Lecture Topic: Western Blot Analysis

Date: Thursday May 6th 2010

Introduction

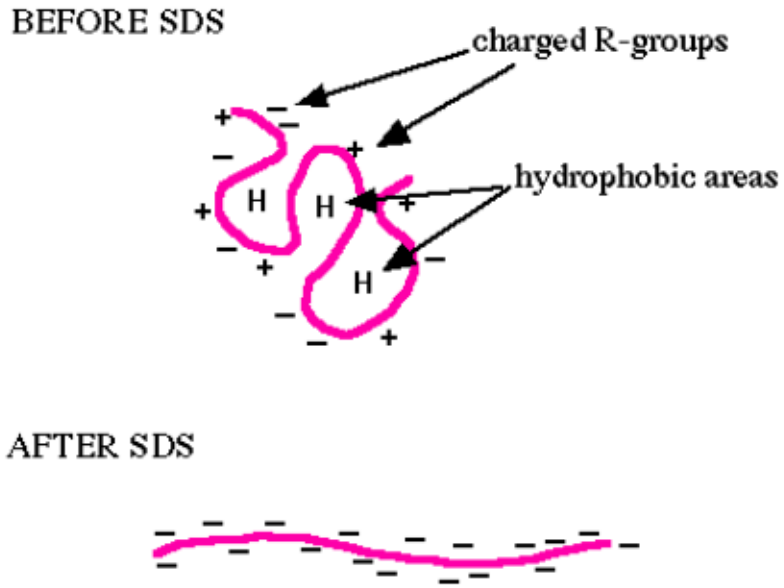
- Immuno (Western) Blotting is a commonly used technique to detect specific proteins from a complex mixture.
- It provides information on:
  - Protein expression (relative to a control sample)
  - Protein size (based on a marker protein run along with your sample)

Steps in Western Blotting

1. Sample Preparation
2. Polyacrylamide Gel Electrophoresis (PAGE)
3. Transfer from gel to membrane
4. Incubation with antibody
5. Detection

Sample Preparation

- Cells are grown to desired density (OD)
- Samples are centrifuged to collect cells and separate media (discard supernatant)
- Wash samples in buffer to remove salts
- Coat samples in SDS-loading buffer
- Boil samples for 5 minutes to denature proteins



SDS dissociates hydrophobic areas and renders proteins highly electronegative so that their migration through the gel is independent of their isoelectric point.

SDS-PAGE

- Discontinuous Gel
  - Top: Stacking Gel
  - Bottom: Resolving Gel
- Proteins run from negative (anode) end to positive (cathode) end
- The percentage of gel used determines the pore size, the larger the percentage the more cross linking and the smaller the pore size

Western Transfer

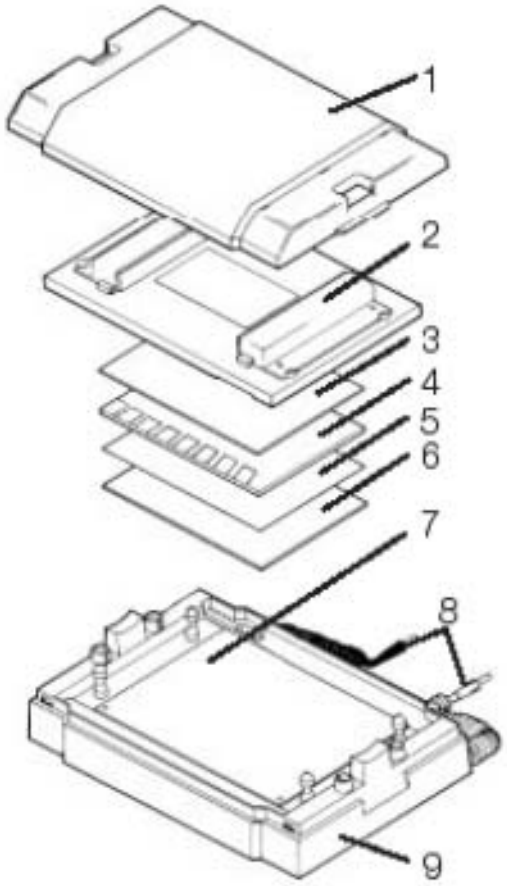
- Transfer from gel to PVDF (polyvinylidene fluoride membrane)
  - PVDF has good protein binding capacity (170-200ug/cm2), physical strength and enhanced binding in the presence of SDS
- Two Types of Transfer Units:

  1. Semi-dry Unit
  2. Mini Trans-Blot

Semi-Dry Electrophoretic Transfer Cell (BioRad)

- How to Set Up Transfer:

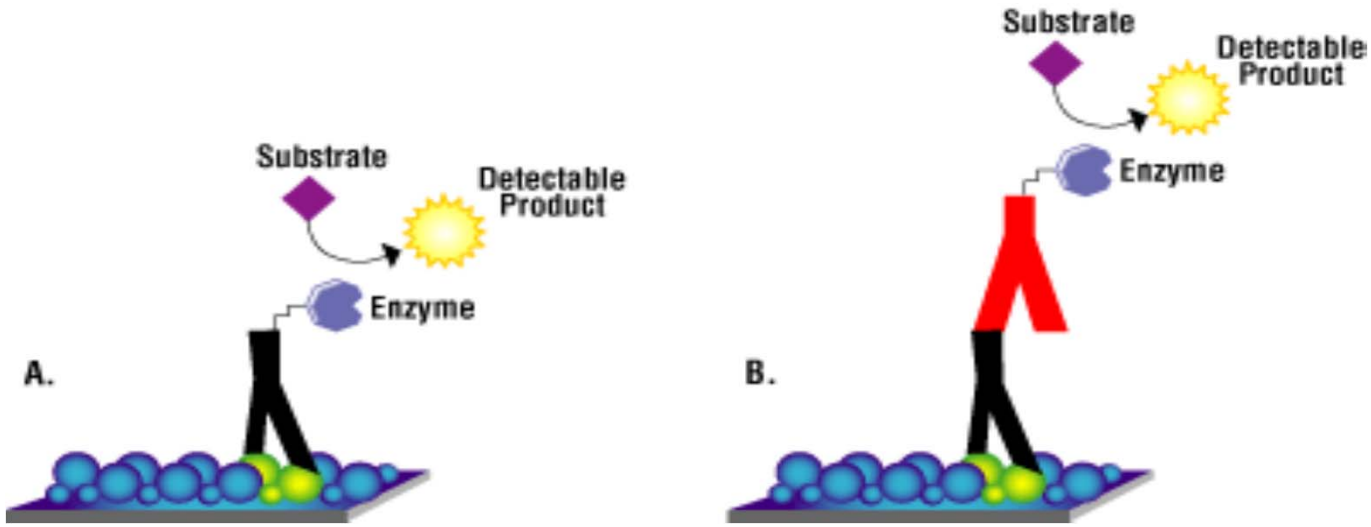
1. Safety Cover
2. Steel Cathode Assembly
3. Thick Blot Paper
4. Gel
5. Membrane (PVDF)
6. Thick Blot Paper
7. Platinum Anode
8. Power Cables
9. Base



Antibody Incubation

- After proteins are transferred from gel to membrane, the membrane is blocked using 5% milk.
- Blocking prevents non-specific interactions
- After blocking, the membrane is incubated in primary antibody

Direct and Indirect Detection



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Detection

- The secondary antibody is attached to HRP (horse radish peroxidase) enzyme
- HRP catalyzes the oxidation of luminol (substrate)
- Oxidation of luminol will put it in an excited state followed by decay to ground state accompanied by the emission of LIGHT
- The light is captured on a special film
- The intensity of the light is correlated with the abundance of protein present
- Enhanced Chemiluminescence occurs in the presence of chemical enhancers such as phenol.
- Signal is increased by 1000 fold

Examples of Western Blots

- Shifted Gel Image
- Overexposed Gel Image
- High Background Gel Image