

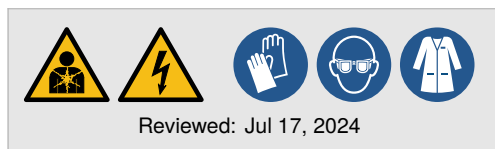
Denaturing SDS polyacrylamide gel electrophoresis

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.

This is a bench card. Full protocol available online.



Procedures

>> Casting SDS polyacrylamide gels

- Gel casting apparatus
- Gel comb, 12 wells
- Gel cassette, 0.5–1.5 mm spacer
- 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!
- (2.) *Optional:* Filter the solutions through a 0.45 μm pore.
- (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.
- (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.

Quality assurance: A sharp interface between the gel and the alcohol overlay indicates complete polymerization.

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- (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-Gly 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

- | | |
|---|--|
| <input type="checkbox"/> Electrophoresis tank | <input type="checkbox"/> 4 × sample buffer |
| <input type="checkbox"/> Power supply | <input type="checkbox"/> 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. ←

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

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

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. 📖

Denaturing SDS polyacrylamide gel electrophoresis

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

<input type="checkbox"/> R0102 0.5 M Tris-Cl, 100 mL	<input type="checkbox"/> R0104 4 × Laemmli sample buffer
<input type="checkbox"/> R0103 1.5 M Tris-Cl, 250 mL	<input type="checkbox"/> R0105 5 × Tris-Gly running buffer, 1.0 L

(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.

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

<input type="checkbox"/> <input checked="" type="checkbox"/> R0106 3.5 × Bis-Tris, 1.0 L	<input type="checkbox"/> <input checked="" type="checkbox"/> R0108 20 × MES running buffer, 0.5 L	<input type="checkbox"/> 1 M Bisulfite, 5 mL
<input type="checkbox"/> <input checked="" type="checkbox"/> R0107 4 × LDS sample buffer, 10 mL	<input type="checkbox"/> <input checked="" type="checkbox"/> R0109 20 × MOPS running buffer, 0.5 L	

(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:

- 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.

⌚ 30–60 min

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List of references

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

[🔗 Recipe \(available online\)](#) [📄 Resources \(available online\)](#) [🔧 Troubleshooting \(available online\)](#) [📖 Notes \(available online\)](#)

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