

## Western Blotting

### A. Electrophoretic transfer of proteins to nitrocellulose

#### Buffer needed:

#### Transfer Buffer

- 14.5 g/l Glycine (192 mM final)
- 3 g/l Tris (25 mM final)
- 20% Methanol

Usually make up 10 liters at a time.

#### Procedure:

1. Place two sheets of Whatman 3MM paper (or equivalent) on top of sponge in transfer cassette. The dark side of the cassette should be face down.
1. Place one sheet of nitrocellulose paper on top of 3MM paper
2. Place gel on top of nitrocellulose paper
3. Place two sheets of 3MM paper on top of the gel. Be sure to remove all bubbles from underneath the gel.
4. Cover with sponge and close cassette. The light gray side of the cassette is the front.
5. Fill the transfer apparatus tank with transfer buffer
6. Place cassette in tank with light gray side facing front.
7. Proteins move to + (back of tank if lid is facing you) so you should arrange your sandwich so that proteins move onto the nitrocellulose (ie. gray side facing you).
8. Transfer about 2 hr at 70 volts
9. Blot can be stored indefinitely (dry) or can be blotted immediately

PVDF can be substituted for nitrocellulose using the same procedure. However, PVDF must be pre-wet in neat methanol before being placed into transfer buffer.

## **B. Blotting**

### **Buffers Needed:**

#### 10x TBS (Tris-buffered saline) – for 2 liters

24.2 g Tris  
175.2 g NaCl  
pH to 7.9

#### TBST (TBS plus Tween 20)

Dilute TBS ten-fold  
Add 0.05% Tween 20 (1 ml/2 l)

#### TBST/BSA

Add 1 mg/ml BSA to TBST

#### 10% powdered milk in TBST

#### ECL Reagents

### **Procedure:**

1. Block blot for ~1 hr in TBST plus 10% powdered milk
2. Rinse to remove traces of milk ( Milk appears to denature some antibodies)
3. Incubate with primary antibody for 1 to 2 hr in TBST/BSA
4. Wash 3 times for 10 min each in TBST/BSA
5. Incubate with secondary antibody for 45 min to 1 hr in TBST/BSA. Be sure to use appropriate (rabbit or mouse) secondary ECL antibody
6. Wash 5 times for 10 min in TBST/BSA

### **C. Developing Blots by ECL**

1. Mix 1 ml of each ECL reagent and add 2 ml water. This gives 4 ml of solution which is usually enough for most blots. More water can be added if a greater volume of solution is desired. Make up this mixture right before you intend to use the solution
2. Incubate blot with ECL solution for 2 minutes by placing in a petri dish and using a pipet to bathe the blot with the solution
3. Wrap in Saran wrap. Expose to film