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 that decreases with their polypeptide length, approximately linearly in the logarithm of the molecular weight.

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. MES running buffer resolves proteins from 2–200 kDa and favors small proteins; MOPS running buffer resolves proteins from 14–200 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: Nov 4, 2026

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. For histones and other proteins below 20 kDa, or to resolve a tag shift of 1–2 kDa, use 15% Tris-glycine or 12% Bis-Tris with MES running buffer and run until the dye front leaves the gel.

> ***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 small proteins and peptides 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. ⊞

> ***Quality assurance:*** 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.

> ***Note:*** 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

> ***Quality assurance:*** 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 months.

›› *Running SDS polyacrylamide gels*

- ☐ Electrophoresis tank
- ☐ Power supply
- ☐ 20% Iodoacetamide, aqueous
- ☐ LMW ◇ R0110
  - *or*: Precision Plus Protein™ Dual Color Standards, Bio-Rad
- ☐ HMW ◇ R0111
  - *or*: HiMark™ Pre-Stained Protein Standard, Invitrogen

(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 20 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 of about 3 g per gram protein. Diluted sample buffer provides 20 µg/µL SDS, enough for the loads above in as little as 5 µL sample.

(5.) Immediately heat to 85 °C for 2–5 min to denature the proteins.

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

> ***Critical:*** Iodoacetamide must exceed the thiol groups of the reductant (one per 2-mercaptoethanol, two per DTT). For this step, reduce with no more than 25 mM DTT instead of 2-mercaptoethanol.

> ***This is why:*** Iodoacetamide caps free thiols, 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, pH 6.8 ⟋ R0102
- ☐ 1.5 M Tris-Cl, pH 8.8 ⟋ R0103
- ☐ 4 × Laemmli sample buffer, pH 6.8 ⟋ R0104
- ☐ 5 × Tris-Gly running buffer ⟋ R0105

(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 the 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.

🔗 [Lae70]

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

- ☐ 3.5 × Bis-Tris, pH 6.8 ◇ R0106
- ☐ 4 × LDS sample buffer, pH 8.5 (at 1 ×) ◇ R0107
- ☐ 20 × MES running buffer, pH 7.3 (at 1 ×) ◇ R0108
- ☐ 20 × MOPS running buffer, pH 7.7 (at 1 ×) ◇ R0109
- ☐ 1 M Sodium bisulfite

(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 the 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.) Run the gel using one of the following conditions: ⏲ 30–60 min

- ○ To resolve 2–200 kDa proteins, particularly small ones, 1 × MES running buffer, 200 V for 35 min.
- ○ To resolve 14–200 kDa proteins, 1 × MOPS running buffer, 200 V for 50 min.

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

Critical: At the neutral pH of Bis-Tris gels, reducing agents such as 2-mercaptoethanol (BME) and dithiothreitol (DTT) remain at the top of the gel and do not migrate with the proteins. To prevent protein reoxidation during electrophoresis, add sodium bisulfite to 2.5 mM to the running buffer of the upper (cathode) chamber only.

🔗 [Inv10]

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% [Bio17].
  - If polymerization is too slow (longer than 120 min), increase APS and TEMED concentration by 50% [Bio17].

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 increase APS and TEMED in the stacking gel by about 20% 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 [Bur09].
  - Peptide bonds, particularly between aspartic acid and proline, cleave when samples are boiled in SDS [Kub95]. For labile proteins, heat the samples at 75 °C for 5 min instead [Bur09; KS12].
- 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.

Materials

Casting SDS polyacrylamide gels

- ☐ **Gel casting apparatus**
  - *e.g.* **Mini-PROTEAN® Tetra Handcast Systems** Bio-Rad
  - *e.g.* **Criterion™ Empty Cassettes for handcasting** Bio-Rad
- ☐ **Gel cassette**, 0.5–1.5 mm spacer
- ☐ **Gel comb**, 12 wells
- ☐ **Isopropyl alcohol**

Running SDS polyacrylamide gels

- ☐ **Electrophoresis tank**
  - *e.g.* **Mini-PROTEAN® Tetra Cell** Bio-Rad
  - *e.g.* **Criterion™ Cell** Bio-Rad
- ☐ **Power supply**
  - *e.g.* **PowerPac™ HC** Bio-Rad
- ☐ **Iodoacetamide**, 20%, aqueous
- ☐ **Molecular weight marker (low)**
  - **Low molecular weight marker (LMW)** ⊘ R0110 Store at −20 °C.
  - *or* **Precision Plus Protein™ Dual Color Standards** Bio-Rad
- ☐ **Molecular weight marker (high)**
  - **High molecular weight marker (HMW)** ⊘ R0111 Store at −20 °C.
  - *or* **HiMark™ Pre-Stained Protein Standard** Invitrogen

Tris-Cl/Tris-glycine SDS PAGE (Laemmli)

- ☐ **Tris hydrochloride (Tris-Cl)**, 0.5 M, pH 6.8 ⊘ R0102 Store at 4 °C.
- ☐ **Tris hydrochloride (Tris-Cl)**, 1.5 M, pH 8.8 ⊘ R0103 Store at 4 °C.
- ☐ **Laemmli sample buffer**, 4 ×, pH 6.8 ⊘ R0104 Store at −20 °C.
- ☐ **Tris-Gly running buffer**, 5 × ⊘ R0105 Store at room temperature.

Bis-Tris/MES and Bis-Tris/MOPS SDS PAGE

- ☐ **Bis-Tris**, 3.5 ×, pH 6.8 ⊘ R0106 Store at 4 °C.
- ☐ **LDS sample buffer**, 4 ×, pH 8.5 (at 1 ×) ⊘ R0107 Stable for 6 months at 4 °C.
- ☐ **MES running buffer**, 20 ×, pH 7.3 (at 1 ×) ⊘ R0108 Stable for 6 months at 4 °C.
- ☐ **MOPS running buffer**, 20 ×, pH 7.7 (at 1 ×) ⊘ R0109 Store at −20 °C. Stable for 6 months at 4 °C.
- ☐ **Sodium bisulfite**, 1 M

Recipes

Low molecular weight marker (LMW)

Amount	Ingredient		Stock	Final
1.25 mL	Sample buffer		4 ×	1 ×
1 mg	Lysozyme, hen egg white	[12650-88-3]	14 kDa	0.2 g/L
	Respiratory sensitization (H334)			
1 mg	Trypsin inhibitor, soybean	[9035-81-8]	22 kDa	0.2 g/L
	Respiratory sensitization (H334)			
1 mg	Carbonic anhydrase, bovine erythrocytes [9001-03-0]		29 kDa	0.2 g/L
	Respiratory sensitization (H334)			
2 mg	Peroxidase, horseradish	[9003-99-0]	40 kDa	0.4 g/L
	Respiratory sensitization (H334)			
1 mg	Albumin, bovine serum	[9048-46-8]	66 kDa	0.2 g/L
1 mg	Lipoxidase, soybean	[9029-60-1]	96 kDa	0.2 g/L
	Respiratory sensitization (H334)			
To 5 mL	Water, reagent-grade			

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

Low molecular weight marker (LMW)

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, 0.2 g/L Lipoxidase

Composition not fully characterised; assess before use.

☐ Hold for EHS review before disposal

Date: Sign: R0110

High molecular weight marker (HMW)

Amount	Ingredient		Stock	Final
1.25 mL	Sample buffer		4 ×	1 ×
2 mg	Peroxidase, horseradish	[9003-99-0]	40 kDa	0.4 g/L
	Respiratory sensitization (H334)			
1 mg	Albumin, bovine serum	[9048-46-8]	66 kDa	0.2 g/L
1 mg	Lipoxidase, soybean	[9029-60-1]	96 kDa	0.2 g/L
	Respiratory sensitization (H334)			
1 mg	β-Galactosidase, *E. coli*	[9031-11-2]	116 kDa	0.2 g/L
1 mg	Myosin, heavy chain, rabbit muscle [1638095-42-7]		205 kDa	0.2 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. ***Note:*** Check the integrity of each component on a gel before combining. Apply 20 µL high molecular weight marker (HMW) per lane.

High molecular weight marker (HMW)

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

Composition not fully characterised; assess before use.

☐ Hold for EHS review before disposal

Date: Sign: R0111

Tris hydrochloride (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
3.5 mL	HCl, 37%	[7647-01-0]	12.0 M	
	Skin corrosion (H314)			
To 100 mL	Water, reagent-grade			

Adjust to pH 6.8 with hydrochloric acid before making up to volume. Store at 4 °C. [Bio24]

0.5 M Tris hydrochloride (Tris-Cl)

pH 6.8

No GHS hazards at the indicated concentrations

☐ Dilute below the discharge limit
☐ Flush to drain with excess water

Date: Sign: R0102

Tris hydrochloride (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
4.5 mL	HCl, 37%	[7647-01-0]	12.0 M	
	Skin corrosion (H314)			
To 250 mL	Water, reagent-grade			

Adjust to pH 8.8 with hydrochloric acid before making up to volume. Store at 4 °C. [Bio24]

1.5 M Tris hydrochloride (Tris-Cl)

pH 8.8



No GHS hazards at the indicated concentrations

- ☐ Dilute below the discharge limit
- ☐ Flush to drain with excess water

Date: Sign: R0103

Laemmli sample buffer, pH 6.8, 4 ×

Amount	Ingredient		Stock	Final
5.0 mL	Tris hydrochloride (Tris-Cl), pH 6.8 R0102		0.5 M	250 mM
0.80 g	SDS	[151-21-3]	288.38 g/mol	8%
	Flammable solid (H228); Serious eye damage (H318)			
2.0 mg	Bromophenol blue	[115-39-9]	670.00 g/mol	0.2 g/L
3.2 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
	Acute dermal toxicity (H310); Serious eye damage (H318); Reproductive toxicity (H361); Organ toxicity, repeated exposure (H373)			
To 10 mL	Water, reagent-grade			

Make up to 9.2 mL with water, dispense into 920 µL aliquots, and complete each to 1 mL with 80 µL 2-mercaptoethanol. Add 2-mercaptoethanol immediately before use. Store at −20 °C. ***Note:*** The high chloride concentration in this sample buffer aids stacking. ***Hint:*** Alternatively, make up with water and add dithiothreitol to 100 mM when diluting samples.

4 × Laemmli sample buffer

63 mM Tris-Cl, 2% SDS, 50 mg/L Bromophenol blue, 10% Glycerol, ☐ 285 mM 2-Mercaptoethanol, pH 6.8



DANGER

Serious eye damage; Skin sensitization; Acute dermal toxicity; Organ damage through prolonged or repeated exposure; Reproductive toxicity; Acute oral toxicity; Aquatic toxicity (long-term)

- ☐ Collect as HAZARDOUS WASTE
- ☐ DO NOT wash into sewer

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	[151-21-3]	288.38 g/mol	0.5%
	Flammable solid (H228); Serious eye damage (H318)			
To 1.0 L	Water, reagent-grade			

Expect pH 8.3. Store at room temperature. ***Critical:*** Do not adjust even if above pH 9.0 since glycine (rather than chloride) must remain the trailing ion in the buffer! [Bio24]

5 × Tris-Gly running buffer

25 mM Tris base, 192 mM Glycine, 0.1% SDS

No GHS hazards at the indicated concentrations

- ☐ Dilute below the discharge limit
- ☐ Flush to drain with excess water

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
	Serious eye damage (H318)			
30 mL	HCl, 37 percent	[7647-01-0]	12.0 M	
	Skin corrosion (H314)			
To 1.0 L	Water, reagent-grade			

Adjust to pH 6.8 with hydrochloric acid before making up to volume; 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



DANGER



Serious eye damage

- ☐ Hold for EHS review before disposal

Date: Sign: R0106

LDS sample buffer, pH 8.5 (at 1×), 4×

Amount	Ingredient		Stock	Final
0.666 g	Tris hydrochloride	[1185-53-1]	157.59 g/mol	423 mM
0.682 g	Tris base	[77-86-1]	121.14 g/mol	563 mM
0.80 g	Lithium dodecyl sulfate (LDS) [2044-56-6]		272.40 g/mol	8%
	Flammable solid (H228); Serious eye damage (H318)			
40 µL	EDTA, pH 8.0	R0017	0.5 M	2 mM
0.75 mL	Brilliant Blue G	[6104-58-1]	10 g/L	0.75 g/L
0.25 mL	Phenol red	[143-74-8]	10 g/L	0.25 g/L
3.2 mL	Glycerol, anhydrous	[56-81-5]	92.09 g/mol	40%
To 10 mL	Water, reagent-grade			

Do not adjust the pH. Stable for 6 months at 4 °C. ***Note:*** 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.

[Inv10]

4 × LDS sample buffer

106 mM Tris hydrochloride, 141 mM Tris base, 2% LDS, 500 µM EDTA, 0.19 g/L Brilliant Blue G, 63 mg/L Phenol red, 10% Glycerol, pH 8.5 (at 1 ×)



DANGER



Serious eye damage

- ☐ Collect as HAZARDOUS WASTE
- ☐ DO NOT wash into sewer

Date: Sign: R0107

MES running buffer, pH 7.3 (at 1×), 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	[151-21-3]	288.38 g/mol	2%
	Flammable solid (H228); Serious eye damage (H318)			
20 mL	EDTA, pH 8.0	R0017	0.5 M	20 mM
To 0.5 L	Water, reagent-grade			

Do not adjust the pH. Stable for 6 months at 4 °C. [Inv10]

20 × MES running buffer

50 mM Tris base, 50 mM MES, 0.1% SDS, 1 mM EDTA, pH 7.3 (at 1 ×)



WARNING



Skin and serious eye irritation; Respiratory irritation

- ☐ Dilute below the discharge limit
- ☐ Flush to drain with excess water

Date: Sign: R0108

MOPS running buffer, pH 7.7 (at 1×), 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	[151-21-3]	288.38 g/mol	2%
	Flammable solid (H228); Serious eye damage (H318)			
20 mL	EDTA, pH 8.0	R0017	0.5 M	20 mM
To 0.5 L	Water, reagent-grade			

Do not adjust the pH. Dispense into 50 mL aliquots. Store at −20 °C. Stable for 6 months at 4 °C.

[Inv10]

20 × MOPS running buffer

50 mM Tris base, 50 mM MOPS, 0.1% SDS, 1 mM EDTA, pH 7.7 (at 1 ×)



WARNING



Skin and serious eye irritation; Respiratory irritation

- ☐ Dilute below the discharge limit
- ☐ Flush to drain with excess water

Date: Sign: R0109

Sodium bisulfite, 1 M

Amount	Ingredient		Stock	Final
10.4 g	Sodium bisulfite	[7631-90-5]	104.06 g/mol	1 M
To 100 mL	Water, reagent-grade			

Prepare freshly before use.

1 M Sodium bisulfite



EPA Safer Choice low-concern

☐ Hold for EHS review before disposal

Date: Sign:

List of references

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Change log

2024-07-17 **Benjamin C. Buchmuller** Version 1, 2022 to 2024. Tris-glycine on the Mini-PROTEAN after the Bio-Rad Mini-PROTEAN and Criterion cell manuals (Bulletins 1658100 and 6040); the Bis-Tris route with MES and MOPS running buffers from the Tom Muir lab "Pouring and running SDS-PAGE gels"; the molecular-weight marker recipes from the C. R. Hauck lab "SDS-PAGE gel recipes". Adapted as an SOP in 2022.

2026-11-04 **Benjamin C. Buchmuller** Version 2. Credited the Invitrogen NuPAGE Technical Guide (IM-1001), the Bio-Rad Mini-PROTEAN Tetra Cell manual, and the Daniel Summerer lab "Glycine-SDS gel electrophoresis"; corrected the sample buffers, SDS in the running buffer, and heating; dropped unsourced claims. The three Tris gel buffers are titrated to their stated pH.

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