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Professional Development

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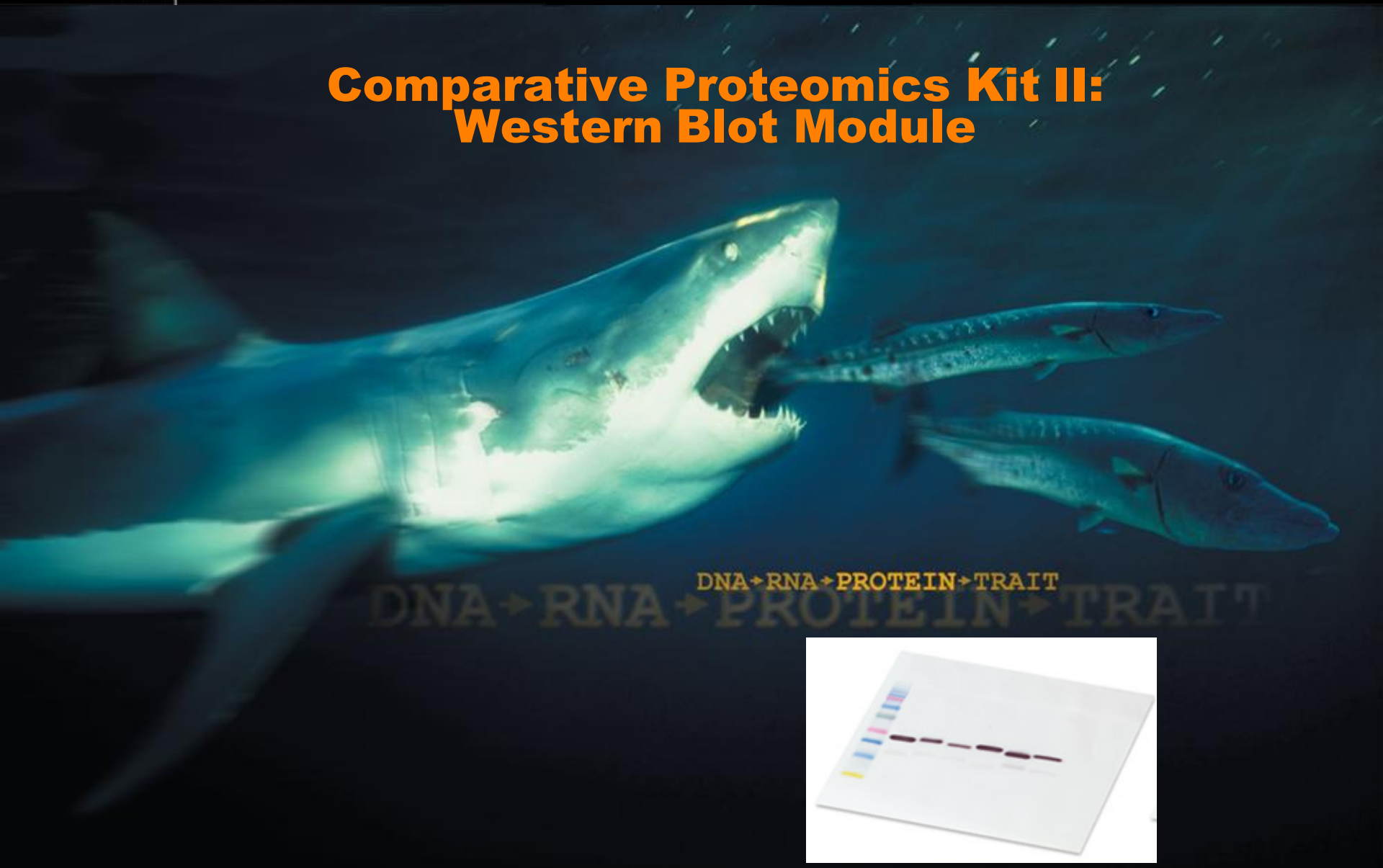
Comparative Proteomics Kit II: Western Blot Module



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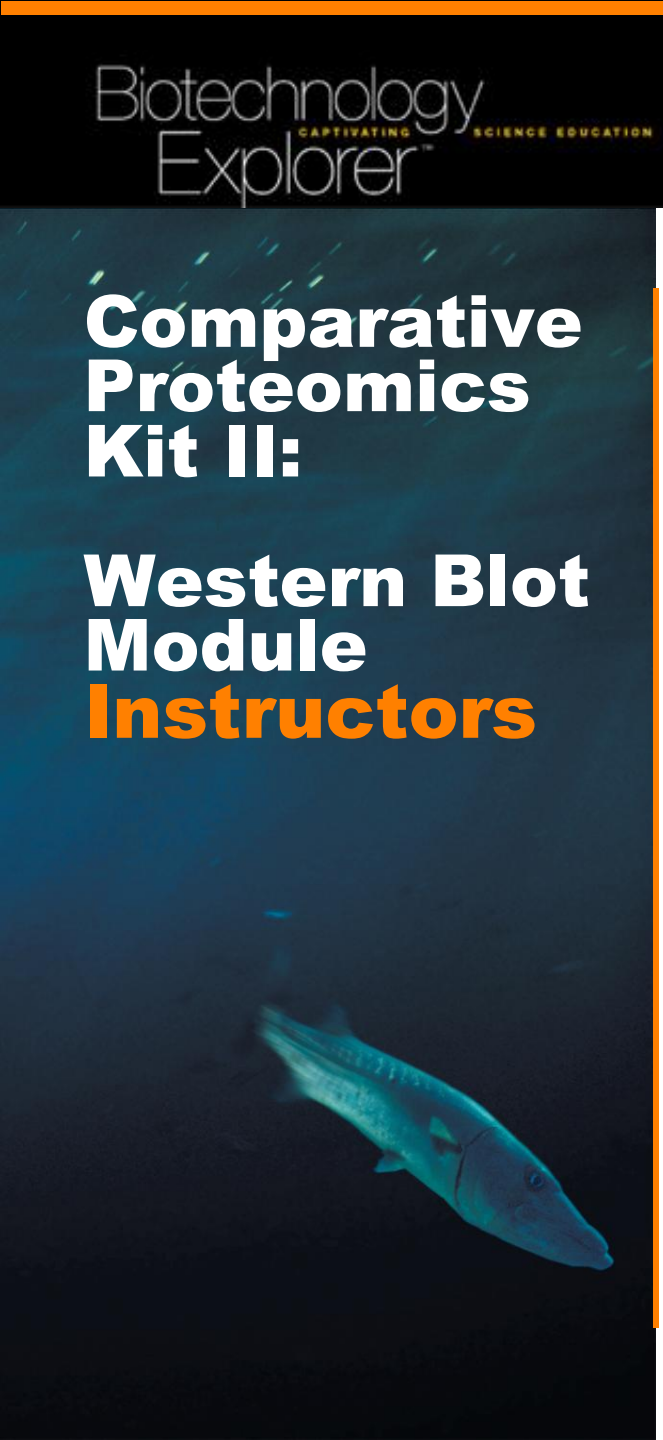
Comparative Proteomics Kit II:

Western Blot Module Instructors



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Why Teach Western Blotting?

- Powerful teaching tool
- Real-world connections
- Laboratory extensions
- Tangible results
- Link to careers and industry
- Standards-based

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Scientific Inquiry

- Using immunodetection to identify specific proteins
- Use of experimental controls
- Creation and uses of standard curves
- Use of bioinformatics databases

Chemistry of Life

- Chemical and physical properties of proteins
- Antibody structure and function
- Protein extraction techniques
- Enzyme-substrate interactions
- Chemistry of electrophoresis and blottings

Genetics

- DNA > RNA > protein > trait
- Antibody production via genetic recombination and gene splicing
- Genetic and epigenetic factors

Evolution

- Molecular variation
- Evolution of adaptive traits
- Environment and natural selection
- Phylogenetic relationships

Cell and Molecular Biology

- Posttranscriptional and posttranslational modification
- Muscle protein structure and function
- Adaptions to environment
- Immunology and virology

Environmental and Health Science

- Ecosystems, symbiosis, and interdependence
- Real-world HIV, mad cow disease, and bird flu test

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Comparative Proteomics Kit II: Western Blot Module

Kit Advantages

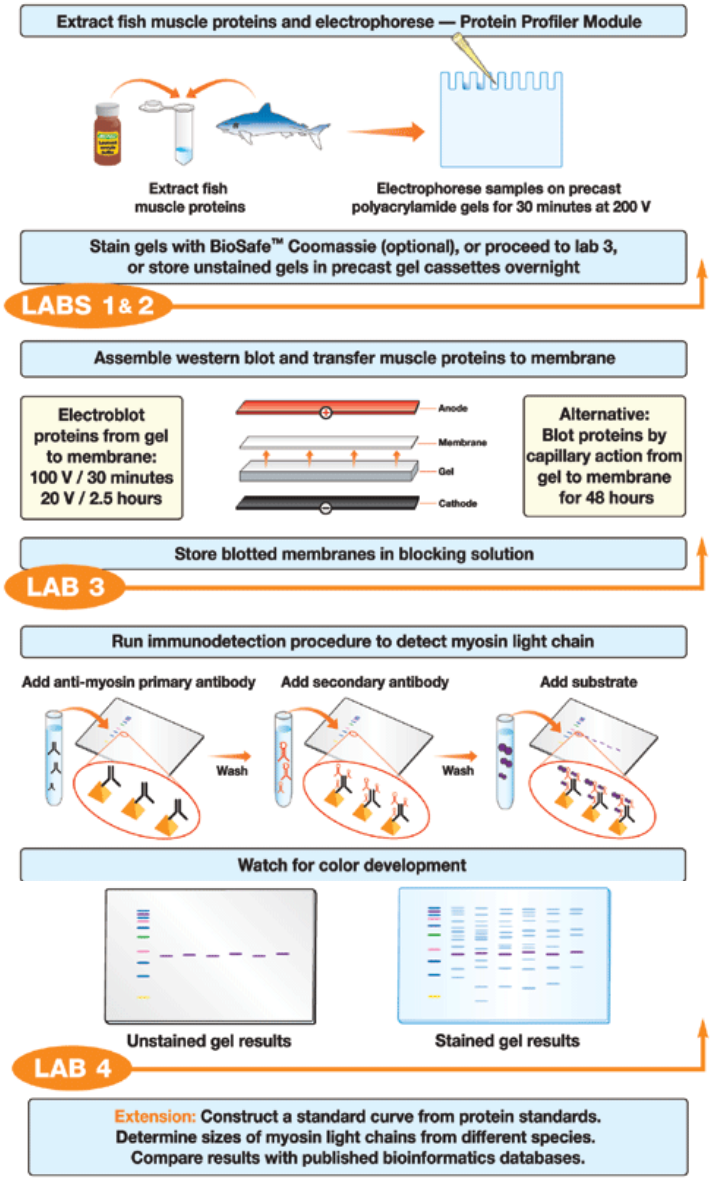
- Explore immunodetection: use of western blot analysis for HIV detection
- Applied immunology activity
- Use antibodies as detection tools
- Laboratory extension to Comparative Proteomics Kit I: Protein Profiler Module
- Includes sufficient materials for 8 student workstations
- Complete lab activity in four 45-minute lab sessions

Workshop Timeline

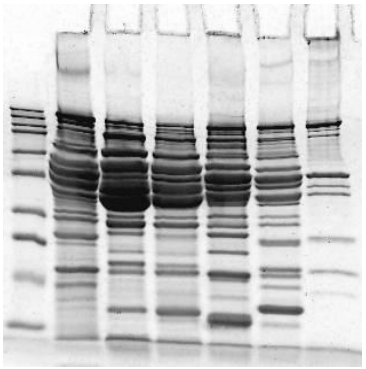
- Introduction
- Prepare the blotting system
- Block nitrocellulose membrane
- Incubation with antibody solutions
- Color development of the blot

Comparative Proteomics Kits I and II

Procedures Overview



From Gel to Blot



- **Polyacrylamide Gel Electrophoresis:**
  - **Break protein complexes into individual proteins**
  - **Separates protein samples based on size**

- **Western Blot Analysis:**
  - **Transfer the proteins to a nitrocellulose membrane**
  - **More stable and permanent**
  - **Identifies proteins by immunodetection: using specific antibodies against the protein of interest**

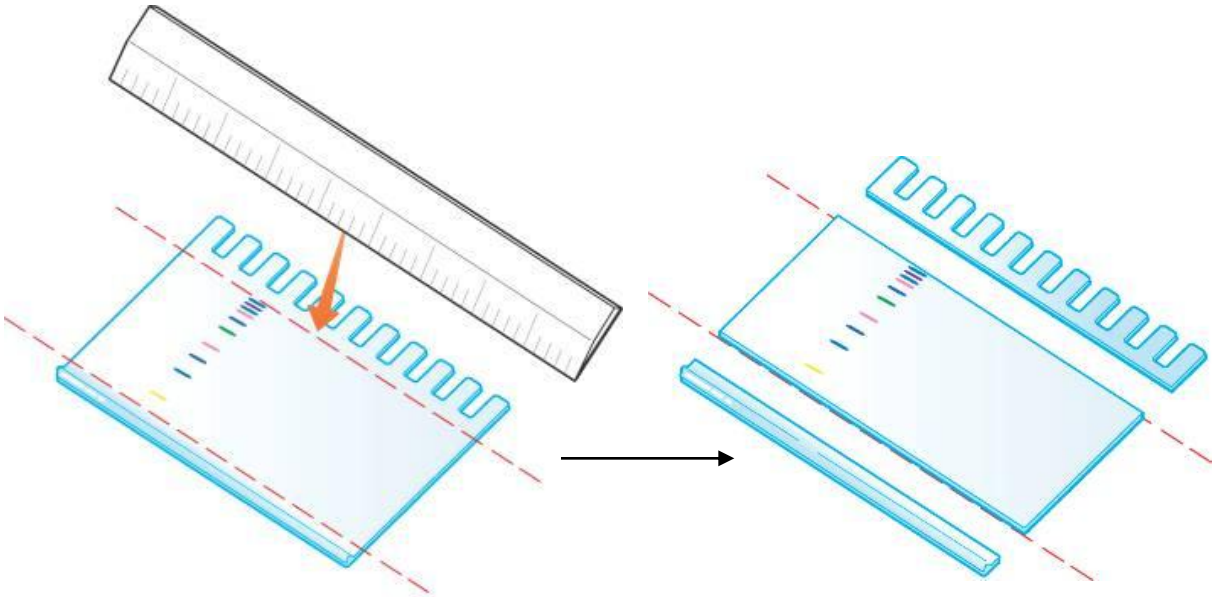
Laboratory Quick Guide

QUICK GUIDE

Lesson 4 Quick Guide

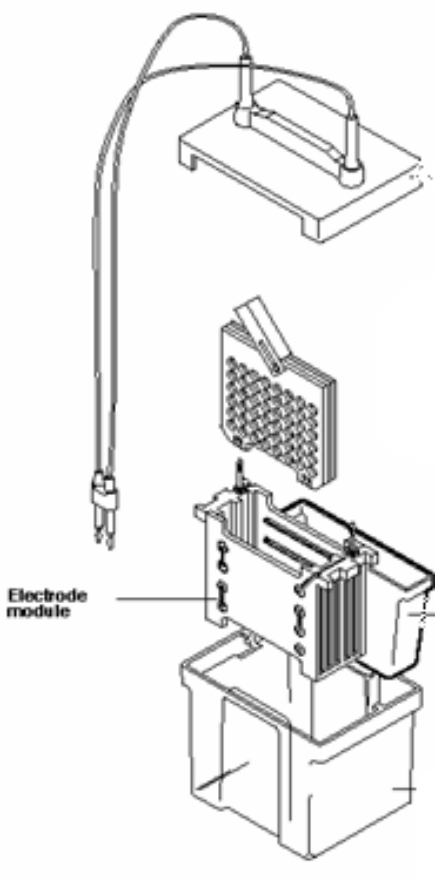
1. If not blocked overnight, immerse membrane in 25 ml blocking solution for 15 minutes to 2 hours at room temperature on a rocking platform.
2. Discard blocking solution and incubate membrane with 10 ml of primary antibody for 10–20 minutes on rocking platform set to a faster setting to ensure constant coverage of the membrane.
3. Quickly rinse the membrane in 50 ml of wash buffer then discard the wash.
4. Add 50 ml of wash buffer to membrane for 3 minutes on rocking platform at a medium speed setting.
5. Discard the wash and incubate membrane with 10 ml of secondary antibody for 5–15 minutes on rocking platform set to a fast setting.
6. Quickly rinse the membrane in 50 ml of wash buffer and discard the wash.
7. Add 50 ml of wash buffer and wash membrane for 3 minutes on rocking platform on a medium speed setting.
8. Discard the wash and add 10 ml of HRP color detection reagent.
9. Incubate 10–30 minutes, either with manual shaking or on a rocking platform, and watch the color development.
10. Rinse the membrane twice with distilled water and blot dry with paper towel.
11. Air dry for 30 minutes to 1 hour and then cover in plastic wrap or tape in lab book.

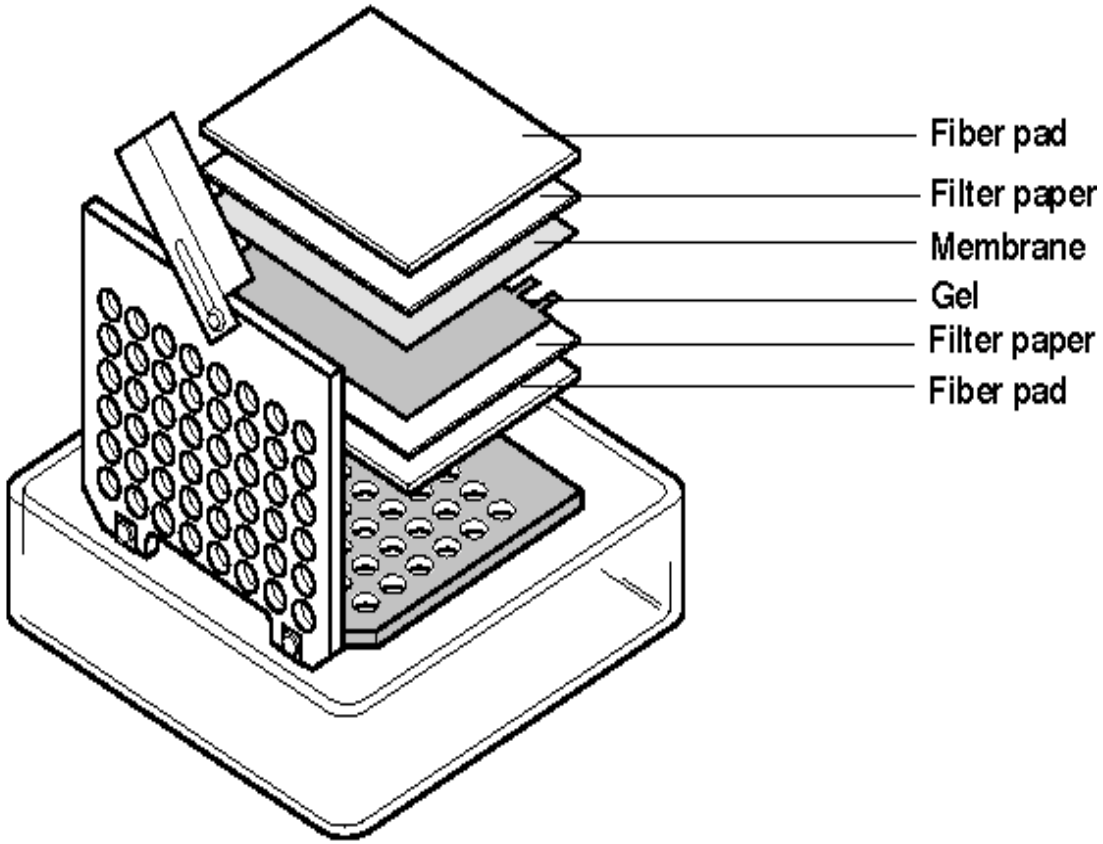
Prepare to transfer proteins to a nitrocellulose membrane



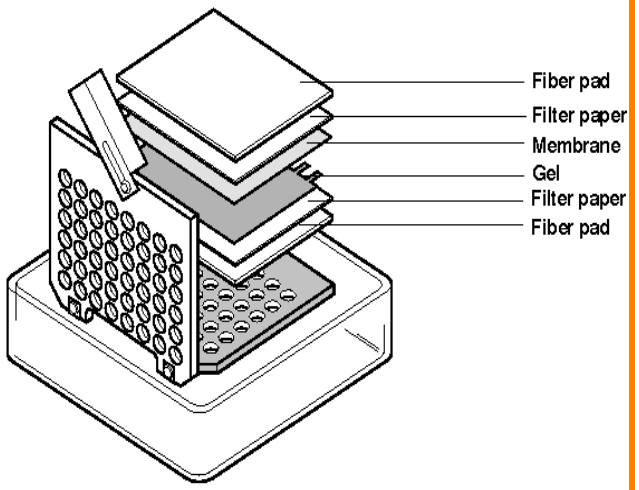
- **Trim gel**

Mini Trans-Blot Transfer Cell



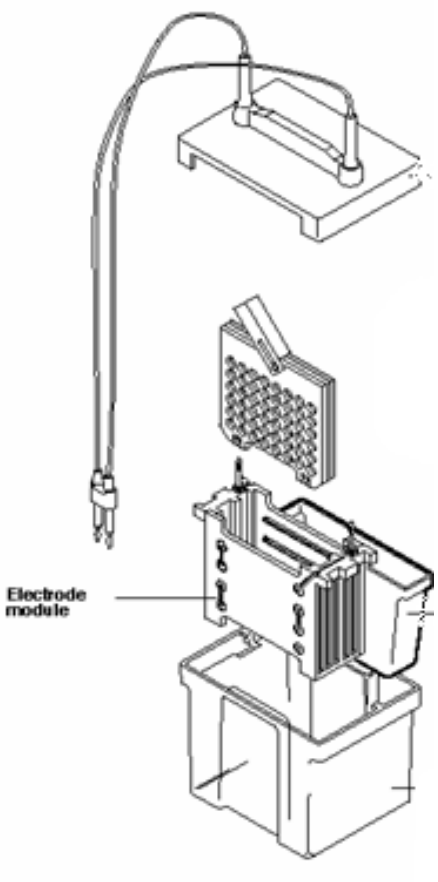


Preparing the Blotting Sandwich

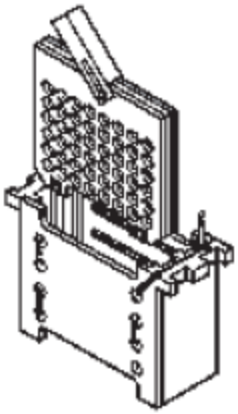


1. Place the cassette with gray side down on clean surface
2. Place one pre-wetted fiber pad on the gray side of the cassette
3. Place a sheet of filter paper on the fiber pad
4. Place gel on filter paper taking care to remove air bubbles
5. Place the pre-wetted nitrocellulose membrane on the gel
6. Place the second fiber pad on top
7. Close the cassette firmly DO NOT move gel/filter sandwich
8. Lock the cassette

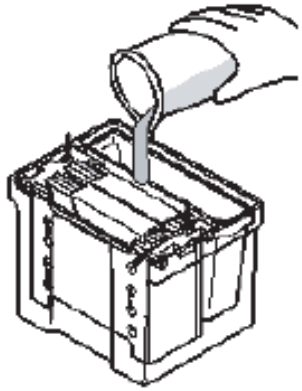
Prepare for Electrophoretic Transfer

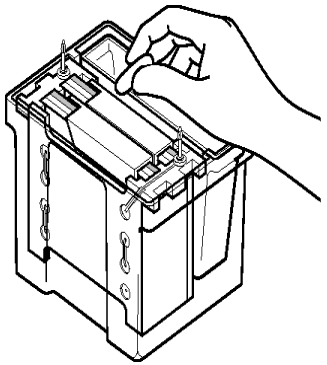


- **Place the closed and locked cassette in the electrode module**



- **Add the frozen Bio-Ice cooling unit and place in tank**
- **Fill the tank with buffer**



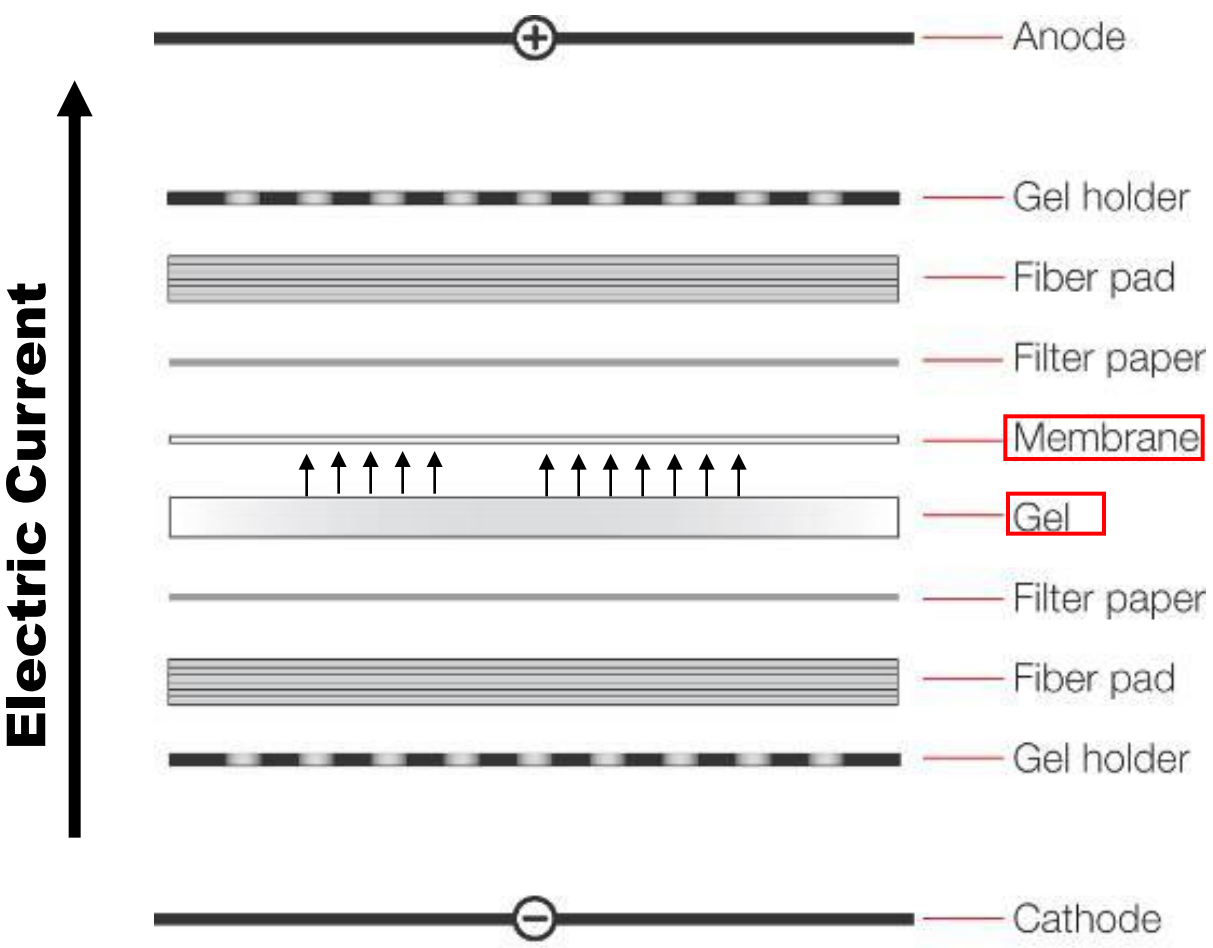


- **A stir bar can be added to help maintain the ion and temperature distribution in the tank even**

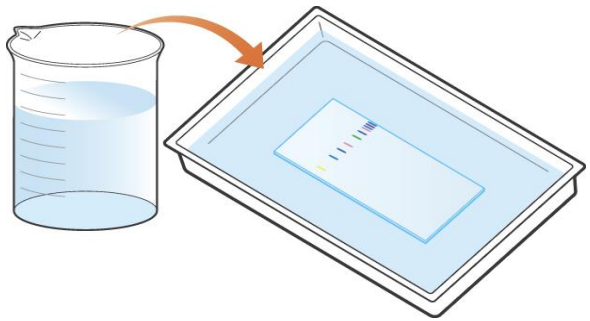
Transfer Proteins from the gel to the nitrocellulose membrane

30 minutes 100V

Blotting buffer 1x Tris glycine with 20% ethanol



Blocking Buffer



Remove membrane from the blotting sandwich and immerse in 25ml of blocking solution for 15minutes

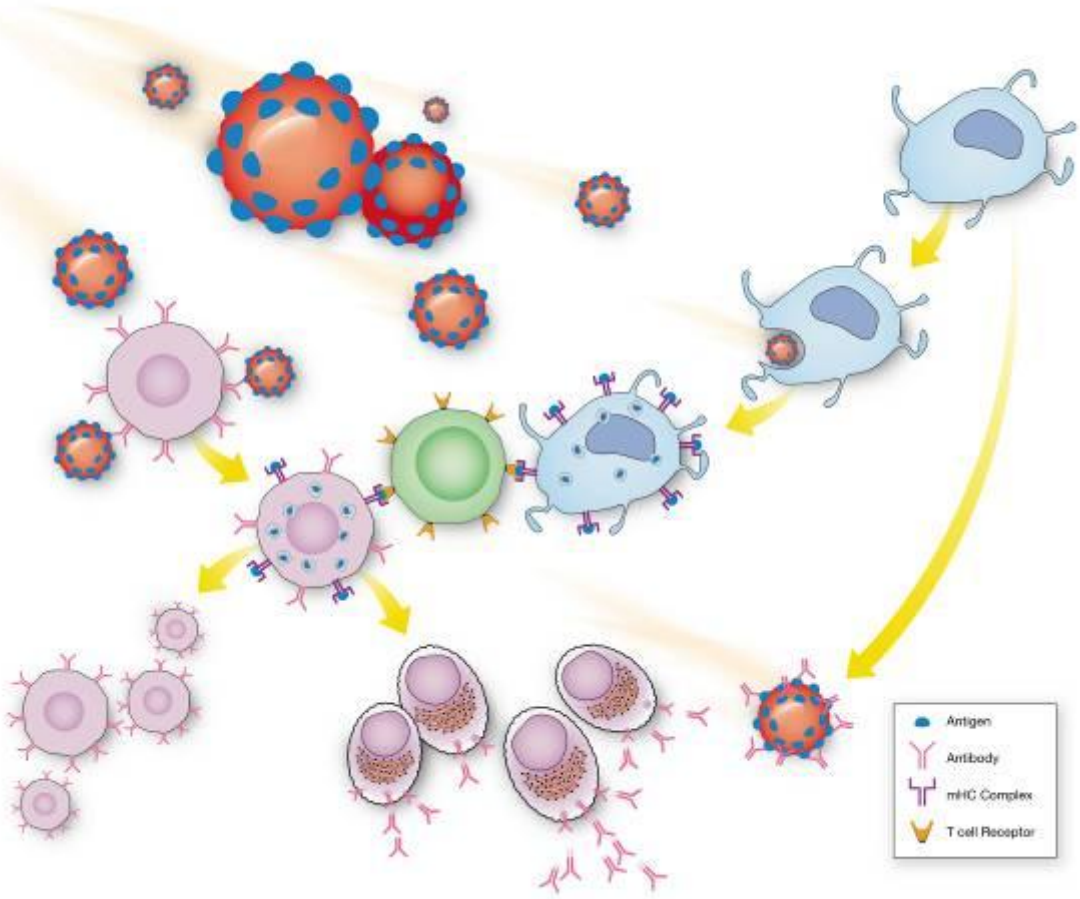
5% non-fat milk: Prevents the primary antibody from binding randomly to the membrane

Phosphate buffered saline (PBS): Provides the correct environment (pH, Salt) to maintain protein shape

0.025% Tween 20: non-ionic detergent that prevents non-specific binding of antibodies to the membrane

Using the mammalian immune system to produce antibodies

These antibodies are specific for our protein of interest



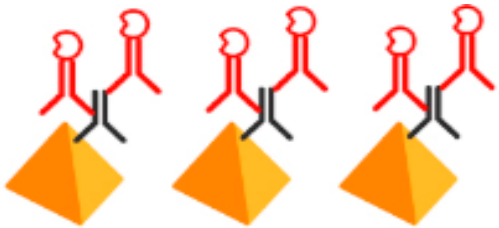
Use of antibodies as a diagnostic tool

- **Molecule of interest is injected into primary animal model**

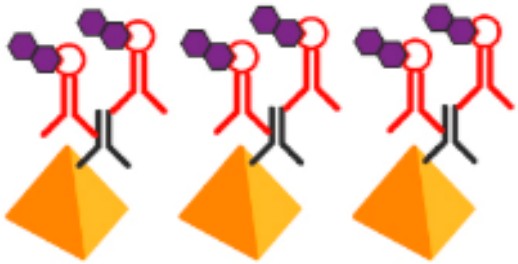
- **Animal makes antibodies against the molecule**
- **Antibodies are purified (primary antibody)**

Use of antibodies as a diagnostic tool

- **Antibodies from the first animal model are injected into a second animal model**
- **The second animal produces antibodies against the first antibody (secondary antibody)**

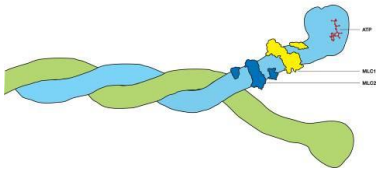


- **The secondary antibody is purified and conjugated to a colorimetric substrate or to an enzyme that can cleave a colorimetric compound**

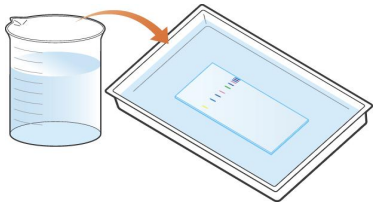


Add the Primary Antibody

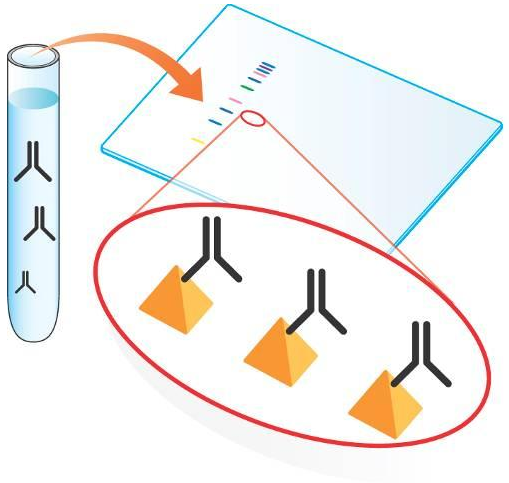
anti- myosin light-chain



Wash



- Discard blocking solution
- Pour 10ml of primary antibody onto the membrane and gently rock for 10 minutes
- Primary antibody will bind to the myosin light-chain



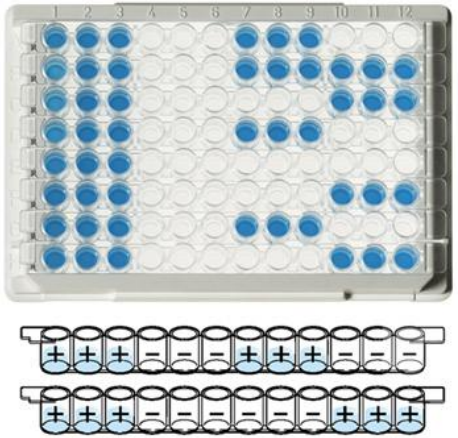
- Quickly rinse membrane in 50ml of wash buffer and discard the wash buffer
- Add 50ml of wash leave for 3 minutes on the rocking platform

ELISA

Enzyme-Linked Immunosorbant Assay

vs.

Western Blot

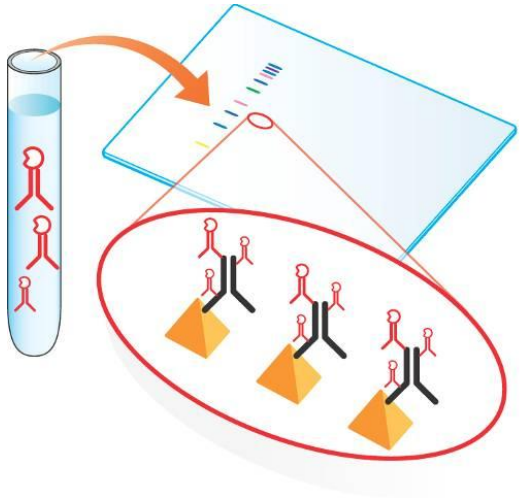


ELISA
- Quick results
- Primary screening
- Identifies proteins by antibody specificity only

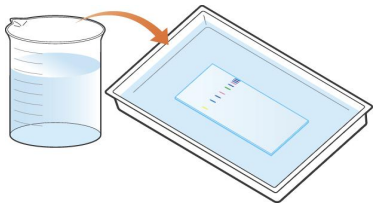
Western Blot
- Confirm ELISA results
- More specific
- Identifies proteins by both antibody specificity and size

Add Enzyme-linked Secondary Antibody

- Discard wash solution
- Pour 10ml of the secondary antibody onto the membrane and gently rock for 10 minutes
- Secondary antibody will bind to the primary antibody



Wash



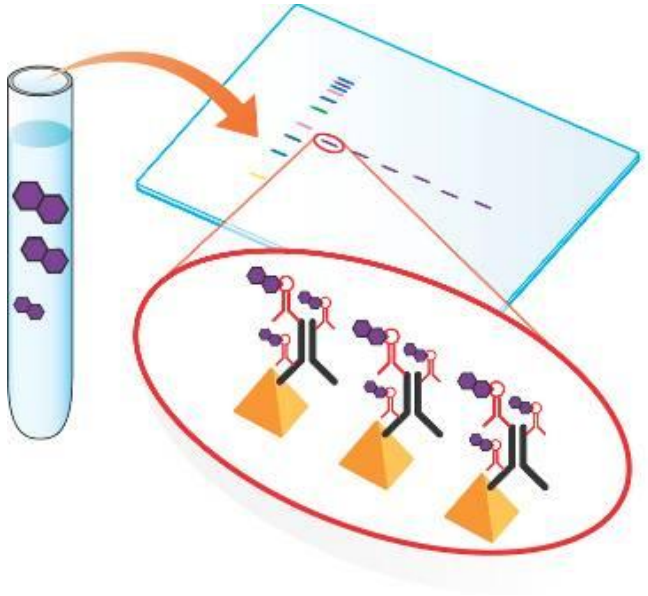
- Quickly rinse membrane in 50ml of wash buffer and discard the wash buffer
- Add 50ml of wash leave for 3 minutes on the rocking platform

Western Blot animation

Add Enzyme Substrate

- Discard wash solution
- Add 10ml of the enzyme substrate (HRP color detection reagent) onto the membrane
- Incubate for 10 minutes
- The colorimetric substrate is cleaved by the enzyme conjugated (attached) to the secondary antibody

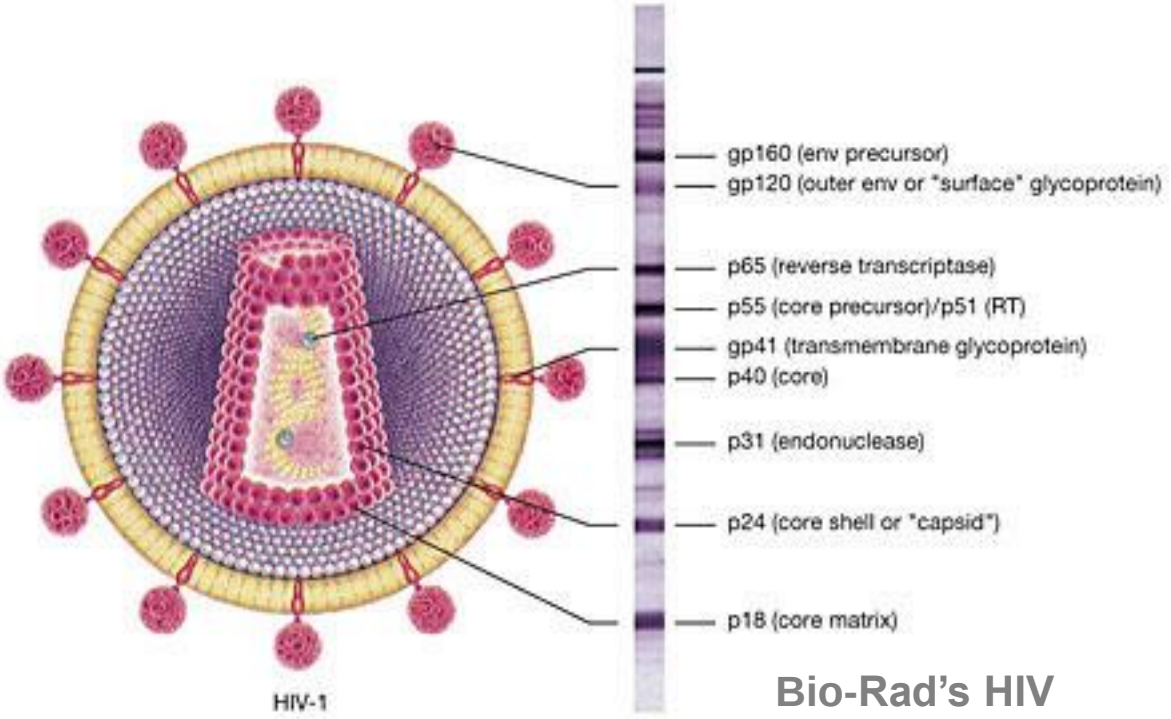
Watch for Color Development



Use of Antibodies in a Clinical Diagnostic Kits

HIV can be detected by ELISA or western blot technology. (Both of which are developed using the basis of the mammalian immune system) ELISA tests are very quick. Western Blot tests are slower and more expensive and are used for confirmatory tests.

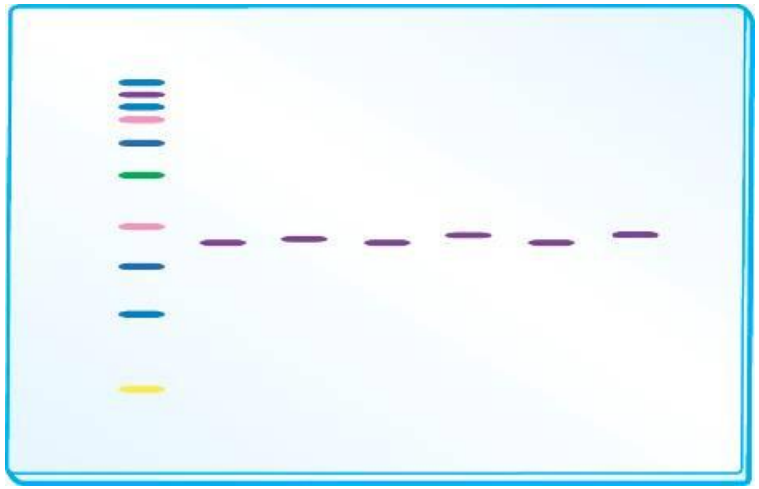
Bio-Rad's HIV-2 ELISA Kit



Bio-Rad's HIV Western Blot Kit

Rinse and Store

- Rinse the developed membrane twice with distilled water and blot dry
- Air dry for 30min-1hr and store in lab notebook



Western Blot Results

Blot from unstained Gel

Blot from stained Gel