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

1 g of protein sticks to 1.4 g of SDS

Sodium dodecylsulphate
Acrylamide gel

- acrylamide (gel itself)
- N,N'-methylene-bis-acrylamide (cross-links linear acrylamide)
- ammonium persulfate (APS) (provides radical)
- N,N,N',N'-tetramethylethylene diamine [TEMED](catalyst)
Stacking and separating gel

- Stacking gel: low concentration. Proteins are not separated, rather are stacked together.
- Separating gel: higher concentration.
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.
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 µl
- TEMED: 8 µl

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


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 µl
- **TEMED**: 4 µl

8. Pour stacking gel solution over the separating gel.

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

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

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 lavers of the Clamping Frame.
5e. Lower the Inner Chamber into the Mini Tank.

Fig. 5. Mini-PROTEAN 3 assembly.
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.

<table>
<thead>
<tr>
<th>Sample loading buffer (4 x)</th>
<th>10 µl</th>
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<tbody>
<tr>
<td>Sample preparation</td>
<td>30 µl</td>
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6. Boil 100 °C for 3 minutes (use tape to prevent the cover to open).
7. Load each 10 µl in to the well by the following order (use 5 µl 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 maker 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.