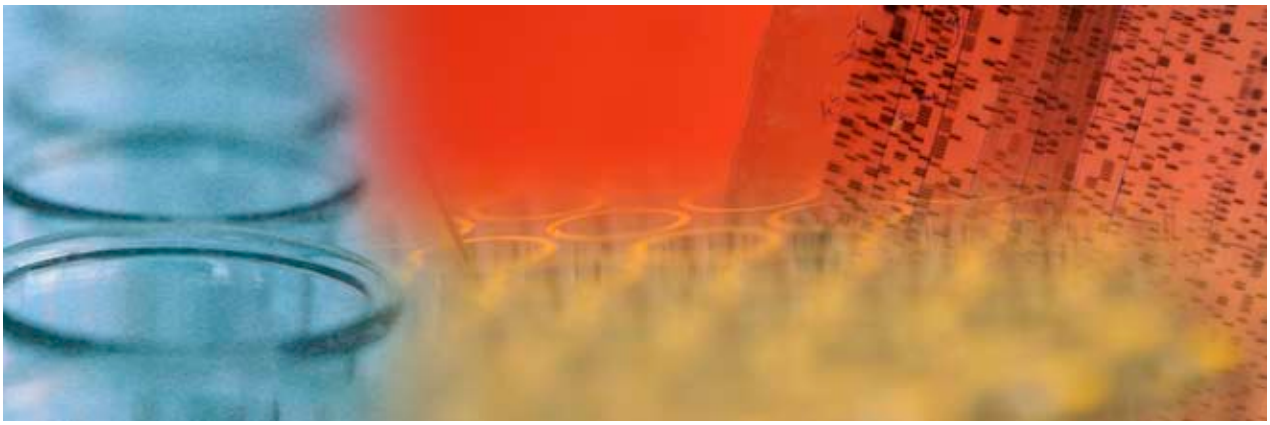

In This Section

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 14 cm × 24 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.

PAGEr® Precast Gels

Q. Which PAGEr® Precast Gels will fit my gel chamber?

A. 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.

Standard Vertical Systems	PAGEr® Gels
PAGEr® Minigel Chamber	9 cm × 10 cm 10 cm × 10 cm gels
Bio-Rad® MiniPROTEAN® II, MiniPROTEAN® 3 or Ready Gel® Cell Systems Reverse the inner core gasket so the flat side faces outward.	9 cm × 10 cm gels
Novex® XCell SureLock® Mini-Cell Request the spacer for the XCell SureLock®, Mini-Cell Chamber from Scientific Support, (Cat. No. 59900).	9 cm × 10 cm 10 cm × 10 cm gels
FisherBiotech® Vertical Minigel FBVE121, Owl Separations Systems Wolverine™ P82 Chamber comes with 2 sets of wedges. Use the thinner wedges for the PAGEr® Gold Gels.	10 cm × 10 cm gels
FisherBiotech® Vertical Minigel FB-VE101, Owl Separations Systems Penguin™ Model P8DS Request adaptor for these chambers from Scientific Support, (Cat. No. 59902).	10 cm × 10 cm gels
Hoefer® Mighty Small (SE250) Replace the buffer chamber with a 'Deep lower buffer chamber for the SE260', order number 80-6148-78, from GE Healthcare.	9 cm × 10 cm 10 cm × 10 cm gels
Daiichi 2, ISS chambers To run one gel: Place one 10 × 10 cm cassette on wedge side of chamber. Use suitable buffer dam on the other side. Use regular Daiichi/ISS wedges. To run two gels: Widen the hole on the yellow port of the inner core. Replace the long arm wedges with modified wedges.This chamber modification and new wedges are available from Scientific Support.	10 cm × 10 cm gels
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
Biometra® Mini V Chamber	9 cm × 10 cm gels
CBS Scientific MGV 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

Q: Do Lonza PAGEr® Precast Gels contain a stacking gel? What is the purpose of the stacking gel?

A. PAGEr® 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 nondenaturing gel. What can I use?

A. PAGEr® 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)

Towbin Transfer Buffer (1X)	Running Buffer (1X)	Sample Buffer (1X)
0.025 M Tris base	25 mM Tris Base	62.5 mM Tris-HCl, pH 6.8
0.192 M Glycine	192 mM Glycine	2% SDS*
0.05 – 0.1% SDS*	0.1% SDS*	10% Glycerol
20% Methanol		0.01% Bromophenol Blue
		2.5% βME (2-mercaptoethanol)*

*Omit for native proteins
For best results use Lonza AccuGENE® Electrophoresis Buffers

Q. What is the difference between gradient vs. homogeneous (single concentration) gels? Which one should I use?

A. Gradient gels are suitable for a wide range of size resolutions. A homogeneous or single concentration gel is appropriate where the proteins of interest are known to be within a narrow size range.

Q. How much protein should I load on the gel?

A. 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.

Protein stain detection limits

Protein Stain	Lower Detection Limit (Protein / Band)
Coomassie® Blue Stain	30 ng
Silver Stain	2 ng
SYPRO® Red Protein Gel Stain	4 ng – 8 ng
SYPRO® Ruby Protein Gel Stain	2 ng – 8 ng
SYPRO® Tangerine Protein Gel Stain	4 ng – 8 ng

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

Q. What is the best membrane to use for Western blotting?

A. Use this table to find a suitable membrane.

Nitrocellulose	PVDF	Nylon
Hydrophobic binding	Hydrophobic binding	Hydrophobic and electrostatic binding
General purpose membrane	SDS tolerant	Stable if baked
Low background	High background	High background
Low strength	High strength	High strength
Becomes brittle if baked	Suitable for protein sequencing	Least suitable for Western transfer

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.

Notes