Neutral Polyacrylamide Gel Electrophoresis

Joseph Sambrook and David W. Russell

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INTRODUCTION

How to pour and run a neutral polyacrylamide gel.

MATERIALS

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 Joulic heating. Other electrophoresis buffers such as 1x TAE (please see Agarose Gel Electrophoresis) 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.

6x Gel-loading buffer

Acrylamide:bisacrylamide (29:1) (% 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.

DNA samples

Ethanol

Optional, please see Step 5.

KOH/methanol solution

Siliconizing fluid (e.g., Sigmacote or Acrylease) (optional)

TEMED
METHOD

1. If necessary, clean the glass plates and spacers with KOH/methanol.

2. Wash the glass plates and spacers in warm detergent solution and rinse them well, first in tap water and then in deionized H₂O. 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 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.

3. (Optional) Treat one surface of one of the two plates with siliconizing fluid (e.g., Sigmacote or Acrylease): Place the glass on a pad of paper in a chemical fume hood and pour a small quantity of siliconizing fluid onto the surface. Wipe the fluid over the surface of the plate with a pad of Kimwipes, and then rinse the plate in deionized H₂O. Dry the plate with paper towels.

This treatment prevents the gel from sticking tightly to one plate and reduces the possibility that the gel will tear when the mold is dismantled after electrophoresis.

4. Assemble the glass plates with spacers:
   Lay the larger (or unnotched) plate flat on the bench and arrange the spacers at each side parallel to the two edges.

   Apply minute dabs of petroleum jelly to keep the spacer bars in position during the next steps.

   Lay the inner (notched) plate in position, resting on the spacer bars.

   Clamp the plates together with binder or "bulldog" paper clips and bind the entire length of the two sides and the bottom of the plates with gel-sealing tape to make a watertight seal.

   Take particular care with the bottom corners of the plates, as these are the places where leaks often occur. An extra band of tape around the bottom of the plates can help to prevent leaks.

   There are many types of electrophoresis apparatuses available commercially, and the arrangement of the glass plates and spacers differs slightly from manufacturer to manufacturer. Whatever the design, the aim is to form a watertight seal between the plates and the spacers so that the unpolymerized gel solution does not leak out. Several manufacturers also sell precast polyacrylamide gels, which are foolproof but expensive and often can be used only in the manufacturer's gel apparatus.

5. Taking into account the size of the glass plates and the thickness of the spacers, calculate the volume of gel required. Prepare the gel solution with the desired polyacrylamide percentage according to the table below, which gives the amount of each component required to make 100 ml.

Volume of Reagents Used to Cast Polyacrylamide Gels
Volumes of Reagents to Cast Polyacrylamide Gels of Indicated Concentrations in 1x TBEₐ
<table>
<thead>
<tr>
<th>Polyacrylamide Gel (%)</th>
<th>29% Acrylamide plus 1% N,N'-Methylenebisacrylamide&lt;sup&gt;b&lt;/sup&gt; (ml)</th>
<th>H&lt;sub&gt;2&lt;/sub&gt;O (ml)</th>
<th>5x TBE (ml)</th>
<th>10% Ammonium Persulfate (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5</td>
<td>11.6</td>
<td>67.7</td>
<td>20.0</td>
<td>0.7</td>
</tr>
<tr>
<td>5.0</td>
<td>16.6</td>
<td>62.7</td>
<td>20.0</td>
<td>0.7</td>
</tr>
<tr>
<td>8.0</td>
<td>26.6</td>
<td>52.7</td>
<td>20.0</td>
<td>0.7</td>
</tr>
<tr>
<td>12.0</td>
<td>40.0</td>
<td>39.3</td>
<td>20.0</td>
<td>0.7</td>
</tr>
<tr>
<td>20.0</td>
<td>66.6</td>
<td>12.7</td>
<td>20.0</td>
<td>0.7</td>
</tr>
</tbody>
</table>

<sup>a</sup>Some investigators prefer to run acrylamide gels in 0.5x TBE. In this case, adjust the volumes of 5x TBE and H<sub>2</sub>O accordingly.

<sup>b</sup>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.

6. (Optional) Place the required quantity of acrylamide:bis solution in a clean sidearm flask with a magnetic stir bar. De-aerate the solution by applying vacuum, gently at first. Swirl the flask during de-aeration until no more air bubbles are released.

*De-aeration of the acrylamide solution is not essential, but it does reduce the chance that air bubbles will form when thick gels (>1 mm) are poured, as well as reduce the amount of time required for polymerization.*

7. Perform the following manipulations over a tray so that any spilled acrylamide:bis solution will not spread over the bench. Wear gloves. Work quickly to complete the gel before the acrylamide polymerizes.

Add 35 µl of TEMED for each 100 ml of acrylamide:bis solution, and mix the solution by gentle swirling.

*Gels can be cast with as much as 1 µl of TEMED per milliliter of gel solution to increase the rate of polymerization.*

Draw the solution into the barrel of a 50-cc syringe. Invert the syringe and expel any air that has entered the barrel. Introduce the nozzle of the syringe into the space between the two glass plates. Expel the acrylamide gel solution from the syringe, filling the space almost to the top.

*Keep the remaining acrylamide solution at 4°C to reduce the rate of polymerization. If the plates have been well cleaned and well sealed, there should be no trapped air bubbles and...*
no leaks. If air bubbles form, they can sometimes be coaxed to the top of the mold by gentle tapping or may be snagged with a bubble hook made of thin polypropylene tubing. If these methods fail, empty the gel mold, thoroughly clean the glass plates, and pour a new gel.

Place the glass plates against a test tube rack at an angle of approx. 10° to the bench top.

8. 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.

9. Allow the acrylamide to polymerize for 30-60 minutes at room temperature, adding more acrylamide:bis gel solution if the gel retracts significantly.

10. 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 and store it at 4°C until needed.

11. When ready to proceed with electrophoresis, squirt 1x TBE buffer around and on top of the comb and carefully pull the comb from the polymerized gel. Use a syringe to rinse out the wells with 1x TBE. Remove the gel-sealing tape from the bottom of the gel with a razor blade or scalpel.

12. Attach the gel to the electrophoresis tank, using large bulldog clips on the sides or clamps built into the apparatus. The notched plate should face inward toward the buffer reservoir.

13. Fill the reservoirs of the electrophoresis tank with electrophoresis buffer prepared from the same batch of 5x TBE used to cast the gel. Use a bent Pasteur pipette or syringe needle to remove any air bubbles trapped beneath the bottom of the 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.**

14. 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 6x gel-loading buffer. Load the mixture into the wells using a Hamilton syringe or a micropipette equipped with a drawn-out plastic tip.

*Usually, approx. 20-100 µl of DNA sample is loaded per well depending on the size of the slot. 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.*

15. Connect the electrodes to a power pack (positive electrode connected to the bottom reservoir), 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. Therefore, with higher voltages, gel boxes that contain a metal plate or extended buffer chamber should be used to distribute the heat evenly. Many types of gel apparatuses are equipped with thermal sensors that monitor the temperature of the gel during the run. These are particularly useful when striving to minimize variation from one gel run to the next. Alternatively, use a gel-temperature-monitoring strip.

16. 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.

17. Detach the glass plates, and use a scalpel or razor blade to remove the gel-sealing tape. Lay the glass plates on the bench (siliconized plate uppermost). Use a spacer or plastic wedge to lift a corner of the upper glass plate. Check that the gel remains attached to the lower plate. Pull the upper plate smoothly away. Remove the spacers.

18. Use one of the methods described in Detection of DNA in Polyacrylamide Gels by Staining or Detection of DNA in Polyacrylamide Gels by Autoradiography to detect the positions of bands of DNA in the polyacrylamide gel.

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**Caution**

**Acrylamide:bisacrylamide**

Acrylamide:bisacrylamide, see Acrylamide; Bisacrylamide

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**Caution**

**Ammonium persulfate**

Ammonium persulfate \((\text{NH}_4)_2\text{S}_2\text{O}_8\) is extremely destructive to tissue of the mucous membranes and upper respiratory tract, eyes, and skin. Inhalation may be fatal. Wear appropriate gloves, safety glasses, and protective clothing. Always use in a chemical fume hood. Wash thoroughly after handling.

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**Caution**

**KOH and KOH/methanol**
KOH and KOH/methanol (Potassium hydroxide) can be highly toxic. It may be harmful by inhalation, ingestion, or skin absorption. Solutions are caustic and should be handled with great care. Wear appropriate gloves.

Caution

TEMED

TEMED \textit{N,N',N',N''-Tetramethylethylenediamine} is extremely destructive to tissues of the mucous membranes and upper respiratory tract, eyes, and skin. Inhalation may be fatal. Prolonged contact can cause severe irritation or burns. Wear appropriate gloves, safety glasses, and other protective clothing. Use only in a chemical fume hood. Wash thoroughly after handling. Flammable: Vapor may travel a considerable distance to source of ignition and flash back. Keep away from heat, sparks, and open flame.

Recipe

\begin{itemize}
  \item \textbf{6x Gel-loading buffer}
  \begin{itemize}
    \item 30\% glycerol
    \item 0.25\% bromophenol blue
  \end{itemize}
\end{itemize}

Recipe

\textbf{Acrylamide:bisacrylamide}

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. Warning: Wear gloves while working with acrylamide.

Recipe

\textbf{Ammonium persulfate}

To prepare a 10\% (w/v) solution: Dissolve 1 g ammonium persulfate in 10 ml of H$_2$O and store at 4$^\circ$C. Ammonium persulfate decays slowly in solution, so replace the stock solution every 2-3 weeks.
Ammonium persulfate is used as a catalyst for the copolymerization of acrylamide and bisacrylamide gels. The polymerization reaction is driven by free radicals generated by an oxido-reduction reaction in which a diamine (e.g., TEMED) is used as the adjunct catalyst.

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**Recipe**

**KOH/methanol solution**

This solution is for cleaning the glass plates used to cast sequencing gels. It is prepared by dissolving 5 g of KOH pellets in 100 ml of methanol. Store the solution at room temperature in a tightly capped glass bottle.

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**Recipe**

**TBE buffer**

Prepare a 5X stock solution in 1 L of H2O:

- 54 g of Tris base
- 27.5 g of boric acid
- 20 mL of 0.5 M EDTA (pH 8.0)

The 0.5X working solution is 45 mM Tris-borate/1 mM EDTA.

TBE is usually made and stored as a 5X or 10X stock solution. The pH of the concentrated stock buffer should be ~8.3. Dilute the concentrated stock buffer just before use and make the gel solution and the electrophoresis buffer from the same concentrated stock solution. Some investigators prefer to use more concentrated stock solutions of TBE (10X as opposed to 5X). However, 5X stock solution is more stable because the solutes do not precipitate during storage. Passing the 5X or 10X buffer stocks through a 0.22-µm filter can prevent or delay formation of precipitates.

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