

Gel Electrophoresis of Proteins in Denaturing Conditions

References:

- Bollag, D. M. and S. J. Edelman, 1991. Protein Methods. Wiley-Liss, Inc., New York, NY.
- Instruction Manual, Mini-V 8-10 Vertical Gel Electrophoresis system. Life Technologies.
- Instruction Manual, Mini-Protean II cell. BioRad.

Materials:

30 % (w/v) Acrylamide/0.8 % (w/v) methylene bisacrylamide

for 100 mls

30.0 g acrylamide

0.8 g methylene bisacrylamide

dH₂O to 100 mls

Filter through a 0.45 µm filter. Store at 4°C in the dark. It can be stored in the refrigerator for months.

NOTE: Unpolymerized acrylamide is a skin irritant and a neurotoxin. Always wear gloves, eye protection, a mask and a lab coat. Weigh the acrylamide in the fume hood. Add the acrylamide to dH₂O in a beaker in the fume hood. Cover the beaker with parafilm and mix until the acrylamide is completely dissolved. Wipe off the equipment and area where you weighed out the acrylamide thoroughly. Thoroughly clean out any glassware that you used to make up your solution.

4 X Lower Tris (1.5 M Tris·HCl pH8.8, 0.4 % SDS)

To 110 mls dH₂O add 36.4 g of Tris base

Add 8 mls of 10 % SDS

Adjust pH to 8.8 with 1N HCl

Adjust the final volume to 200 mls with dH₂O

This solution can be stored in the refrigerator for months.

4 X Upper Tris (0.5 M Tris·HCl pH6.8, 0.4 % SDS)

To 110 mls dH₂O add 12.12 g of Tris base

Add 8 mls of 10 % SDS

Adjust pH to 6.8 with 1N HCl

Add dH₂O to a final volume of 200 ml

This solution can be stored in the refrigerator for months.

Temed

10 % AP (ammonium persulfate solution)

0.1 g ammonium persulfate

1 ml dH₂O

Stable for up to a month in the refrigerator.

4 X Sample Buffer

4 mls glycerol

2 mls 2-mercaptoethanol

1.2 g SDS

5 mls 4 X Upper Tris

0.03 g Bromophenol blue

Aliquot into 1.5 ml microcentrifuge tubes. Store at -20°C. One tube can be stored at 4°C for up to a month.

4 X Tris-glycine reservoir buffer - SDS

36 g Tris base

172.8 g glycine

dH₂O to 3 L

Stable indefinitely at room temperature.

Reservoir buffer

750 mls of 4 X Tris-glycine reservoir buffer - SDS

30 mls of 10 % SDS

dH₂O to 3 L

Stable indefinitely at room temperature.

Prestained molecular weight standards

1 X Sample buffer

Protocol:*Preparation of the separating gel:*

1. Clean a set of glass plates for each gel first with dH₂O and then with 70 % ethanol.
2. Assemble the casting assembly. Be sure that the bottom of the gel plates and the spacers are perfectly aligned. Place a mark on the glass plates 1.5 cm below where the top of the gel will be.
3. Have ready:
 - a Pasteur pipette with a bulb
 - A P-1000 set to 1 ml and a tip
 - ethanol
4. Prepare the separating gel solution (See Tables 1 and 2)

Table 1: Preparation of the separating gel solution for 2 gels using 0.75 mm spacers.

	5 %	7.5 %	10 %	15 %
dH ₂ O	5.8 mls	5 mls	4.2 mls	2.5 mls
30 % acrylamide/0.8 % methylene bisacrylamide	1.7 mls	2.5 mls	3.3 mls	5.0 mls
4 X Lower Tris	2.5 mls	2.5 mls	2.5 mls	2.5 mls
10 % AP	100 µl	100 µl	100 µl	100 µl

Table 2: Resolution ranges for gels of different acrylamide concentrations.

Acrylamide Percentage	Separating Resolution
5 %	60 - 212 kd
7.5 %	30 - 120 kd
10 %	18 - 75 kd
15 %	15 -45 kd

5. Gently mix the separating gel solution. **Note:** Excess aeration will interfere with the polymerization of the gel.
6. Add 10 µl of Temed to the separating gel solution. Mix well. Use a Pasteur pipette to fill the glass plates with separating gel solution up to the mark on the glass plates. **Note:** Work rapidly as soon as the Temed is added because the gel will begin to polymerize upon addition of the Temed.

7. Over lay the gel with 1 ml of the 95 % ethanol.
8. Let the gel separating gel polymerize for 30-60 minutes minutes.

Preparation of the stacking gel:

1. While the lower gel is polymerizing, prepare the stacking gel (see Table 3).

Table 3: Preparation of stacking gel solution for 2 gels using 0.75 mm spacers:

Component	Volume
dH ₂ O	3.25 mls
30 % acrylamide/0.8 % methylene bisacrylamide	0.5 mls
4 X Upper Tris	1.25 mls
10 % AP	50 μ l

2. Gently mix the stacking gel solution. **Note:** Excess aeration will interfere with the polymerization of the gel.
3. Get ready a Pasteur pipette with a bulb.
4. Pour the ethanol off the separating gel. Rinse the gel assembly out with several changes of dH₂O.
5. Add 5 μ l of Temed to the stacking gel solution. Mix well. Use a Pasteur pipette to fill the glass plates up to the top with stacking gel solution.
6. Carefully insert the comb. Be sure that no air bubbles are trapped on the ends of the teeth.
7. Let the stacking gel polymerize for 30 minutes.

Preparation of protein samples for loading:

1. While the stacking gel is polymerizing, prepare the protein samples for loading. The volume of sample depends on the capacity of the wells (See Table 4), the type of protein sample and the use of the gel.

Table 4: Capacity per well

Apparatus	Number of wells	Thickness	Volume of sample
BioRad	10 well	0.75 mm	24 μ l
	15 well	0.75 mm	14 μ l
Life Technologies	10 well	0.75 mm	20 μ l
	15 well	0.75 mm	10 μ l

- For pure protein samples, use 2 ng if the gel will be silver stained or 0.1 μ g if the gel will be coomassie stained.
 - Use a maximum of 20 - 40 μ g for mixed protein samples.
2. Thaw the protein samples and molecular weight standard at room temperature.
 3. Combine the protein sample and 4 X sample buffer in a 1.5 ml microcentrifuge tube. Prepare protein standard according to accompanying directions.
 3. Heat the samples in a boiling water bath for 2 minutes.
 4. Centrifuge the samples in a microcentrifuge for 20 seconds at 12,000 rpm. The protein samples are now ready to load.

Loading of the samples and running of the gel:

1. Place the gel(s) into the electrophoresis assembly. Fill the assembly with reservoir buffer.
2. Use a Pasteur pipette to rinse out the wells of the gel with reservoir buffer.
3. Introduce the marker and the samples into the wells using a protein gel loading tip. Be careful not to introduce air into the wells. This can allow some sample to flow over into the adjacent wells. Fill any unused wells with 1 X sample buffer.
4. Note the order in which the samples were loaded.
5. Attach the electrodes.
6. Turn on the power supply to 200 V, constant voltage.
7. Turn the power supply off when the Bromophenol blue reaches the bottom of the gel and remove the electrodes.
8. Remove the gels. Carefully remove a spacer. Place that spacer between a corner of the plates. Gently pry apart the glass plates. The gel will usually stick to one of the plates.
9. Cut off the stacking gel and discard. Mark the orientation of the gel by cutting off one corner.
10. The gel is now ready to be stained with Coomassie or the proteins in the gel can be transferred to nitrocellulose by electroblotting.