Denaturing SDS polyacrylamide gel electrophoresis

In the presence of anionic detergents like sodium dodecyl sulfate (SDS), proteins lose their tertiary structure and acquire a uniform negative charge. Since most secondary structures are also disrupted, the proteins migrate through a gel matrix at a rate proportional to their polypeptide length.

Protein resolution in SDS-PAGE depends on multiple factors, including acrylamide concentration, crosslinker ratio, and the buffer system used. Lower acrylamide or crosslinker concentrations form larger pores that better resolve high molecular weight proteins, while smaller proteins separate better in high-percentage acrylamide gels. To concentrate the sample in a possibly small volume before separation, a stacking gel with larger pores is often poured on top of a finer resolving gel.

Discontinuous buffer systems further enhance resolution by creating sharp voltage gradients. Laemmli's original Tris-HCl/glycine system (Laemmli, 1970), *Alternative A*, remains the most widely used for proteins between 5 kDa and 250 kDa, although the buffer can reach pH values above pH 9.5 during electrophoresis, potentially leading to deamidation or alkylation of the samples. For sensitive applications such as mass spectrometry, Bis-Tris gels with MES or MOPS running buffers (*Alternative B*) provide improved pH stability and are gentler on proteins. These buffers are especially suitable for small and mid-sized proteins, respectively, but tend to perform poorly for proteins above 100 kDa.

Risk assessment

- **Acrylamide is a KNOWN NEUROTOXIN and LIKELY CARCINOGENIC!**
- **Work with high voltage power sources**

▷ Wear gloves, safety glasses, lab coat

☐ Collect acrylamide monomer solutions as HAZARDOUS WASTE

☐ DO NOT wash into sewer

Reviewed: Jul 17, 2024

Procedures

›› *Casting SDS polyacrylamide gels*

☐ Gel casting apparatus
☐ Gel cassette, 0.5–1.5 mm spacer
☐ Gel comb, 12 wells
☐ Isopropyl alcohol

(1.) Prepare resolving and stacking gel solutions in the order of the recipes below, but omit ammonium persulfate and TEMED. Invert the solution several times to mix the ingredients. Avoid foaming!

> ***Hint:*** Gel percentage determines how far and how sharply proteins migrate. There is a reference chart at the end of this protocol for approximate resolution ranges. 12% is a good starting point for most proteins in the 20–100 kDa range.

> ***This is why:*** The crosslinker ratio describes the proportion of acrylamide monomer to the crosslinker bis-acrylamide. A ratio of 37.5:1 (2.6% crosslinker) is the most common choice and suitable for most applications. Lower ratios such as 19:1 (5% crosslinker) produce a tighter mesh that improves resolution of large proteins but makes gels more brittle. Higher ratios such as 100:1 create softer gels with larger pores.

> ***This is why:*** Ammonium persulfate (APS) initiates the polymerization of the acrylamide/bis-acrylamide mixture catalyzed by TEMED. Solutions without APS and TEMED can be stored for several months. If TEMED is present, store the premixed solution preferably at a cold place in the dark.

(2.) *Optional:* Filter the solutions through a 0.45 µm pore.

> ***This is why:*** Filtration removes keratin, a common contaminant in mass spectrometry.

(3.) *Optional:* Degas under vacuum for 15 min at room temperature.

> ***This is why:*** For reproducible polymerization, the dissolved oxygen must be removed.

(4.) Assemble the gel cassette. Mark the front plate about 1 cm below the bottom of the comb to indicate the top of the resolving gel. Remove the comb.

(5.) Add ammonium persulfate and TEMED to the resolving gel solution, invert gently several times to mix. Work quickly since polymerization begins immediately.

> ***Quality assurance:*** TEMED aliquots should be replaced every three months, as they slowly oxidize.

(6.) Pour the resolving gel solution into the assembled gel cassette with the comb removed.

Gel fraction	Spacer	Mini Gel (7.2 cm × 8.6 cm)	Midi Gel (13.3 cm × 8.7 cm)
Resolving gel	1.0 mm	5.0 mL	9.0 mL
Stacking gel	1.0 mm	1.5 mL	2.5 mL

(7.) Gently overlay the resolving gel with a few drops of isopropyl alcohol to create a smooth interface.

(8.) Allow 45–90 min for the gel to polymerize. This ensures reproducible pore size. 60 min

> ***Hint:*** A sharp interface between the gel and the alcohol overlay indicates complete polymerization.

(9.) Pour off the alcohol, rinse with water, dry the area above the resolving gel by touching a strip of filter paper to the edge of the cassette.

(10.) Add ammonium persulfate and TEMED to the stacking gel solution. Invert gently to mix.

(11.) Pour the stacking gel solution in the gel cassette. To avoid spilling, place the pipet tip at an inclined angle and dispense slowly. Fill to the rim before inserting the comb.

(12.) Store gels flat at 4 °C with the comb inserted. Wrap in a wet paper towel and seal in a plastic bag.

> ***Critical:*** Run handcast gels with discontinuous buffer systems right after casting since the discontinuity gradually disappears. Basic Tris-Cl SDS-PAGE gels must be used within a couple of weeks; Bis-Tris gels are stable for up to six months.

›› *Running SDS polyacrylamide gels*

- ☐ Electrophoresis tank
- ☐ Power supply
- ☐ 4 × sample buffer
- ☐ 20% Iodoacetamide, 1 mL

(1.) Remove comb by pulling straight up, slowly and gently. Rinse wells carefully with water.

(2.) Assemble the electrophoresis cell.

(3.) Add fresh running buffer to the reservoir that connects to the stacking gel. Fill the other reservoir with fresh or used running buffer.

> ***Critical:*** Always fill both inner and outer chambers with the recommended volume of running buffer. Incomplete filling may cause uneven band migration or excessive heating.

> ***Hint:*** The stacking gel chamber must always be filled with fresh buffer. The buffer in the other chamber may be reused once or twice, especially for short runs. Avoid reuse if previous samples were high in guanidinium, or reducing agent, or if gels are destined for downstream mass spectrometry. Discoloration of the tracking dye during the run may indicate excessive reuse.

(4.) Prepare samples in 1 × sample buffer. Load 0.1–5 µg of purified protein or up to 25 µg crude extract per lane.

> ***Hint:*** Concentrate samples by lyophilization, spin concentration, gel filtration, or dialysis against polyethylene glycol. To remove potassium, guanidine hydrochloride, or ionic detergents, precipitate with trichloroacetic acid or acetone.

> ***Quality assurance:*** Ensure an excess of SDS; typically 1.5–3.0 µg SDS per microgram protein. Diluted sample buffer provides approximately 10 µg/µL SDS, which is sufficient for most applications up to 5 µg protein per lane.

(5.) Immediately heat to 80–90 °C for 2 min (5 min for pellets) to denature the proteins.

(6.) *Optional:* Bring to 60 °C, add iodoacetamide to 2% (w/v). Incubate for 30 min at room temperature.

> ***This is why:*** Iodoacetamide caps free sulfides, preventing back-folding and protein aggregation, which leads to sharper bands and less artifacts such as double bands, blurred zones, or horizontal lines across the gel from excess reductant.

(7.) Remove insoluble material at 17 000 × *g* for 2 min.

> ***Hint:*** Certain proteins like histones and membrane proteins may not completely dissolve by heating in SDS sample buffer. Add 6–8 M urea or a nonionic detergent such as Triton™ X-100.

> ***Quality assurance:*** Cleared SDS-PAGE samples stored at 4 °C overnight or frozen for longer periods, should be briefly warmed at 37 °C to redissolve SDS. Remove insoluble material by centrifugation.

(8.) Load the appropriate volume of each sample per well. For midi gels, wells typically accommodate 15–40 µL. Use a long pipette tip for loading. Viscous samples often fail to settle into the well and can cause smears.

(9.) Close the lid, connect tank and power supply, perform electrophoresis at constant voltage. 30–60 min

- Run at 5–10 V per centimeter of gel (70–80 V for Mini and Midi gels) for about 10 min until the sample is concentrated at the starting point of the resolving gel.
- Increase the voltage for the resolving phase as recommended. Do not exceed the limits set by the manufacturer for the electrophoresis system.

(10.) Turn the power supply off and disconnect the electrical leads.

(11.) Pop open the gel cassette. Gently float the gel off the plate into a water basin for staining or transfer.

> ***Hint:*** Wet gloves before handling the gel to minimize sticking and tearing. Lift one edge with a dampened plastic wedge.

A › *Tris-Cl/Tris-glycine SDS PAGE (Laemmli)*

- ☐ 0.5 M Tris-Cl, 100 mL ⟨*R*⟩
- ☐ 1.5 M Tris-Cl, 250 mL ⟨*R*⟩
- ☐ 4 × Laemmli sample buffer ⟨*R*⟩
- ☐ 5 × Tris-Gly running buffer, 1.0 L ⟨*R*⟩

(1.) Prepare the gel solutions.

- Combine the ingredients below to prepare 24 mL resolving gel solution sufficient for two Midi gels (9 mL each) or four Mini gels (5 mL each) with 1.0 mm spacer. Select the column matching your desired acrylamide percentage.

  With 30% acrylamide-bis acrylamide solutions (other ingredients as below):

Ingredient	**Stock**	**Final**	**7.0%**	**8.0%**	**10.0%**	**12.0%**	**15.0%**	**16.0%**
Water, reagent-grade			12.2 mL	11.4 mL	9.8 mL	8.2 mL	5.8 mL	5.0 mL
Acrylamide/bis-acrylamide (37.5:1)	30%		5.6 mL	6.4 mL	8.0 mL	9.6 mL	12.0 mL	12.8 mL

  With 40% acrylamide-bis acrylamide solutions:

Ingredient	**Stock**	**Final**	**7.0%**	**8.0%**	**10.0%**	**12.0%**	**15.0%**	**16.0%**
Water, reagent-grade			13.6 mL	13.0 mL	11.8 mL	10.6 mL	8.8 mL	8.2 mL
Acrylamide/bis-acrylamide (37.5:1)	40%		4.2 mL	4.8 mL	6.0 mL	7.2 mL	9.0 mL	9.6 mL
Tris-Cl, pH 8.8	1.5 M	375 mM	6.0 mL	6.0 mL	6.0 mL	6.0 mL	6.0 mL	6.0 mL
SDS	20%	0.1%	120 µL	120 µL	120 µL	120 µL	120 µL	120 µL
TEMED	100%	0.1%	24 µL	24 µL	24 µL	24 µL	24 µL	24 µL
Ammonium persulfate (APS)	10%	0.1%	240 µL	240 µL	240 µL	240 µL	240 µL	240 µL

- For 8 mL stacking gel solution.

  With 30% acrylamide-bis acrylamide solutions (others below):

Ingredient	**Stock**	**Final**	**4.0%**	**5.0%**
Water, reagent-grade			4.8 mL	4.6 mL
Acrylamide/bis-acrylamide (37.5:1)	30%		1.1 mL	1.3 mL

  With 40% acrylamide-bis acrylamide solutions:

Ingredient	**Stock**	**Final**	**4.0%**	**5.0%**
Water, reagent-grade			5.1 mL	4.9 mL
Acrylamide/bis-acrylamide (37.5:1)	40%		0.8 mL	1.0 mL
Tris-Cl, pH 6.8	0.5 M	125 mM	2.0 mL	2.0 mL
SDS	20%	0.1%	40 µL	40 µL
TEMED	100%	0.1%	8 µL	8 µL
Ammonium persulfate (APS)	10%	0.1%	80 µL	80 µL

(2.) Dilute samples with 4 × Laemmli sample buffer.

(3.) Run gels in 1 × Tris-Gly running buffer at 180–200 V for 40–60 min.

B › *Bis-Tris/MES and Bis-Tris/MOPS SDS PAGE*

- ☐ 3.5 × Bis-Tris, 1.0 L ⟨*R*⟩
- ☐ 4 × LDS sample buffer, 10 mL ⟨*R*⟩
- ☐ 20 × MES running buffer, 0.5 L ⟨*R*⟩
- ☐ 20 × MOPS running buffer, 0.5 L ⟨*R*⟩
- ☐ 1 M Bisulfite, 5 mL

(1.) Prepare the gel solutions.

- Combine the ingredients below to prepare 24 mL resolving gel solution sufficient for two Midi gels (9 mL each) or four Mini gels (5 mL each) with 1.0 mm spacer. Select the column matching your desired acrylamide percentage.

  With 30% acrylamide-bis acrylamide solutions (other ingredients as below):

Ingredient	Stock	Final	7.0%	8.0%	10.0%	12.0%	15.0%	16.0%
Water, reagent-grade			11.4 mL	10.6 mL	9.0 mL	7.4 mL	5.0 mL	4.2 mL
Acrylamide/bis-acrylamide (37.5:1)	30%	(*var.*)	5.6 mL	6.4 mL	8.0 mL	9.6 mL	12.0 mL	12.8 mL

  With 40% acrylamide-bis acrylamide solutions:

Ingredient	Stock	Final	7.0%	8.0%	10.0%	12.0%	15.0%	16.0%
Water, reagent-grade			12.8 mL	12.2 mL	11.0 mL	9.8 mL	8.0 mL	7.4 mL
Acrylamide/bis-acrylamide (37.5:1)	40%	(*var.*)	4.2 mL	4.8 mL	6.0 mL	7.2 mL	9.0 mL	9.6 mL
Bis-Tris, pH 6.8	3.5 ×	360 mM	6.9 mL	6.9 mL	6.9 mL	6.9 mL	6.9 mL	6.9 mL
TEMED	100%	0.1%	24 µL	24 µL	24 µL	24 µL	24 µL	24 µL
Ammonium persulfate (APS)	10%	0.06%	144 µL	144 µL	144 µL	144 µL	144 µL	144 µL

- For 8 mL stacking gel solution.

  With 30% acrylamide-bis acrylamide solutions (others below):

Ingredient	Stock	Final	4.0%	5.0%
Water, reagent-grade			4.6 mL	4.4 mL
Acrylamide/bis-acrylamide (37.5:1)	30%	(*var.*)	1.1 mL	1.3 mL

  With 40% acrylamide-bis acrylamide solutions:

Ingredient	Stock	Final	4.0%	5.0%
Water, reagent-grade			4.9 mL	4.7 mL
Acrylamide/bis-acrylamide (37.5:1)	40%	(*var.*)	0.8 mL	1.0 mL
Bis-Tris, pH 6.8	3.5 ×	360 mM	2.3 mL	2.3 mL
TEMED	100%	0.1%	8 µL	8 µL
Ammonium persulfate (APS)	10%	0.06%	48 µL	48 µL

(2.) Dilute samples with 4 × Laemmli sample buffer or 4 × LDS sample buffer.

(3.) Typical run conditions: 30–60 min

- To resolve 5–75 kDa proteins, 1 × MES running buffer, 200 V for 35 min, 4 °C.
- To resolve 50–200 kDa proteins, 1 × MOPS running buffer, 200 V for 50 min, room temperature.

> ***Critical:*** Replace running buffer as soon as the pH indicator in the LDS sample buffer turns yellow.

> ***Critical:*** Reducing agents such as 2-mercaptoethanol (BME) and dithiothreitol (DTT) are not ionized in Bis-Tris and therefore do not enter the gel. To prevent protein reoxidation during electrophoresis, add 5 mM bisulfite (final) to the running buffer.

Troubleshooting

Casting SDS polyacrylamide gels

In Step 8:

- Swirls in gel; disturbed protein separation due to unequal or incomplete polymerization of the gel.
  - Ensure alcohol is poured off within one hour to avoid dehydration.
  - For more consistent results, let the resolving gel polymerize overnight at room temperature. Pour off the alcohol overlay within one hour to prevent dehydration of the gel surface.
  - If polymerization is too fast (less than 10 min), reduce APS and TEMED by 25%.
  - If polymerization is too slow (longer than 120 min), increase APS and TEMED concentration by 50%.

In Step 11:

- Air bubbles trapped in the resolving or stacking gel
  - Pour gel solutions slowly along the glass plate. Tilt the cassette slightly to allow bubbles to escape before the gel sets.
  - If bubbles appear after pouring, dislodge by gently tapping the cassette or inserting a thin needle.
- Poor well formation.
  - Degas the monomer solution to remove dissolved oxygen, which inhibits polymerization.
  - Use fresh catalyst stocks and adjust concentration to 0.12% TEMED and 0.06% APS if necessary.
- Webbing; excess acrylamide behind the comb teeth.
  - Use fresh APS and TEMED or slightly increase their concentration to improve polymerization at the interface.

Running SDS polyacrylamide gels

In Step 3:

- Bands are uneven or wonky.
  - Ensure the gel is covered entirely with buffer during running.
  - Check that the gel cassette is properly seated and there are no leaks between chambers.
  - Use a recirculating cooler or run at lower voltage to reduce heating.

In Step 5:

- Protein degradation during sample preparation.
  - Heat samples immediately after adding SDS sample buffer. SDS easily unfolds most proteins while many proteases remain active unless denatured by heating.
  - For labile proteins, particularly if aspartic acid–proline peptide bonds are present, heat the samples for a shorter time or at a lower temperature such as 75 °C for 10 min.
- Unusual band patterns such as doublets or unexpected fast or slow migration
  - Add urea or nonionic detergent to remove residual secondary structure and complete denaturation

In Step 8:

- Extremely viscous samples due to high DNA or RNA content such as crude cell extracts.
  - Treat samples with Benzonase® Nuclease. This recombinant endonuclease lacks proteolytic activity.
  - Vigorously vortex the heated sample or shear the nucleic acids through sonication to reduce viscosity.

In Step 9:

- Smile effect on bands, distorted lanes, or poor band resolution.
  - Reduce voltage and dissipate heat created during the electrophoresis using a recirculating cooler.
- Frown effect (bands curve downward) at the edges
  - Ensure even gel thickness and uniform polymerization. Check that the gel cassette is level during casting.
  - Verify that the stacking gel has polymerized completely before loading samples.

Recipes

Tris-Cl, pH 6.8, 0.5 M

Amount	Ingredient		Stock	Final
6.0 g	Tris base	[77-86-1]	121.14 g/mol	0.5 M
5 mL	HCl, 37%	[7647-01-0]	11.65 M	
To 100 mL	Water, reagent-grade			

Store at 4 °C.

0.5 M Tris-Cl

pH 6.8

Date: Sign: R0102

Tris-Cl, pH 8.8, 1.5 M

Amount	Ingredient		Stock	Final
45.4 g	Tris base	[77-86-1]	121.14 g/mol	1.5 M
5 mL	HCl, 37%	[7647-01-0]	11.65 M	
To 250 mL	Water, reagent-grade			

Store at 4 °C.

1.5 M Tris-Cl

pH 8.8

Date: Sign: R0103

Laemmli sample buffer, pH 6.8, 4 ×

Amount	Ingredient		Stock	Final
5.0 mL	Tris-Cl, pH 6.8	RA007	0.5 M	250 mM
0.80 g	SDS	[141-21-3]	288.38 g/mol	8%
2.0 mg	Bromophenol blue	[115-39-9]	670.00 g/mol	0.2 g/L
4.0 mL	Glycerol, anhydrous	[56-81-5]	92.09 g/mol	40%
0.8 mL	2-Mercaptoethanol □	[60-24-2]	78.14 g/mol	1.14 M

Add reducing agent freshly; dispense into 920 µL aliquots and make up to 1 mL with 80 µL 2-mercaptoethanol just before use. Alternatively, make up with water and add dithiothreitol to 100 mM when diluting samples. Store at −20 °C. ***Note:*** Bromophenol blue is saturated at 1.0 g/L. ***This is why:*** The high chloride concentration in this sample buffer aids stacking.

4 × Laemmli sample buffer

62.5 mM Tris-Cl, 2% SDS, 0.05 g/L Bromophenol blue, 10% Glycerol, □ 285 mM 2-Mercaptoethanol, pH 6.8 *[At 1 × dilution]*

Date: Sign: R0104

Tris-Gly running buffer, 5 ×

Amount	Ingredient		Stock	Final
15.1 g	Tris base	[77-86-1]	121.14 g/mol	125 mM
72.0 g	Glycine	[56-40-6]	75.07 g/mol	0.96 M
5.0 g	SDS	[141-21-3]	288.38 g/mol	5%
To 1.0 L	Water, reagent-grade			

Expect pH 8.3. Store at room temperature.

5 × Tris-Gly running buffer

25 mM Tris base, 192 mM Glycine, 1% SDS

[At 1 × dilution]

Date: Sign: R0105

Bis-Tris, pH 6.8, 3.5 ×

Amount	Ingredient		Stock	Final
261.6 g	Bis-Tris	[6976-37-0]	209.24 g/mol	1.25 M
40 mL	HCl	[77-86-1]	37%11.65 M	
To 1.0 L	Water, reagent-grade			

Filter sterilize. Store at 4 °C. ***Note:*** This buffer can be prepared at pH 6.5 to aid resolution. Bis-Tris strongly interacts with copper, lead, and other heavy metal cations.

3.5 × Bis-Tris

pH 6.8

Date: Sign: R0106

LDS sample buffer, pH 8.5, 4 ×

Amount	Ingredient		Stock	Final
1.21 g	Tris base	[77-86-1]	121.14 g/mol	1 M
0.80 g	Lithium dodecyl sulfate (LDS) [2044-56-6]		272.40 g/mol	8%
40 µL	EDTA, pH 8.0	R0017	0.5 M	2 mM
0.2 mL	Brilliant Blue G	[6104-58-1]	10 g/L	0.2 g/L
0.2 mL	Phenol red	[143-74-8]	10 g/L	0.2 g/L
4.0 mL	Glycerol, anhydrous	[56-81-5]	92.09 g/mol	40%
To 10 mL	Water, reagent-grade			

Stable for 6 months at room temperature. ***Note:*** 8% LDS can be substituted with up to 4% SDS.. Brilliant Blue G gives a sharper dye front in MES and MOPS running buffers and migrates more closely to the moving ion front than bromophenol blue. Phenol red will turn yellow when the running buffer was poorly made or recycled too many times. Commercialized in XT or NuPAGE® systems.

4 × LDS sample buffer

250 mM Tris base, 2% LDS, 0.5 mM EDTA, 75 mg/L Brilliant Blue G, 50 mg/L Phenol red, 10% Glycerol, pH 8.5 *[At 1 × dilution]*

Expiry: Sign: R0107

MES running buffer, pH 7.3, 20 ×

Amount	Ingredient		Stock	Final
60.6 g	Tris base	[77-86-1]	121.14 g/mol	1 M
97.6 g	2-(*N*-Morpholino)ethanesulfonic acid (MES)	[4432-31-9]	195.24 g/mol	1 M
10.0 g	SDS	[141-21-3]	288.38 g/mol	2%
20 mL	EDTA, pH 8.0	R0017	0.5 M	20 mM
To 0.5 L	Water, reagent-grade			

expect pH 7.3, do not adjust. Stable for 6 months at 4 °C.

20 × MES running buffer

50 mM Tris base, 5 mM MES, 0.1% SDS, 1 mM EDTA, pH 7.3 *[At 1 × dilution]*

Expiry: Sign: R0108

MOPS running buffer, pH 7.7, 20 ×

Amount	Ingredient		Stock	Final
60.6 g	Tris base	[77-86-1]	121.14 g/mol	1 M
104.6 g	4-Morpholinepropanesulfonic acid (MOPS)	[1132-61-2]	209.27 g/mol	1 M
10.0 g	SDS	[141-21-3]	288.38 g/mol	2%
20 mL	EDTA, pH 8.0	R0017	0.5 M	20 mM
To 0.5 L	Water, reagent-grade			

Expect pH 7.7, do not adjust. Stable for 6 months at 4 °C. ***Note:*** Aliquots of 50 mL can be stored at −20 °C.

20 × MOPS running buffer

50 mM Tris base, 5 mM MOPS, 0.1% SDS, 1 mM EDTA, pH 7.7 *[At 1 × dilution]*

Expiry: Sign: R0109

Low molecular weight marker

Amount	Ingredient		Stock	Final
1.25 mL	Sample buffer		4 ×	1 ×
1 mg	Lysozyme, hen egg white	[9001-63-2]	14 kDa	0.2 g/L
1 mg	Trypsin inhibitor, soybean	[9035-81-8]	22 kDa	0.2 g/L
1 mg	Carbonic anhydrase, from bovine erythrocytes	[9001-03-0]	29 kDa	0.2 g/L
2 mg	Peroxidase, horseradish	[9003-99-0]	40 kDa	0.4 g/L
1 mg	Albumin, bovine serum	[9048-46-8]	66 kDa	0.2 g/L
2 mg	Lipoxidase, soybean	[9029-60-1]	96 kDa	0.4 g/L
To 5 mL	Water, reagent-grade			

Dilute each components in 500 µL 1 × sample buffer. Dispense into 20 µL aliquots. Store at −20 °C. ***Hint:*** Apply 20 µL low molecular weight marker (LMW) per lane. ***Quality assurance:*** Check the integrity of each component on a gel before combining.

Low molecular weight marker

1 × Sample buffer, 0.2 g/L Lysozyme, 0.2 g/L Trypsin inhibitor, 0.2 g/L Carbonic anhydrase, 0.4 g/L Peroxidase, 0.2 g/L Albumin, 0.4 g/L Lipoxidase

Date: Sign: R0110

High molecular weight marker

Amount	Ingredient		Stock	Final
1.25 mL	Sample buffer		4 ×	1 ×
2 mg	Peroxidase, horseradish	[9003-99-0]	40 kDa	0.4 g/L
1 mg	Albumin, bovine serum	[9048-46-8]	66 kDa	0.2 g/L
2 mg	Lipoxidase, soybean	[9029-60-1]	96 kDa	0.4 g/L
2 mg	β-Galactosidase, from *E. coli*	[9031-11-2]	116 kDa	0.4 g/L
2 mg	Myosin, heavy chain, from rabbit muscle	[1638095-42-7]	205 kDa	0.4 g/L
To 5 mL	Water, reagent-grade			

Dilute each component in 500 µL 1 × sample buffer. Dispense into 20 µL aliquots. Store at −20 °C. ***Hint:*** Apply 20 µL high molecular weight marker (HMW) per lane. ***Quality assurance:*** Check the integrity of each component on a gel before combining.

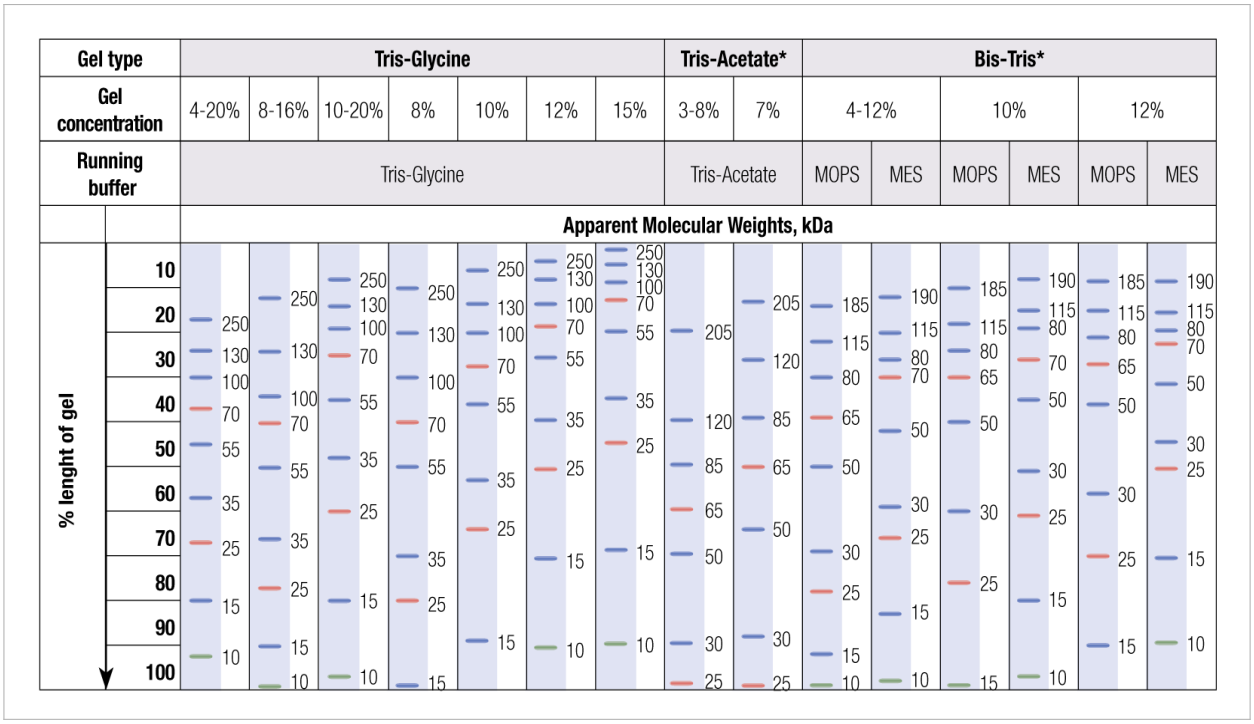
High molecular weight marker

1 × Sample buffer, 0.4 g/L Peroxidase, 0.2 g/L Albumin, 0.4 g/L Lipoxidase, 0.4 g/L β-Galactosidase, 0.4 g/L Myosin

Date: Sign: R0111

Resources

Casting SDS polyacrylamide gels



In Step 1: Migration and resolution of denatured proteins in different buffer systems and varying acrylamide concentrations established by a commercial protein ladder. Prestained proteins can exhibit different mobility than the unstained standard. Reproduced from Thermo Scientific Pub. No. MAN0011773.

List of references

U. Laemmli, *Nature* **227**(5259), 680—685 (1970).

Change log

2022-09-20	Benjamin C. Buchmuller	Adaptation as SOP; added SDS/Laemmli and LDS sample buffer recipes.
2023-01-13	Benjamin C. Buchmuller	Added low and high molecular weight marker recipes from C. R. Hauck lab.
2024-03-11	Benjamin C. Buchmuller	Corrected bromophenol blue content in loading dye concentrate to 0.02% (w/v); literature ranges 0.01–0.50%; less is better.
2024-04-21	Benjamin C. Buchmuller	Corrected diluted molar concentrations of MES and MOPS running buffers to 50 mM; added a section on crosslinker ratios.
2024-07-17	Benjamin C. Buchmuller	Adjusted volumes of resolving gels to accomodate three gels with 1.5 mm spacer or four gels with 1.0 mm.