommended press blot procedure. |  
 | | • Concentration of stain too high | • Extend time of fixing, rinsing and destaining. |  
 | | • Stain is old | • Reduce concentration. |  
 | | • Gel allowed to settle in bottom of staining dish | • Make fresh stain and be sure all dye is in solution. |  

Delamination (peeling) of gel from film support on staining after drying | • Residual fixative in dried gel | • Float gel side down in stain for 15-30 minutes. |  

### References


Section XIII: Protein Separation in Agarose Gels

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Section XIII: Protein Separation in Agarose Gels

Introduction

Protein electrophoresis in agarose gels is an alternative approach to using polyacrylamide gels and provides several benefits. Gels can be run using a vertical system or a horizontal system and unlike polyacrylamide gels, agarose gels can be used effectively to separate proteins larger than 600,000 Da.

Advantages

— Separate high-molecular-weight proteins (>600,000 Da)
— Easy to prepare and handle
— Efficient recovery of proteins
— Excised proteins can be used to immunize animals directly for antibody production
— Non-toxic

Recommended agaroses for protein electrophoresis

The table below is a list of Lonza Agaroses that are recommended for protein electrophoresis. For performing routine separations, we recommend a standard melting temperature agarose such as MetaPhor® Agarose or SeaKem® Gold Agarose. When proteins are to be recovered for further analysis, use a low melting temperature agarose such as SeaPlaque® GTG® or NuSieve® GTG® Agarose.

<table>
<thead>
<tr>
<th>Protein Size Range (kDa)</th>
<th>Agarose</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 - 200</td>
<td>MetaPhor® or NuSieve® GTG®</td>
<td>5%</td>
</tr>
<tr>
<td>150 - 300</td>
<td>MetaPhor® or NuSieve® GTG®</td>
<td>3%</td>
</tr>
<tr>
<td>300 - 600</td>
<td>MetaPhor® or NuSieve® GTG®</td>
<td>2%</td>
</tr>
<tr>
<td>600 - 1,000</td>
<td>SeaKem® Gold or SeaPlaque®</td>
<td>1.5%</td>
</tr>
<tr>
<td>1,000 - 5,000</td>
<td>SeaKem® Gold or SeaPlaque®</td>
<td>1.0%</td>
</tr>
</tbody>
</table>
The buffer systems used for agarose electrophoresis are similar to those used for polyacrylamide electrophoresis. When performing horizontal electrophoresis, we have found that a Tris-borate gel and running buffer provide greater resolution than using the standard Laemmli Buffer system.

Vertical and horizontal gel

<table>
<thead>
<tr>
<th>1X Stack Buffer, pH 8.0</th>
<th>g/l for 1X Stack Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>125 mM Tris-HCl</td>
<td>19.7 g Tris-HCl in 1 liter distilled water</td>
</tr>
</tbody>
</table>

**NOTE:** Horizontal gels do not require the use of a stack gel.

<table>
<thead>
<tr>
<th>1X Resolving Buffer, pH 8.5</th>
<th>g/l for 1X Resolving Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 mM Tris base</td>
<td>60.55 g Tris base</td>
</tr>
<tr>
<td>160 mM Boric acid</td>
<td>9.90 g Boric acid</td>
</tr>
<tr>
<td>1 M Urea</td>
<td>60.06 g Urea</td>
</tr>
</tbody>
</table>

Adjust volume to 1 liter with distilled water

<table>
<thead>
<tr>
<th>1X Running Buffer, pH 8.5</th>
<th>amount for 1X Running Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>90 mM Tris base</td>
<td>10.90 g Tris base</td>
</tr>
<tr>
<td>90 mM Boric acid</td>
<td>5.57 g Boric acid</td>
</tr>
<tr>
<td>0.1% SDS</td>
<td>10 ml of 10% SDS</td>
</tr>
</tbody>
</table>

Adjust volume to 1 liter with distilled water

**Laemmli Buffer**

— Gels can be made with standard Laemmli Buffer (see page 156).
— To avoid excess foaming during agarose dissolution; only add SDS to the cathodal buffer and not to the gel buffer. The SDS in the cathodal buffer will migrate faster than the proteins during electrophoresis, maintaining protein denaturation.
— To prevent buffer depletion in vertical systems, use 10X Laemmli Buffer without SDS in the anodal buffer.

**Tips for buffer preparation**

— Add SDS to the cathodal buffer only.
— Do not add SDS to the gel prior to dissolution.
— If other buffer systems are used, the pH should be between pH 5-9.
— Denaturants such as urea and formamide should only be added at low concentrations (4 M-6 M Urea).
— For buffers more alkaline than pH 9, dissolve and cast the agarose in distilled water, allow to gel. Soak horizontal gels in the alkaline gel buffer for 30 minutes prior to electrophoresis. For vertical gel systems, prerun the gel to equilibrate the gel with the alkaline buffer.

**Caution:** Materials and methods shown here present hazards to the user and the environment. Refer to the safety information on page 210 before beginning these procedures.
The procedures for dissolving and casting agarose gels for protein separation are the same as the procedures used for nucleic acid separation. Refer to Dissolving Agarose and Casting Agarose Gels (see Section II).

**Tips for casting horizontal agarose gels**

- Use of a stacking gel is not necessary for horizontal submarine electrophoresis.
- The resolving gel buffer and running buffer should be the same.
  - Dissolve the agarose in running buffer without SDS.
  - For denaturing electrophoresis, add SDS to the sample buffer and the running buffer.
- Let the gel set for 20-30 minutes at room temperature.
- For MetaPhor® and SeaPlaque® Agarose, chill the gel at 4°C for 20-30 minutes before removing comb.
- If gels are to be dried, cast the gels onto GelBond® Film.

**Tips for casting vertical agarose gels**

- **Stacking gel for vertical gels**
  - Prepare a 1% SeaKem® Gold Agarose gel in stacking gel buffer.
  - For proteins >100 kDa, the use of a stacking gel may be omitted. It will not enhance band resolution.
  - After the stacking gel is set, place cassette at 4°C for 30 minutes prior to removing the comb.
- **Resolving gel for vertical gels**
  - Dissolve the agarose in running buffer without SDS.
  - Refer to Vertical Gel Casting Instructions (see page 86).
  - Allow resolving gel to set approximately 3 minutes at room temperature then cast the stacking gel.
- **To facilitate comb removal from a vertical gel**
  - The teeth of the comb can be tapered so the width at the bottom is slightly smaller than at the top. A slight rounding of the edges is all that is needed so that the end is U-shaped. Tapering the teeth in this way will not affect the pattern of the protein bands.
- Flood the comb area with running buffer prior to removing the comb.
- If clamps are used, remove the clamps at the top of the gel cassette and gently loosen the comb by moving it forward and back before removal.

**Preparation and Loading of Protein Samples**

Sample preparation and amount of protein that can be loaded on agarose gels is essentially the same as for polyacrylamide gels and is largely dependent on your application and detection method.

**Guidelines**

- Suspend protein samples in 2X sample buffer, 1:1 (v:v).
- If denatured proteins are required, incubate at 95°C-100°C for 5 minutes.
- Load the samples into the sample wells.
- The minimal amount of protein detectable by Coomassie® Brilliant Blue stain is about 1.0 μg; and may vary depending on the protein.
- Larger amounts of protein can be loaded, but band thickness increases accordingly.
- For a 0.8 cm wide well, 25 ml (50 μg total protein) is recommended for a complex mixture, if staining with Coomassie® Blue, and 1 ml (10 μg total protein) is needed for samples containing one or a few proteins.
- For vertical electrophoresis, load empty wells with sample buffer.

**2X Tris-Glycine SDS sample buffer for agarose electrophoresis of proteins**

<table>
<thead>
<tr>
<th>2X concentrate</th>
<th>Amount to add for 10 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>126 mM Tris-HCl, pH 6.8</td>
<td>2.5 ml of 0.5 M Tris-HCl, pH 6.8</td>
</tr>
<tr>
<td>15% Ficoll® Type 400</td>
<td>1.5 g Ficoll® Type 400</td>
</tr>
<tr>
<td>4% SDS</td>
<td>4 ml of 10% SDS</td>
</tr>
<tr>
<td>0.002% Bromophenol Blue</td>
<td>0.2 ml of 0.1% Bromophenol blue</td>
</tr>
</tbody>
</table>

Adjust volume to 10 ml with distilled water

Before use: add 1 ml β-Mercaptoethanol (βME) to 10 ml of 2X Tris-Glycine SDS sample buffer
Section XIII: Protein Separation in Agarose Gels

Optimal Voltage and Electrophoretic Times

- Avoid higher power settings as the heat generated may melt the agarose.
- Thick vertical gels (>1 mm) will require proportionally higher current settings to complete the electrophoresis run within the times indicated.
- Electrophorese the gel until the tracking dye travels to the bottom of the resolving gel.

Prestained molecular weight markers such as Lonza’s ProSieve® Color Protein Marker can be used to monitor electrophoresis. The gels can be electrophoresed longer, but care should be taken that smaller proteins do not travel off the gel.

<table>
<thead>
<tr>
<th>Gel Type</th>
<th>Gel Size</th>
<th>Power Setting</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horizontal Gel</td>
<td>5.3 cm x 8.5 cm x .4 cm</td>
<td>100 volts</td>
<td>3 - 4 hours</td>
</tr>
<tr>
<td>Vertical Gel</td>
<td>14.5 cm x 16.5 cm x .1 cm</td>
<td>25 mA (constant)</td>
<td>3 - 3.5 hours</td>
</tr>
<tr>
<td>Mini-Vertical</td>
<td>8 cm x 10 cm x .1 cm</td>
<td>20 mA</td>
<td>1.5 - 2 hours</td>
</tr>
</tbody>
</table>

Section XIV: Protein Separation in Agarose Gels

Detection of Proteins in Agarose Gels

The procedures for staining agarose gels with Coomassie® Blue Stain are essentially the same as they are for polyacrylamide gels with some modifications (listed below). For detailed procedures on Coomassie® Brilliant Blue staining (see page 168), using the modifications listed below. For detailed procedures on Silver staining, refer to Staining Proteins with Silver Stain (see page 186 in Isoelectric Focusing of Proteins on Agarose Gels).

Tips
- Agarose gels require more time to process than polyacrylamide gels of similar dimensions.
- Staining and destaining times will vary depending on the gel concentration, thickness and protein concentration.
- Place container on shaker with gentle motion during staining and destaining procedures.

Staining proteins with Coomassie® Brilliant Blue Stain

<table>
<thead>
<tr>
<th>Coomassie® Blue Stain solution</th>
<th>Destain Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>40% Methanol</td>
<td>20% Methanol</td>
</tr>
<tr>
<td>10% Glacial Acetic Acid</td>
<td>5% Glacial Acetic Acid</td>
</tr>
<tr>
<td>0.25% Coomassie® Brilliant Blue R-250</td>
<td></td>
</tr>
</tbody>
</table>

Room temperature staining
- A 14.5 cm x 16.5 cm, 1 mm thick agarose gel will stain in approximately 1 to 2 hours.
- Destain for 1-4 hours with gentle shaking at room temperature.

Overnight staining
- Use 0.125% Coomassie® Blue R-250 with the same concentrations of methanol and acetic acid as in the stain solution.
- Destain approximately 4 hours.

Accelerated staining
- Stain gels using standard stain solutions at 50°C.
- A 1 mm thick gel takes approximately 1 hour to stain and 1 hour to destain.
- Change the destaining solution 1 time.
- Agarose gels become softer at 50°C - use a support to transfer between solutions.

Storage
- Do not store agarose gels in destain solution, they may become brittle and fracture.
- Store gels in a 5% glycerol solution or dried.
Agarose gels can be dried overnight at room temperature, dried in a forced hot-air oven or dried using a standard vacuum gel dryer. When not using a vacuum gel dryer, the gel must first have been cast onto GelBond® Film to prevent the gel from shrinking during the drying process. The procedures for drying protein agarose gels are the same as drying DNA agarose gels.

### Proteins Separation in Agarose Gels

**Autoradiography**

After drying, agarose gels can be exposed directly to X-ray film.

**Fluorography**

Do not immerse agarose gels into any fluorography solution if the gels are attached to GelBond® Film. Solutions containing high concentrations (>50%) of DMSO must not be used, as they will dissolve the agarose. Commercially prepared solutions which precipitate the fluor within the agarose gel matrix (e.g., EN³HANCE® from Perkin Elmer) work best. Follow the manufacturer’s instructions. The fluor-impregnated gel can then be dried onto filter paper under vacuum at <50°C in a slab-gel dryer and then exposed directly to X-ray film.

**Electroblotting proteins from agarose gels**

Proteins can be electroblotted out of agarose gels onto membranes (nitrocellulose, PVDF, etc.) by using the same methods used for polyacrylamide gels. Refer to Blotting Proteins from Polyacrylamide Gels for detailed procedures (see page 171). It is important to note that agarose gels adhered to GelBond® Film cannot be electroblotted because GelBond® Film is nonporous. The time required for optimal transfer of specific proteins will need to be determined experimentally. In general, proteins transfer 15% faster out of agarose gels than from a polyacrylamide gel.
Proteins can be readily recovered from agarose gels. When protein is to be recovered, the use of a low melting temperature agarose such as NuSieve® GTG®, MetaPhor® or SeaPlaque® Agarose is recommended.

Protein Recovery Tips

Identifying the protein to be recovered from the gel can be accomplished by several methods. If the gel is fixed and stained with Coomassie® Blue as detailed earlier, then 1% SDS should be added to the dilution buffer. Recovery of proteins in the native state requires that they not be fixed prior to recovery. Methods for detecting proteins which do not require fixing include:

- Using prestained molecular weight markers as a guide for the relative position of the desired protein in the gel.
- Performing a short [<10 minutes] pressure/capillary blot of the gel so that small [<5%] amounts of the proteins are transferred to a membrane and then gold stained. If prestained molecular weight markers are used, it is possible to place the stained membrane under the gel and identify the region of interest.

- We recommend the use of 50 mM Tris-HCl, 1 mM EDTA at pH 8.0 as the extraction/dilution buffer. The buffer may require modification depending upon the particular protein to be recovered and what further work is planned after it is recovered. The buffer should be one in which the protein of interest will be stable.

- The amount of extraction/dilution buffer added is directly related to the amount of protein that can be recovered: the more the agarose is diluted, the more protein will be recovered. Greater than 90% of a protein can be recovered with a dilution to 0.5% agarose. A second dilute-freeze-spin cycle can be performed to recover additional protein.

Materials
- Spatula
- 1.5 ml microfuge tube(s)
- Heat block
- -70°C freezer
- Microcentrifuge at 4°C

Reagents
- Extraction buffer (50 mM Tris-HCl, 1 mM EDTA at pH 8.0)
- Ice
Section XIII: Proteins Separation in Agarose Gels

Protein Separation in Agarose Gels — continued

Procedure

1. Identify the region of the gel which contains the protein to be recovered.
2. Excise a gel slice containing the protein of interest.
3. Place the gel slice in a 1.5 ml microfuge tube.
4. Determine the volume of the gel slice by weight or size.
5. Add the appropriate amount of extraction buffer so the final concentration of agarose is 0.5%.
6. Melt the gel slice by heating to 70°C for SeaPlaque® or NuSieve® GTG® Agarose or 80°C for MetaPhor® Agarose.
7. Mix thoroughly.

NOTE: Ensure the gel is completely melted and diluted by the buffer.

8. Incubate on ice for 30 minutes.

NOTE: It is important that the agarose mixture has gelled as much as possible at this step.

9. Freeze the mixture at -70°C for 1 to 2 hours.
10. Allow the mixture to thaw on ice.
11. Centrifuge the mixture at 13,000 rpm in a microcentrifuge for 10 to 20 minutes at 4°C.
12. Remove the supernatant; this contains the recovered protein.

NOTE: Each step of this procedure should be carefully followed to obtain quantitative results. Care should be taken to COMPLETELY gel the protein-agarose solution. If the procedure cannot be completed at one time, we recommend keeping the mixture at -70°C, as prolonged freezing will not affect the protein. Protein recovered from MetaPhor® Agarose by this technique will co-purify with some residual agarose. The amount of agarose present is between 1% and 3% of the original amount of agarose present in the gel slice. All of the residual agarose will pass through a 0.1 mm pore filter, about 83% will pass through a 100 kDa molecular-weight cutoff filter, and 50% will pass through a 30 kDa molecular-weight cutoff filter. The recovered protein can be separated from the residual agarose by conventional chromatography.

References

FMC BioProducts RESOLUTIONS, 8[1]: 6 - 7, 1992.
Litz, J.S., FMC BioProducts RESOLUTIONS, 6[3]: 6, 1990.
Appendix A: Electrophoretic Theory

In This Section

<table>
<thead>
<tr>
<th>Voltage, Current and Power; Interactive Effects on Gel</th>
<th>204</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrophoresis</td>
<td>204</td>
</tr>
<tr>
<td>Electrophoretic Parameters</td>
<td>205</td>
</tr>
</tbody>
</table>
Appendix A: Electrophoretic Theory

Voltage, Current and Power: Interactive Effects on Gel Electrophoresis

Introduction

Electrophoresis is defined as the movement of ions and charged macromolecules through a medium when an electric current is applied. Agarose and polyacrylamide are the primary stabilizing media used in the electrophoresis of macromolecules.

Macromolecules are separated through the matrix based on size, charge distribution and structure. Proteins will separate through the matrix based on size, structure and charge. In general, nucleic acids migrate through a gel based on size, with little influence from base composition or sequence.

Two equations are relevant to the use of power supplies for electrophoresis of macromolecules: Ohm’s Law and the Second Law of electrophoresis. These two laws and the interactions of these parameters (watts, volts, current) are critical to understanding electrophoresis.

Ohm’s Law

Current (I) = Voltage (V) / Resistance (R)

Ohm’s Law states that current is directly proportional to the voltage and is inversely proportional to the resistance. Resistance of the system is determined by the buffers used, the type and configurations of the gels being run, and the total volume of all the gels being run.

Second Law

Watts (W) = Current (I) x Voltage (V)

The Second Law states that power or watts (a measure of the heat produced) is equal to the product of the current and voltage. Since V = I x R, this can also be written as Watts = I^2 x R.
During electrophoresis, one of the parameters is held constant and the other two are allowed to vary as the resistance of the electrophoretic system changes. In vertical systems, the resistance of the gel increases as highly conductive ions like Cl are electrophoresed out of the gel. As these ions are removed from the gel, the current is carried by less conductive ions like glycine, borate, acetate, etc. Under normal conditions in horizontal systems, there is little change in resistance. However, with high voltage or extended runs in horizontal systems, resistance can decrease.

**Introduction**

There are advantages and disadvantages for setting each of the critical parameters as the limiting factor in electrophoresis. Sequencing gels are usually run at constant wattage to maintain a uniform temperature. Agarose and acrylamide gels for protein and DNA separation are run at constant voltage or constant current.

**Constant wattage**

In a vertical system when wattage is held constant, the velocity of the samples will decrease because the current, which is in part carried by the DNA, decreases to compensate for the increase in voltage. The generation of heat will remain uniform.

If the current should decrease disproportionately (from a buffer problem, a buffer leak or a hardware problem), the power supply will increase the voltage to compensate.

Since voltage and current vary over time at a constant wattage, it is not possible to predict mobility of samples from the calculation of watt-hours.

**Constant current**

When the current is held constant, the samples will migrate at a constant rate. Voltage and wattage will increase as the resistance increases, resulting in an increase in heat generation during the run.

If a break occurs in the system, such as a damaged lead or electrode or a buffer leak, the resistance of the gel will be greatly increased. This will cause a large increase in wattage and voltage resulting in the generation of excessive heat. It is even possible for the system to get hot enough to boil, or start the apparatus to scorch or burn.

**Constant voltage**

When voltage is set constant, current and wattage will decrease as the resistance increases, resulting in a decrease of heat and DNA migration.

Since the heat generated will decrease, the margin of safety will increase over the length of the run. If a problem develops and the resistance increases dramatically, the current and wattage will fall since the voltage cannot increase. Even if the apparatus fails, the worst that is likely to happen is that the resistance will increase so much that the power supply will not be able to compensate, and it will shut off.

**Reference**

Appendix B: Agarose Physical Chemistry

Agarose is a polysaccharide consisting of 1,3-linked β-D-galactopyranose and 1,4-linked 3,6-anhydro-α-L-galactopyranose [Figure 1]. This basic agarobiose repeat unit forms long chains with an average molecular mass of 120,000 daltons, representing about 400 agarobiose units [1]. There are also charged groups present on the polysaccharide, most notably pyruvate and sulfate. These residues are responsible for many agarose properties, and by careful selection of raw materials, these properties can be controlled to meet specific needs.

![Figure 1: Agarobiose – Basic repeating unit of agarose.](image)

Electroendosmosis (EEO) is a functional measure of the number of these sulfate and pyruvate residues present on the agarose polysaccharide (Figure 2). Electroendosmosis [2,3] is a phenomenon that occurs during electrophoresis when the anticonvective medium (the agarose in this case) has a fixed negative charge. In an electric field, the hydrated positive ions associated with the fixed anionic groups in the agarose gel migrate toward the cathode. Water is thus pulled along with the positive ions, and migration of negative molecules such as DNA is retarded (Figure 3). Electroendosmosis is quantitated by subjecting a mixture of dextran and albumin to electrophoresis, then visualizing them and measuring their respective distances from the origin. The amount of EEO [-mr] is calculated by dividing the migration distance of the neutral dextran (OD) by the sum of the migration distances of the dextran and the albumin (OD + OA): 

\[
\text{EEO} = \frac{\text{OD}}{\text{OD} + \text{OA}} \times \text{mr}
\]

![Figure 3: Illustration of EEO. Anionic groups are fixed to the matrix and cannot move. Corresponding cations can move and sweep toward the cathode with their associated water of hydration.](image)

The mechanism for gelation of agarose was first suggested by Rees [4] and later demonstrated by Arnott, et al. [5]. It involves a shift from a random coil in solution to a double helix in the initial stages of gelation, and then to bundles of double helices in the final stage [Figure 4]. The average pore size varies with concentration and type of agarose, but is typically 100 to 300 nm [6].

![Figure 4: Gelation of agarose by formation of double helices connected in three dimensions by zones of random coil configuration.](image)

One of the most important factors contributing to the success of agarose as an anticonvection medium is its ability to exhibit high gel strength at low concentrations (≤6%). Gel strength is defined as the force, expressed in g/cm³, that must be applied to fracture an agarose gel of a standard concentration. As there are several test methods used to measure gel strength, a direct comparison of gel strength values between different manufacturers is sometimes difficult.

The gel strength of a specific lot of agarose will decrease over time because of the spontaneous hydrolysis of the agarose polysaccharide chains. This loss of gel strength can be particularly noticeable after 5 years from the manufacturing date.

Agents that disrupt hydrogen bond formation (chaotropic agents such as urea and potassium iodide) will decrease the melting temperature, gelling temperature and gel strength of agarose gels, or even inhibit the formation of the gel. Since some electrophoretic procedures call for the incorporation of urea into the gel, it is important to be aware of this effect.
The energy needed to melt an agarose gel increases as the gel concentration increases. The gelling temperature of an agarose gel is also influenced by the gel concentration (Figure 5). For this reason, gelling or remelting temperatures are expressed at a given agarose concentration. This property is of practical value since it is possible to vary gelling and melting parameters by using lower or higher concentrations of agarose. The dependence of gelling and melting temperatures on concentration is most pronounced at concentrations less than 1%.

The agarose polysaccharide also contains uncharged methyl groups. The extent of natural methylation is directly proportional to the gelling temperature. Unexpectedly, synthetically methylated agaroses have lower, rather than higher, gelling temperatures (Figure 6, lower curve), and the degree of synthetic methylation is inversely proportional to the melting temperature. A subsequent study demonstrated that a number of simple derivatives have the same effect as synthetic methylation on gelling temperature (Figure 7). Because of manufacturing concerns, hydroxyethylation is preferred for large scale derivatization of agarose.

The major use of low gelling temperature agarose, such as SeaPlaque® GTG® and NuSieve® GTG® Agarose, is to take advantage of the low melting point rather than the low gelling temperature. Because these agarose types have a melting temperature of ≤65°C, it is possible to remelt the agarose without melting the DNA double helix. Thus, slices of SeaPlaque® GTG® or NuSieve® GTG® Agarose gels containing DNA can be melted and the nucleic acids manipulated directly in the remelted agarose.

References
Appendix C: Safety and Environmental Precautions

In general, working with Nucleic Acids and Proteins does not present significant hazards to humans so long as precautions are taken to protect against certain harmful materials.

Throughout The Sourcebook are references to materials and methods which are hazardous to humans and the environment. Specific hazards and protection steps are summarized here, and it is recommended that trained users follow these precautions when performing the operations outlined in this manual.

These precautions are not a substitute for proper health and safety training, nor are they a substitute for the user institution's standards and procedures, or local governmental requirements. In each case, the user should be aware of and follow local guidelines for handling and disposal of these materials.

Specific Chemical Hazards

Ethidium Bromide

Ethidium bromide (EtBr) is a known mutagen and a suspected carcinogen. Care should be taken to prevent exposure. Recommended personal protective equipment includes: nitrile gloves, lab coats, and safety glasses. Use ethidium bromide solutions in a well ventilated area, and prevent inhalation of vapors. Electrophoresis tanks should be kept covered during electrophoresis of gels containing ethidium bromide. Ethidium bromide powder should be handled in a fume hood.

Decontamination and disposal of ethidium bromide should be done according to local government and institutional regulations.

GelStar® and SYBR® Green Nucleic Acid Gel Stains

GelStar® and SYBR® Green Stains contain a component which can penetrate cells (including skin), and is a potential mutagen. Therefore care should be taken to prevent exposure. Recommended personal protective equipment includes: (non nitrile) gloves, lab coats, and safety glasses.

Decontamination and disposal of these stains should be done according to local government and institutional regulations.

Formaldehyde and Formamide

Formaldehyde and Formamide are known carcinogens, and exposure should be limited to as low as feasible. Gloves, safety glasses (or goggles when pouring liquid), and lab coats should be worn. Operations using formaldehyde or formamide should be conducted with the use of respiratory protection, or in a fume hood or other well ventilated area to prevent inhalation.

Disposal of these materials should be done according to local government and institutional regulations.

DMSO

Dimethyl Sulfoxide (DMSO) can penetrate cells and DNA, and is a carrier of other substances in solutions into those cells. Care should be taken to prevent exposure, including the use of non nitrile gloves (natural rubber are recommended), safety glasses and lab coat.

Disposal of these materials should be done according to local government and institutional regulations.

Phenol

Phenol is toxic by inhalation, ingestion, and contact. It will burn eyes. Appropriate gloves, safety glasses (or goggles), and lab coats are required. Proper ventilation should be utilized.

Disposal of these materials should be done according to local government and institutional regulations.
Indices

Chapter 4
### Numerical Index

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description</th>
<th>Page Number</th>
</tr>
</thead>
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