Native polyacrylamide gel electrophoresis

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.

Risk assessment

- **Acrylamide is a KNOWN NEUROTOXIN and LIKELY CARCINOGENIC!**
- **Boric acid may damage fertility or the unborn child**
- **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: Mar 30, 2025

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

Hint: Higher acrylamide percentages or different acrylamide-bis acrylamide ratios such as 19:1 or 29:1 alter the mobility of DNA, proteins, and dyes. Some investigators prefer to run native TBE acrylamide gels generally at 1 × TBE. In this case, the voltage must typically be reduced. Adjust the following recipes accordingly.

Note: If used, the stacking step is typically run at 40–50 V for the first 10–20 min before increasing the resolving voltage.

Hint: For membrane-associated proteins, mild detergents (such as 0.05% Triton™ X-100) may be included to maintain solubility while preserving interactions. However, excessive detergent concentrations can alter migration patterns by disrupting charge-based mobility shifts.

›› *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

> ***This is why:*** Ammonium persulfate initiates the polymerization of the acrylamide/bis-acrylamide mixture catalyzed by TEMED. Solutions without APS or TEMED can be kept refrigerated and be used later the same day.

> ***This is why:*** Polymerization speed depends on monomer and catalyst concentrations. Rapid polymerization may lead to nonuniform pore structure due to excessive heat. For native PAGE, TEMED concentration is thus below 0.35 µL/mL gel solution.

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

> ***This is why:*** 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.

> ***Note:*** Replace TEMED aliquots every three months, they slowly oxidize.

(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.

> ***Hint:*** In high-percentage acrylamide gels (greater than 10% acrylamide) or when sample volumes are low, the resolving gel can provide sufficient focusing such that a stacking gel can be omitted.

(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
- ☐ 10 × Tris-borate-EDTA
- ☐ 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

> ***This is why:*** This helps to stabilize the buffer system in low-ionic strength buffers and removes residual unpolymerized acrylamide and excess APS and TEMED which can interfere with migration. Thicker gels (1.5 mm) may benefit from a longer prerun to fully equilibrate buffer ions.

> ***Hint:*** Pre-running is not necessary for Tris-Gly buffers or high-ionic strength buffers such as 1 × TBE.

(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.

> ***Hint:*** The detection limit per protein band is about 40 ng for a Coomassie Brilliant Blue R-250 stain or 0.5–1.0 ng for fluorescent dyes. For DNA, at least 1.0 ng is needed per band when stained with ethidium bromide.

(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.

> ***Note:*** Since TAE does not provide as much buffering capacity as TBE, TAE gels must be run more slowly.

Troubleshooting

Casting native polyacrylamide gels

In Step 7:

- 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.
  - Let the resolving gel polymerize overnight at room temperature; pour off alcohol latest after one hour to avoid dehydration.
  - Let the gel polymerize overnight at room temperature for more consistent results.
  - 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%.
- Gel is brittle or too soft.
  - Cross-linker (bis-acrylamide) content is too high or too low. Get a new batch of acrylamide-bis acrylamide solution.

In Step 10:

- Poor well formation.
  - Degas the monomer solution to remove dissolved oxygen, which inhibits polymerization.
  - Use fresh catalyst stocks and adjust concentration to 0.06% TEMED and 0.08% 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.

Loading and running of native TBE acrylamide gels

In Step 2:

- Bands are uneven or wonky.
  - Ensure the gel is covered entirely with buffer while it is setting.
  - Pre-chill the running buffer to 4 °C or run in a cold room to prevent overheating in long runs.

Change log

2022-07-23	Benjamin C. Buchmuller	Initial version.
2025-03-18	Benjamin C. Buchmuller	Revised and expanded.
2025-03-30	Jeremy A. Owen	Optimized APS and TEMED concentration for stacking layer.