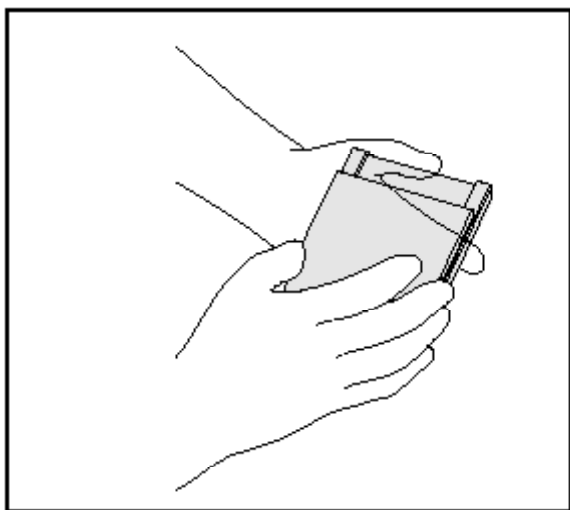


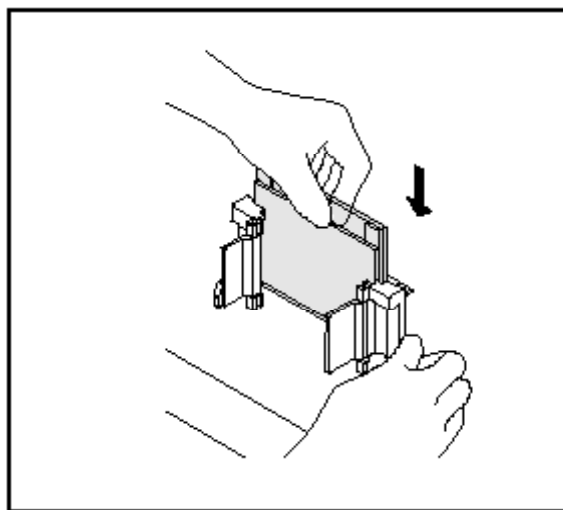
9.12 SDS-Polyacrylamide Gel Electrophoresis and Western Blotting

I. Prepare glass

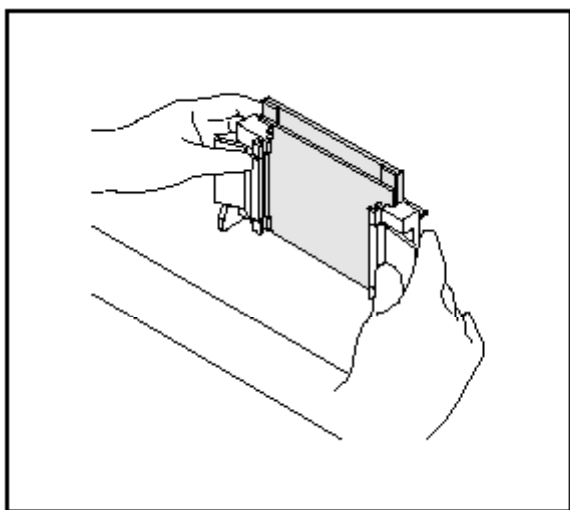
1. Rinse glass with distilled water and then place on a paper towel. Use glove.
2. Dry one surface with paper towel.
3. Spray 70% ethanol on glass and then wipe.
4. Once dry, assemble on casting frame as follows.



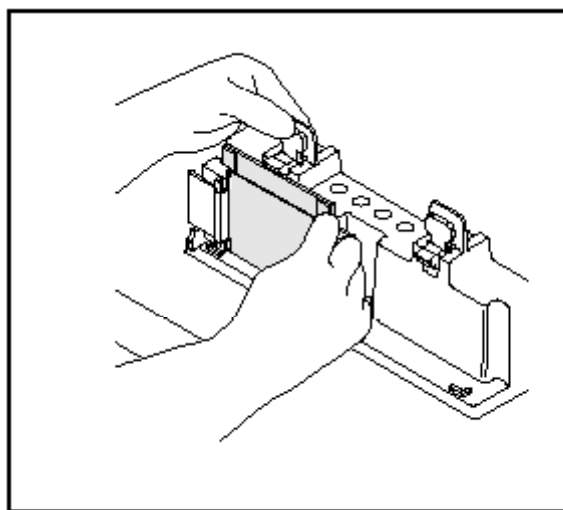
4a. Place a Short Plate on top of the Spacer Plate.



4b. Slide the two plates into the Casting Frame keeping the Short Plate facing front.



4c. Lock the pressure cams to secure the glass plates.



4d. Secure the Casting Frame in the Casting Stand by engaging the spring loaded lever.

II Prepare gel

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 μ l
TEMED	8 μ l

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

4. Overlay gently ~500 μ l of 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. Use paper towel to remove residual 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 μ l
TEMED	4 μ l

8. Pour stacking gel solution over the separating gel.

9. Insert comb carefully avoiding air bubbles.

II. Brain protein preparation (one animal per day)

1. Use dry use to euthanize the animal. Dissect out forebrain and cerebellum.

2. Homogenize in ~10 volume of 0.32 M sucrose (~10 ml) in 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 μ l
Sample preparation	3 μ l
H ₂ O	27 μ l

6. Boil 100 °C for 3 minutes (use tape to prevent the lid to open).

7. Load each 10 μ l (5 μ l for marker) in to the well by the following order. Use thin pipette tip.

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.

10 x electrophoresis buffer. It will make pH 8.3 at 1 x (provided as 1 X solution).

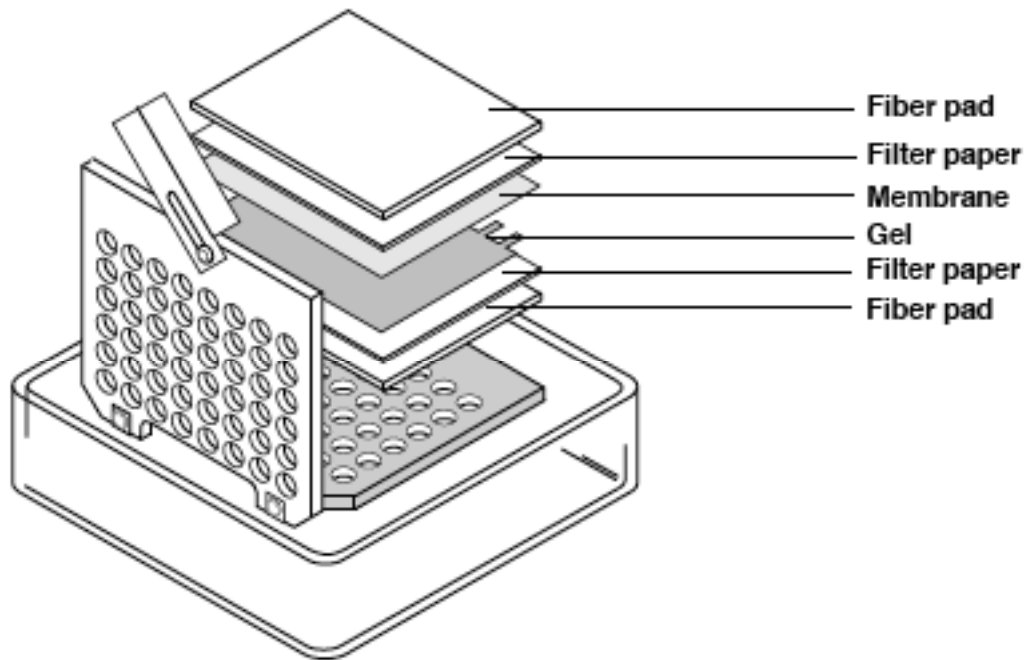
Tris-base	30.2 g	(0.25 M at 10 x)
glycine	144.2 g	(1.29 M at 10 x)
SDS	10 g	(1 % at 10 x)

9. After the electrophoresis is done, take out the gel, separate the glass. Cut the gel on half using scalpel. Float half in CBB staining solution (provided) in plastic case. Float the other half on transfer solution in an emptied pipet tip box.

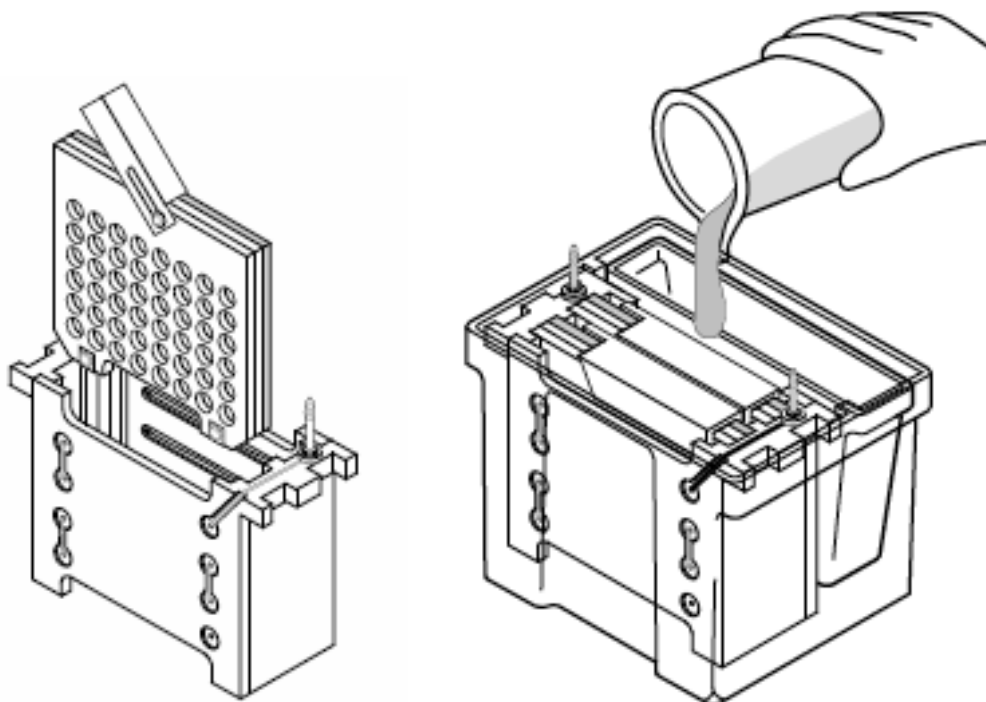
III. Transfer to PDVF Membrane.

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.