

Protein Electrophoresis 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 kDa.

Advantages:

- Separate high-molecular-weight proteins (>600 000 kDa)
- 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 Agaroses that are recommended for protein electrophoresis. For performing routine separations, we recommend a standard melting temperature agarose such as SFRlow melting#. When proteins are to be recovered for further analysis, use a low melting temperature agarose.

<u>Protein Size Range</u>	<u>Agarose</u>	<u>Concentration</u>
20 – 200 kDa	Standard Agarose #31272L	5%
150 – 300 kDa	SFR Agarose	3%
300 – 600 kDa	SFR Agarose	2%
600 – 1 ,000 kDa	SFR Agarose (Low Melting Agarose)	1.5%
1 000 – 5 000 kDa	SFR Agarose (Low Melting Agarose)	1.0%

Buffers for Protein Separation in Agarose

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:

1X **STACK BUFFER**, pH 8.0 *g/l for 1X STACK BUFFER*
 125 mM Tris-HCl 19.7 g Tris-HCl in 1 liter distilled water

NOTE: Horizontal gels do not require the use of a stack gel.

1X **RESOLVING BUFFER**, pH 8.5 *g/l for 1X RESOLVING BUFFER*
 500 mM Tris base 60.55 g Tris base
 160 mM Boric acid 9.90 g Boric acid
 1 M Urea 60.06 g Urea
 Adjust volume to 1 liter with distilled water

1X **RUNNING BUFFER**, pH 8.5 *amount for 1X RUNNING BUFFER*
 90 mM Tris base 10.90 g Tris base
 90 mM Boric acid 5.57 g Boric acid
 0.1% SDS 10 ml of 10% SDS
 Adjust volume to 1 liter with distilled water

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Laemmli buffer:

- Gels can be made with standard Laemmli buffer.
- 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.

Casting Agarose Gels for Protein Separation

The procedures for dissolving and casting agarose gels for protein separation are the same as the procedures used for nucleic acid separation.

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 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% low melting temperature 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.
- 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.

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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:

<i>2X concentrate:</i>	<i>Amount to add for 10 ml:</i>
126 mM Tris-HCl, pH 6.8	2.5 ml of 0.5 M Tris-HCl, pH 6.8
15% Ficoll® Type 400	1.5 g Ficoll Type 400
4% SDS	4 ml of 10% SDS
0.002% Bromophenol Blue	0.2 ml of 0.1% Bromophenol blue
	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

Optimal Voltage and Electrophoretic Times

Tips:

- 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 Cooled Protein Markers #[L77151](#) 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.

Gel Type	Horizontal Gel	Vertical Gel	Mini-Vertical
Gel Size	5.3 cm x 8.5 cm x .4 cm	14.5 cm x 16.5 cm x .1 cm	8 cm x 10 cm x .1 cm
Power Setting	100 volts	25 mA (constant) (approximately 80 volts at the start and 120 volts at the end)	20mA (approximately 50 volts at the start and 200 volts at the end)
Time	3 - 4 hours	3 - 3.5 hours	1.5 - 2 hours

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 staining refer to Detection of Proteins on Polyacrylamide Gels (see page 193), using the modifications listed below. For detailed procedures on Silver staining refer to Staining Proteins with Silver Stain (see page 218 in Isoelectric Focusing of Proteins on Agarose Gels).

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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:

COOMASSIE® BLUE STAIN SOLUTION DESTAIN SOLUTION

40% Methanol 20% Methanol

10% Glacial Acetic Acid 5% Glacial Acetic Acid

0.25% Coomassie brilliant blue R-250

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.

Gel Drying and Preservation

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. For detailed procedures on drying agarose gels by one of the three methods listed, refer to Drying Agarose Gels without a Vacuum Gel Dryer or Drying Agarose Gels with a Vacuum Gel Dryer.

Processing Agarose Gels Following Electrophoresis

Autoradiography:

After drying, agarose gels can be exposed directly to X-ray film.

[List of X-Ray products](#) (i.e. [Radiographic Films #67895A](#), [UnDO X-ray #T89171](#)); [ECL HRP Substrates](#) (UptiLight Luminol)

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. 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.

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