Native polyacrylamide gel electrophoresis

Reviewed: Mar 30, 2025

Unlike SDS-PAGE, which denatures proteins by disrupting non-covalent interactions, native PAGE preserves protein–protein or protein–nucleic acid interactions, allowing proteins to migrate in their folded state, and to retain their quaternary structure and functional interactions. These interactions often alter the electrophoretic mobility of protein complexes, resulting in shifted bands that reflect their altered charge or mass-to-charge ratio. The complexes are therefore not separated purely by molecular weight; rather, the direction and magnitude of the shift depend on the specific properties of the complex.
The choice of the running buffer significantly impacts migration patterns and resolution. TBE is commonly used for small protein–nucleic acid complexes due to its high buffering capacity, whereas TAE or Tris-Gly buffers are preferred for larger complexes. Very large complexes may migrate inefficiently, requiring extended run times or specialized buffer conditions.
The gels are typically prepared in 0.25 ×, 0.5 ×, or 1 × TBE (pH 7.5), TAE (pH 8.0) or Tris-Gly (acc. Towbin) and are resolved slowly at 1–8 V/cm to prevent denaturation of the complexes.

This is a bench card. Full protocol available online.

Procedures

Choosing a native PAGE system

(1.) Starting conditions for further optimization:

Protein complex	Buffer system	Gel composition		Run conditions		
		Ratio	Percent	Field	Temperature	Runtime
Protein–nucleic acid complexes	0.25–0.5 × TBE	19:1 or 29:1	5–8%	4–6 V/cm	4 °C	2–5 h
Nucleosomes, 1- to 4-mer	0.5 × TBE	37.5:1	5%	15 V/cm	25 °C	0.5–1.5 h
TALE nucleases	0.5 × TAE	29:1	6–10%	8 V/cm	25 °C	1–2 h
Methyl-CpG-binding domains	0.25 × TBE	29:1	12%	20 V/cm	4 °C	3 h
Protein complexes, 50–500 kDa	1 × Tris-Gly	37.5:1	6–10%	6–8 V/cm	25 °C	2–4 h
Protein complexes, larger than 500 kDa	0.5 × TBE	19:1	3–6%	1–4 V/cm	4 °C	4–12 h
Membrane protein complexes	1 × Tris-Gly	37.5:1	6–10%	3–5 V/cm	4 °C	4–8 h

›› *Casting native polyacrylamide gels*

- ☐ Gel casting apparatus
- ☐ Gel cassette, 0.5–1.5 mm spacer
- ☐ Gel comb, 15 wells
- ☐ Isopropyl alcohol

(1.) Prepare resolving and stacking gel solutions, but omit ammonium persulfate and TEMED. Invert the solution several times to mix the ingredients.

- These 24 mL resolving gel solution serve two Midi gels (9 mL each) or four Mini gels (5 mL each) with 1.0 mm spacer. Combine according to the desired acrylamide percentage.

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

Ingredient	Stock	Final	5.0%	7.0%	8.0%	10.0%	12.0%	15.0%
Water, reagent-grade			18.8 mL	17.2 mL	16.4 mL	14.8 mL	13.2 mL	10.8 mL
Acrylamide/bis-acrylamide (37.5:1)	30%		4.0 mL	5.6 mL	6.4 mL	8.0 mL	9.6 mL	12.0 mL

With 40% acrylamide-bis acrylamide solutions:

Ingredient	Stock	Final	5.0%	7.0%	8.0%	10.0%	12.0%	15.0%
Water, reagent-grade			19.8 mL	18.6 mL	18.0 mL	16.8 mL	15.6 mL	13.8 mL
Acrylamide/bis-acrylamide (37.5:1)	40%		3.0 mL	4.2 mL	4.8 mL	6.0 mL	7.2 mL	9.0 mL
TBE, pH 7.5	10 ×	0.5 ×	1.2 mL	1.2 mL	1.2 mL	1.2 mL	1.2 mL	1.2 mL
TEMED	100%	0.03%	7 µL	7 µL	7 µL	7 µL	7 µL	7 µL
Ammonium persulfate (APS)	10%	0.04%	108 µL	108 µL	108 µL	108 µL	108 µL	108 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			6.2 mL	5.9 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			6.4 mL	6.2 mL
Acrylamide/bis-acrylamide (37.5:1)	40%		0.8 mL	1.0 mL
TBE, pH 7.5	10 ×	1 ×	0.8 mL	0.8 mL
TEMED	100%	0.08%	6 µL	6 µL
Ammonium persulfate (APS)	10%	0.05%	40 µL	40 µL

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

> ***Quality assurance:*** For reproducible polymerization, the dissolved oxygen must be removed. In this case, polymerization should be allowed to proceed for at least 120 min.

(3.) 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.

(4.) Add ammonium persulfate and TEMED to the resolving gel solution, invert.

(5.) 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

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

(7.) Allow 60–120 min for the gel to polymerize. This ensures reproducible pore size. 90 min

(8.) Pour off the alcohol, rinse with water, dry the area above the resolving gel with a filter paper.

(9.) Add ammonium persulfate and TEMED to the stacking gel solution, invert.

(10.) 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.

(11.) Store gels flat at 4 °C with the comb inserted for up to three months. Wrap in a wet paper towel and seal in a plastic bag.

›› *Loading and running of native TBE acrylamide gels*

- ☐ Electrophoresis tank
- ☐ Power supply
- ☐ R0052 10 × Tris-borate-EDTA
- ☐ R0066 2.5 M Sucrose

(1.) Assemble the electrophoresis cell.

(2.) Place the gel in the electrophoresis tank, fill with TBE buffer from the same batch used for casting. Remove air bubbles trapped beneath the gel with a bent Pasteur pipette or syringe needle.

> ***Critical:*** Always fill the reservoirs with the recommended amount of running buffer to prevent excessive heating.

(3.) Remove comb by pulling straight up, slowly and gently. Flush out the wells with running buffer.

(4.) Pre-run the gels at 4–6 V/cm for 20–30 min before loading the samples. 30 min

(5.) Prepare samples in 1 × sample buffer containing 10–15% sucrose and load into the wells.

> ***Critical:*** Do not attempt to expel all of the sample from the loading device and complete loading quickly.

(6.) Connect the electrodes to the power device. Turn on the power and begin the electrophoresis run. 60 min

- For the first 10 min allow the samples to stack using a reduced field strength of 5–10 V/cm gel length, typically 40–50 V.
- Continue at the recommended voltage until separation is complete.

> ***Critical:*** Non-denaturing polyacrylamide gels are usually run at voltages between 1–8 V/cm gel length. If electrophoresis is carried out at higher voltage, differential heating in the center of the gel may cause bowing of bands or even melting of the protein and DNA complexes. In this case, run gels at 4 °C.

Recipe (available online) Troubleshooting (available online) Notes (available online)