

9.12 Seventh Week  
SDS-PAGE and Western  
Blotting-1

# SDS-PAGE

- Separate protein according to the size.
- Several issues to cope with
  - Amino acid composition (basic, acidic, etc)
  - Folding (linear, globular etc)
  - S-S bond
- How to equalize these difference?

# Principle of SDS-PAGE

QuickTime™ and a  
TIFF (Uncompressed) decompressor  
are needed to see this picture.

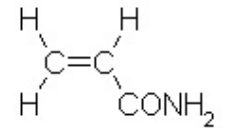
^ and a  
decompressor  
his picture.

1 g of protein  
sticks to 1.4 g  
of SDS

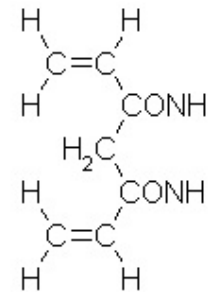
Sodium  
dodecylsulpho  
nate

# Acrylamide gel

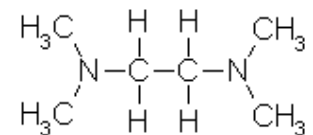
- acrylamide (gel itself)
- N,N'-methylene-bis-acrylamide (cross-links linear acrylamide)
- ammonium persulfate (APS) (provides radical)
- N,N,N',N'-tetramethylethylenediamine [TEMED](catalyst)



Acrylamide



N,N'-methylenebisacrylamide



TEMED

N,N,N',N'-tetramethylethylenediamine

# Stacking and separating gel

- Stacking gel: low concentration. Proteins are not separated, rather are stacked together.
- Separating gel: higher concentration.

QuickTime™ and a  
TIFF (Uncompressed) decompressor  
are needed to see this picture.

1. Rinse glass with distilled water and then place on a paper towel. Use glove.
2. Dry one surface with paper towel.
3. Spray 70% ethanol on glass and then wipe.

Images removed due to copyright restrictions.  
Instructions on using the SDS-PAGE equipment.

1. Separating gel (mixture will be provided)

30 % Acrylamide+ 0.8 % Bisacrylamide	6.7 ml
1.5 M Tris-HCl (pH 8.8)	5 ml
10 % SDS	0.2 ml
distilled water.	7.9 ml

2. Add

10 % Ammonium persulfate (APS)	200 $\mu$ l
TEMED	8 $\mu$ l

3. Pour immediately. Leave ~2 cm from the top of glass.

4. Overlay gently ethanol on the acrylamide solution before it polymerizes.  
Bubbles will disappear. Do not disturb acrylamide solution.

5. After polymerization of acrylamide (20-30 min), remove ethanol by water.

6. Stacking gel (provided).

30 % Acrylamide+ 0.8 % Bisacrylamide	2.7 ml
1 M Tris-HCl (pH 6.8)	0.5 ml
10 % SDS	0.04 ml
distilled water.	2.7 ml

7. Add

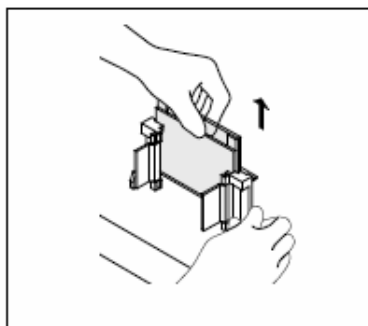
30 % Ammonium persulfate (APS)	40 $\mu$ l
TEMED	4 $\mu$ l

8. Pour stacking gel solution over the separating gel.

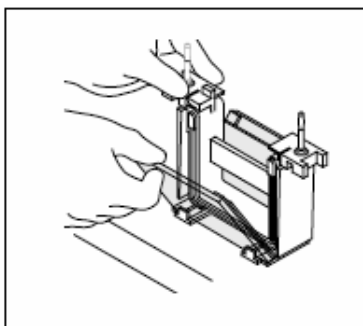
9. Insert comb carefully avoiding air bubble.



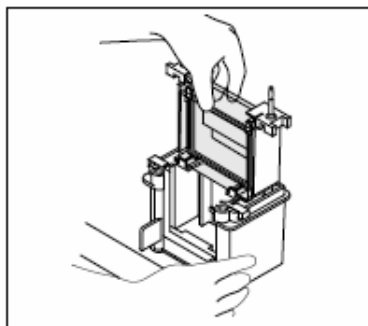
- After the solution became gel, set up gel in tank.



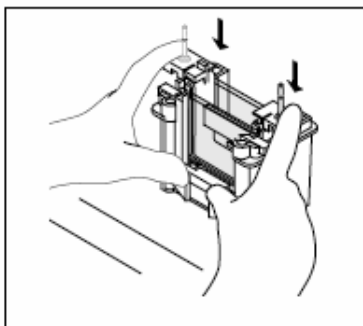
5a. Remove the Gel Cassette Sandwich from the Casting Frame.



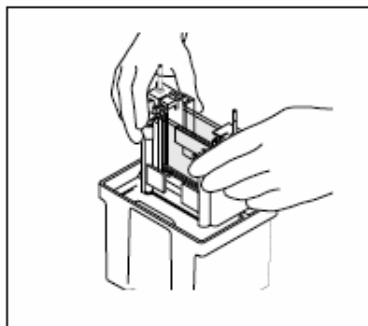
5b. Place Gel Cassette Sandwich into the Electrode Assembly with the Short Plate facing inward.



5c. Slide Gel Cassette Sandwiches and Electrode Assembly into the clamping frame.



5d. Press down on the Electrode Assembly while closing the two cam levers of the Clamping Frame.



5e. Lower the Inner Chamber into the Mini Tank.

Fig. 5. Mini-PROTEAN 3 assembly.

We only have two sets of this. In each set you can run two gels.

1. Dissect out forebrain and cerebellum.
2. Homogenize in ~10 volume of 0.32 M sucrose. (forebrain 5 ml and cerebellum 1ml) un glass-teflon homogenizer
3. Centrifuge at 1000 rpm 3 min.
4. Use supernatant for next step.
5. Mix sample and loading buffer.

Sample loading buffer (4 x)	10 $\mu$ l
Sample preparation	30 $\mu$ l

6. Boil 100 °C for 3 minutes (use tape to prevent the cover to open).
7. Load each 10  $\mu$ l in to the well by the following order (use 5  $\mu$ l for marker)

Marker – Forebrain – Cerebellum-blank-blank-Marker – Forebrain – Cerebellum-blank-blank

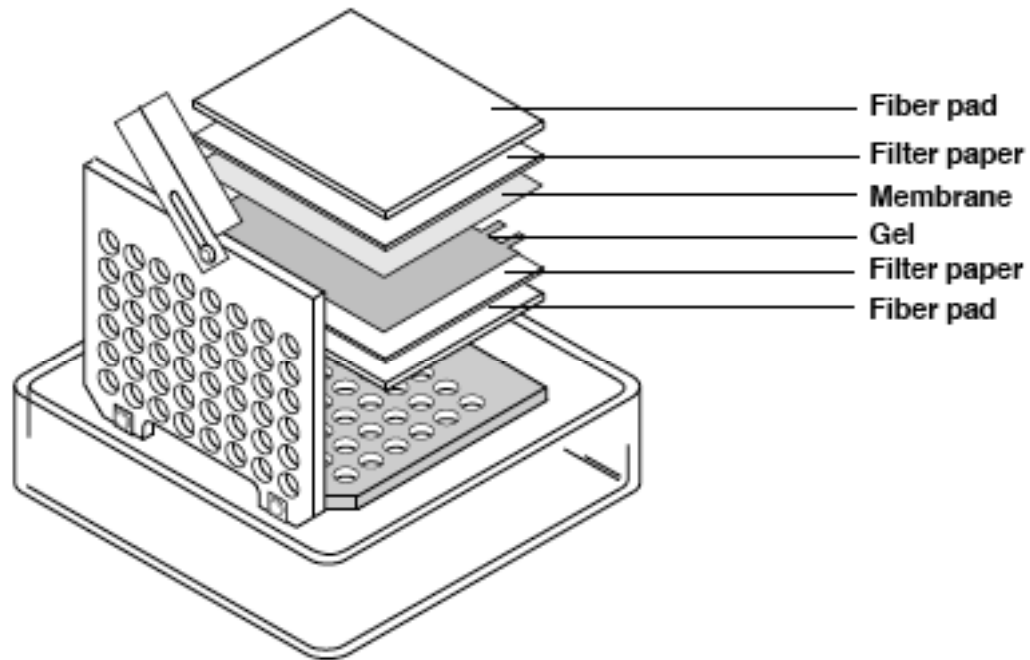
8. Electrophoresis in SDS buffer at 100~200 V until BPB reaches lower end of the gel.
9. After the electrophoresis is done, take out the gel, separate the glass. Cut the gel on half. Float half in CBB staining solution (provided) in plastic case. Float the other half on transfer solution.

# Transfer to PVDF Membrane

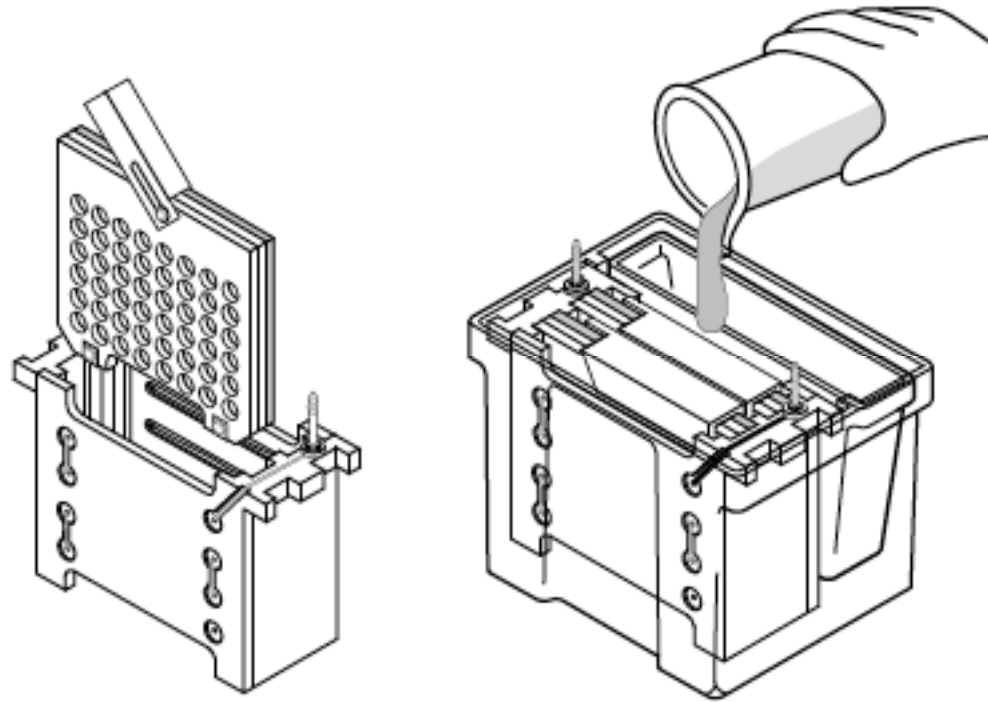
- SDS-PAGE gel is fragile and not easy to handle
- Transfer to PVDF membrane (rigid) by applying current perpendicular to the gel.

1. Cut PDVF into ~5 x 5 cm and moist with 100 % methanol. Place in a container with transfer buffer (emptied pipet tip box). Cut six 3MM filter papers into the same size and place in the same buffer container. Use glove.

2. Make a sandwich as in Figure.



3. Place the sandwich and ice holder in the tank. *Be careful on the orientation.* Fill the tank with transfer buffer. Place whole tank in large container with ice to cool down while running.



6. Electroblot at 100 V (350 mA) for 30 min. Make sure that the unit does not heat up.

7. After blotting, make sure that you see the marker is transferred to the PVDF membrane. Cut upper left corner of the membrane (with protein side up).

8. Place the membrane in 10 ml of 5 % milk in Tris-buffered saline in a pipette box.