

DNA Polyacrylamide Gel Electrophoresis

How to pour and run a neutral polyacrylamide gel.

Buffers and Solutions

Acrylamide:bisacrylamide (29:1) (30% w/v)

Ammonium persulfate (10% w/v)

Ammonium persulfate is used as a catalyst for the copolymerization of acrylamide and bisacrylamide gels. The polymerization reaction is driven by free radicals that are generated by an oxido-reduction reaction in which a diamine (e.g., TEMED) is used as the adjunct catalyst.

5x TBE electrophoresis buffer

Polyacrylamide gels are poured and run in 0.5x or 1x TBE at low voltage (1-8 V/cm) to prevent denaturation of small fragments of DNA by heating. Other electrophoresis buffers such as 1x TAE can be used, but they are not as good as TBE. The gel must be run more slowly in 1x TAE, which does not provide as much buffering capacity as TBE. For electrophoresis runs greater than 8 hours, we recommend that 1x TBE buffer be used to ensure that adequate buffering capacity is available throughout the run.

1. Clean the glass plates and spacers thoroughly. Hold the plates by the edges or wear gloves, so that oils from the hands do not become deposited on the working surfaces of the plates. Rinse the plates with deionized water and ethanol and set them aside to dry.

The glass plates must be free of grease spots to prevent air bubbles from forming in the gel.

2. Assemble the glass plates with spacers in gel caster.
3. Prepare the gel solution with the desired polyacrylamide percentage according to the table below, which gives the amount of each component required to make 12 ml (sufficient for 2 Hoefer minigels of 1 mm thickness):

Volume of Reagents Used to Cast Polyacrylamide Gels

<u>Gel %</u>	<u>30% Acrylamide (29:1)</u>	<u>H₂O (ml)</u>	<u>5x TBE (ml)</u>	<u>10% APS (μl)</u>	<u>TEMED (μl)</u>
8 %	3.2 ml	6.4	2.4	200	10
10%	4.0 ml	5.6	2.4	200	10
12%	4.8 ml	4.8	2.4	200	10

Some investigators prefer to run acrylamide gels in 0.5x TBE. In this case, adjust the volumes of 5x TBE and H₂O accordingly.

Stock solutions other than 29:1 (% w/v) acrylamide:bisacrylamide can be used to cast polyacrylamide gels. However, it is then necessary to recalculate the appropriate amount of stock solution to use. Gels can be cast with acrylamide solutions containing different acrylamide:bisacrylamide (cross-link) ratios, such as 19:1 and 37.5:1, in place of the 29:1 ratio recommended here. The mobility of DNA and dyes in such gels will be different from those given in this protocol.

4. Wear gloves. Work quickly after addition of TEMED to complete the gel before the acrylamide polymerizes.
5. Immediately insert the appropriate comb into the gel, being careful not to allow air bubbles to become trapped under the teeth. The tops of the teeth should be slightly higher than the top of the glass. Clamp the comb in place with bulldog paper clips. If necessary, use the remaining acrylamide gel solution to fill the gel mold completely. Make sure that no acrylamide solution is leaking from the gel mold.
6. Allow the acrylamide to polymerize for 30-60 minutes at room temperature.
7. After polymerization is complete, surround the comb and the top of the gel with paper towels that have been soaked in 1x TBE. Then seal the entire gel in Saran Wrap or plastic bag and store it at 4°C until needed.
8. When ready to proceed with electrophoresis, remove gels from gel caster, carefully clean spilled gel from back of white plates and insert gels into Hoefer gelbox. Add running buffer and carefully pull the combs from the polymerized gel.

It is important to use the same batch of electrophoresis buffer in both of the reservoirs and in the gel. Small differences in ionic strength or pH produce buffer fronts that can greatly distort the migration of DNA.

9. Use a Pasteur pipette or a syringe to flush out the wells once more with 1x TBE. Mix the DNA samples with the appropriate amount of gel-loading buffer. Load the mixture into the wells using a micropipette equipped with a drawn-out plastic tip.

Do not attempt to expel all of the sample from the loading device, as this almost always produces air bubbles that blow the sample out of the well. In many cases, the same device can be used to load many samples, provided it is thoroughly washed between each loading. However, it is important not to take too long to complete loading the gel; otherwise, the samples will diffuse from the wells.

10. Connect the electrodes to a power pack, turn on the power, and begin the electrophoresis run.

Nondenaturing polyacrylamide gels are usually run at voltages between 1 V/cm and 8 V/cm. If electrophoresis is carried out at a higher voltage, differential heating in the center of the gel may cause bowing of the DNA bands or even melting of the strands of small DNA fragments.

11. Run the gel until the marker dyes have migrated the desired distance. Turn off the electric power, disconnect the leads, and discard the electrophoresis buffer from the reservoirs.
12. Detach the glass plates. Lay the glass plates on the bench. Use a spacer or plastic wedge to lift a corner of the upper glass plate. Check that the gel remains attached to the lower (white) plate. Pull the upper plate smoothly away. Remove the spacers.
13. Stain gels with EtBr or SYBR gold or dry gels and expose to film or PhosphorImager screen.