Cihan University/ Sulaymaniya

College of Health Science

Medical Laboratory Analysis

4th Stage- 1st Semester

Pr. Clinical Immunology

Lab- 8: Western Blotting, or Immunoblotting

2023- 2024

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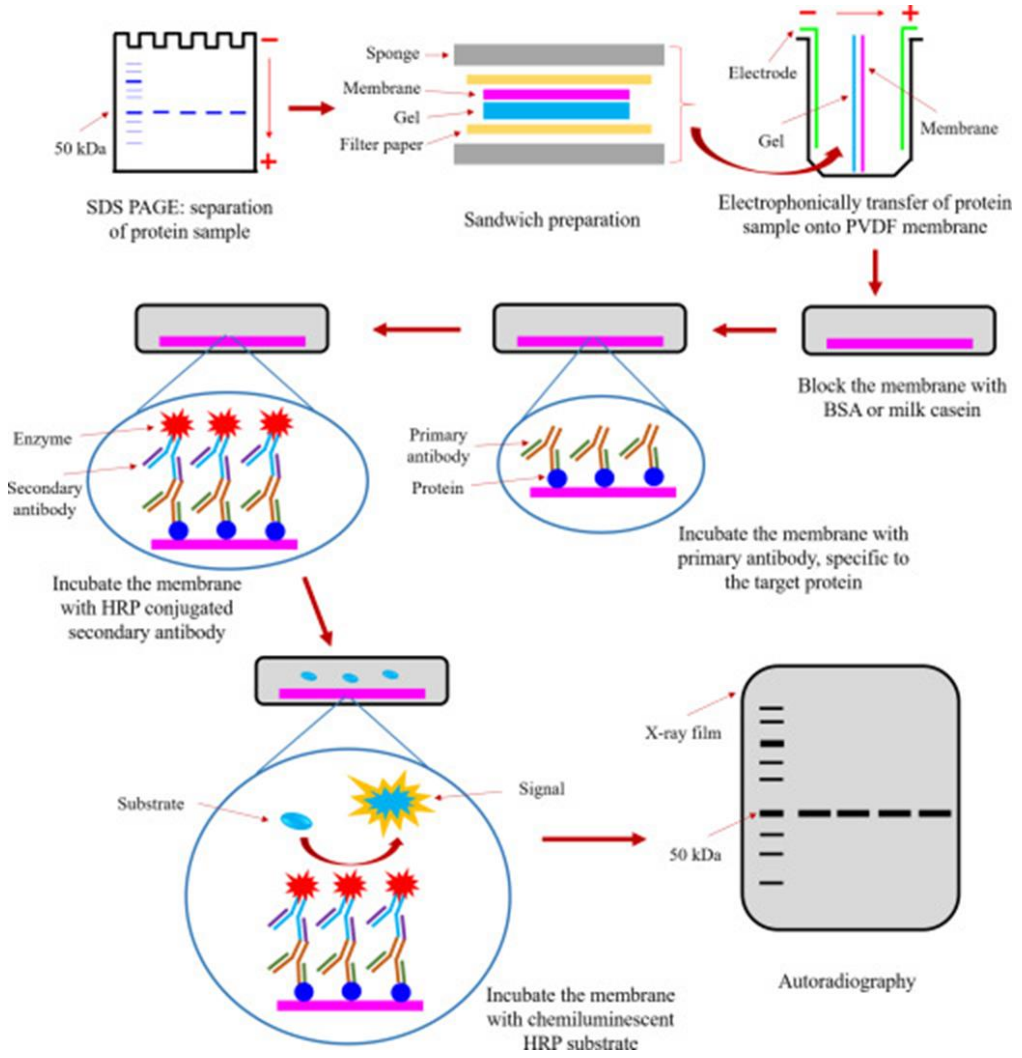
Western Blotting, or Immunoblotting

Western blotting is an important technique used in cell and molecular biology to identify specific proteins from a complex mixture of proteins extracted from cells.

The technique uses three elements to accomplish this task:

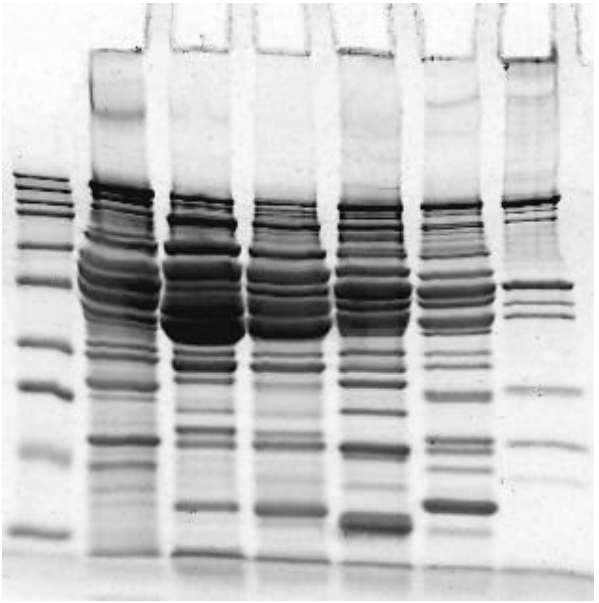
1. Separation by size,
2. Transfer to a solid support, and
3. Marking target protein using a proper primary and secondary antibody to visualize.



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

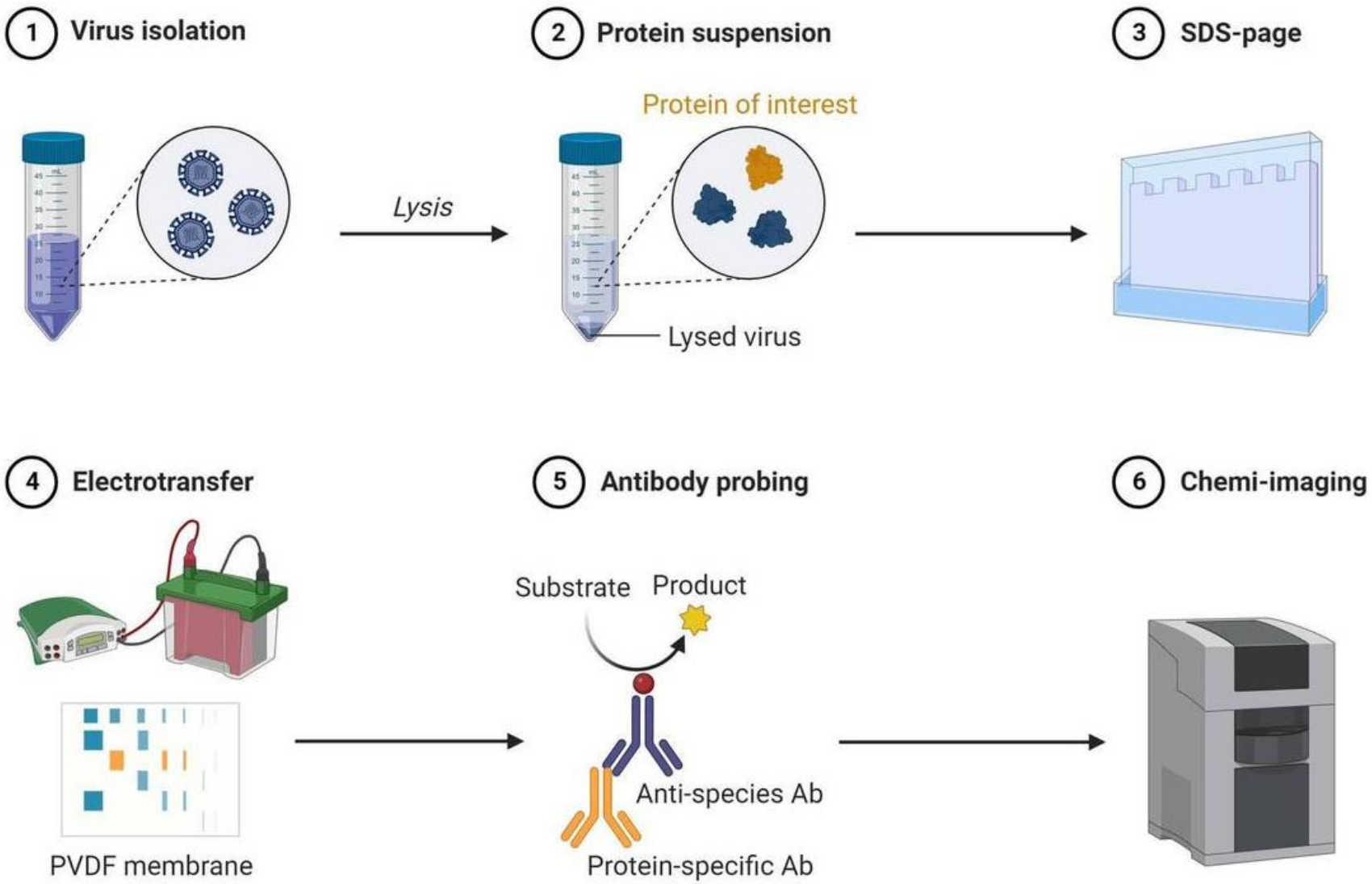
Laboratory Quick Guide

Western blotting is performed in six stages:

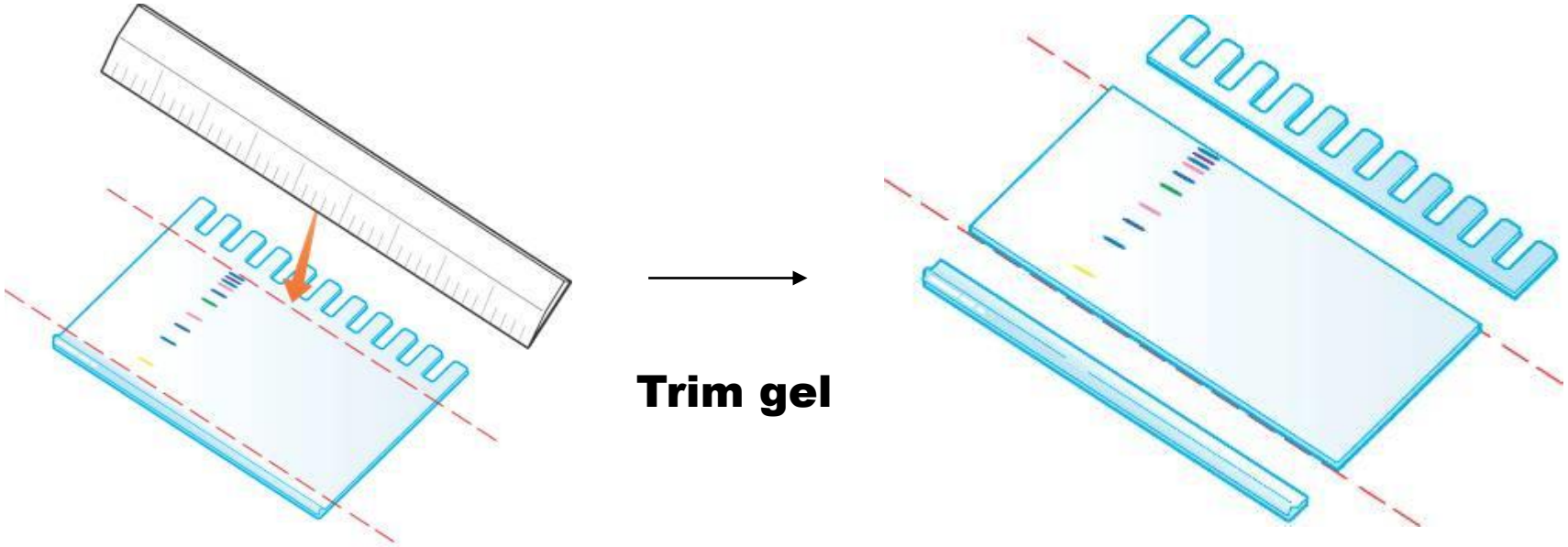
1. Sample preparation.
2. Protein separation by gel electrophoresis.
3. Protein transfer (electroblotting) onto a membrane.
4. Membrane blocking.
5. Immunodetection.
6. Visualization.

Western Blotting Experiment

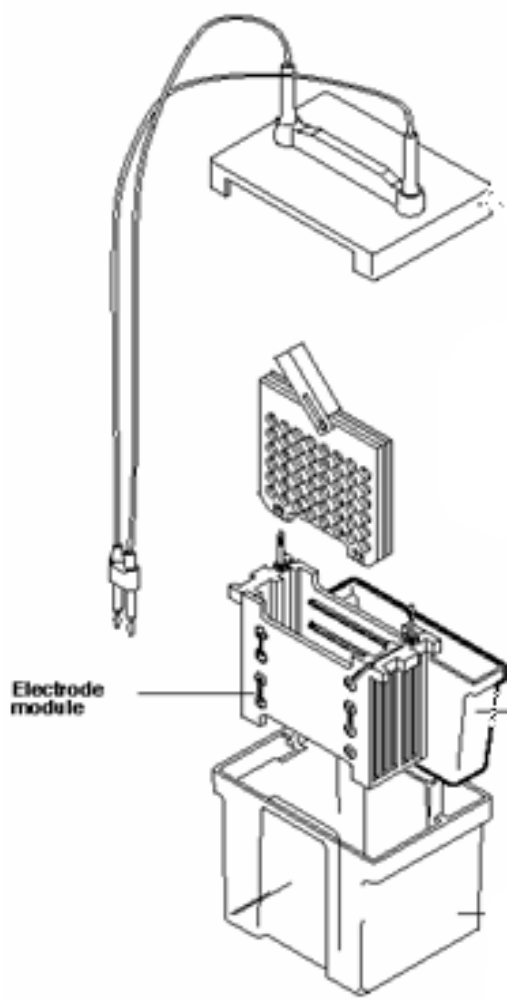
microbenotes.com

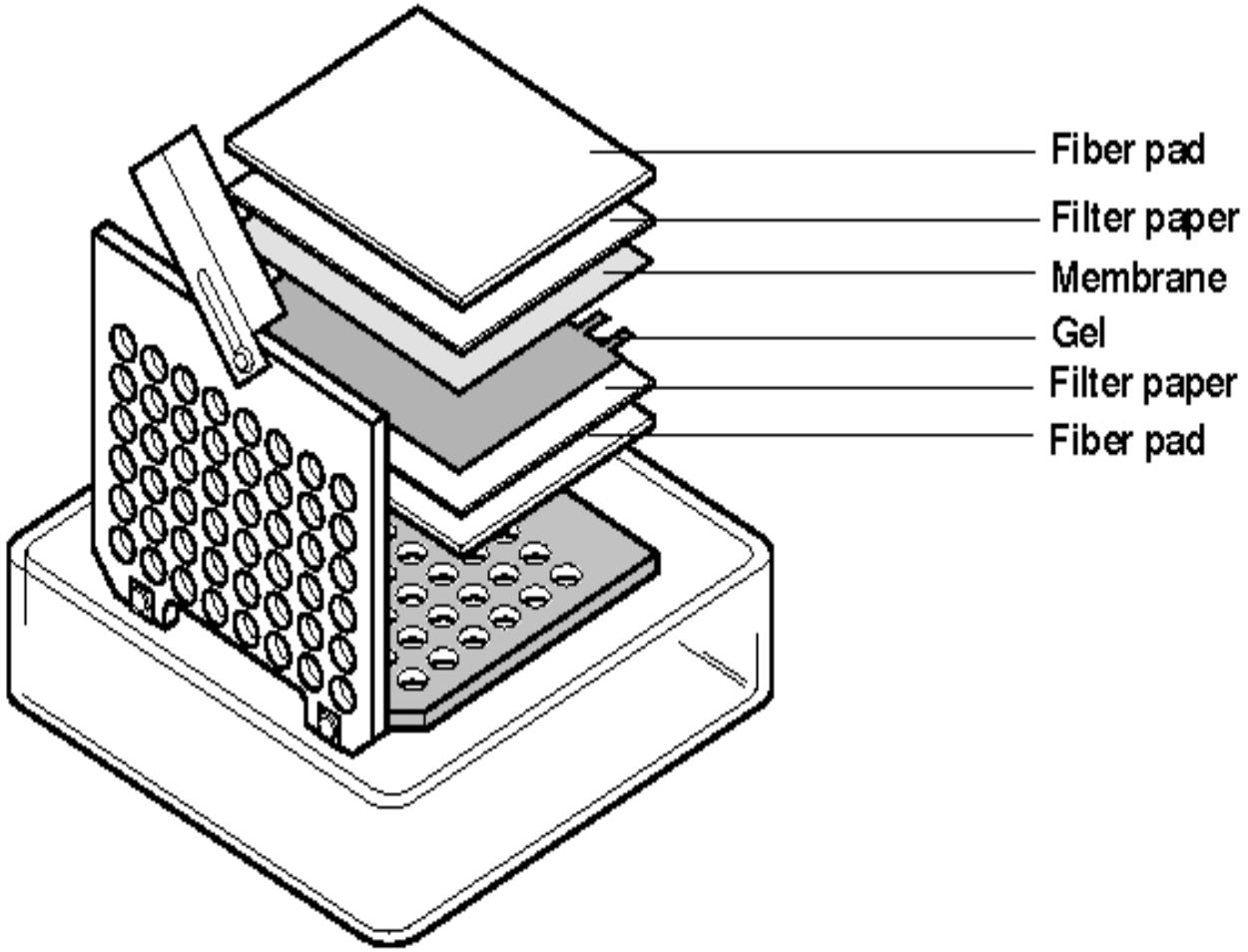


Prepare to transfer proteins to a Nitrocellulose membrane



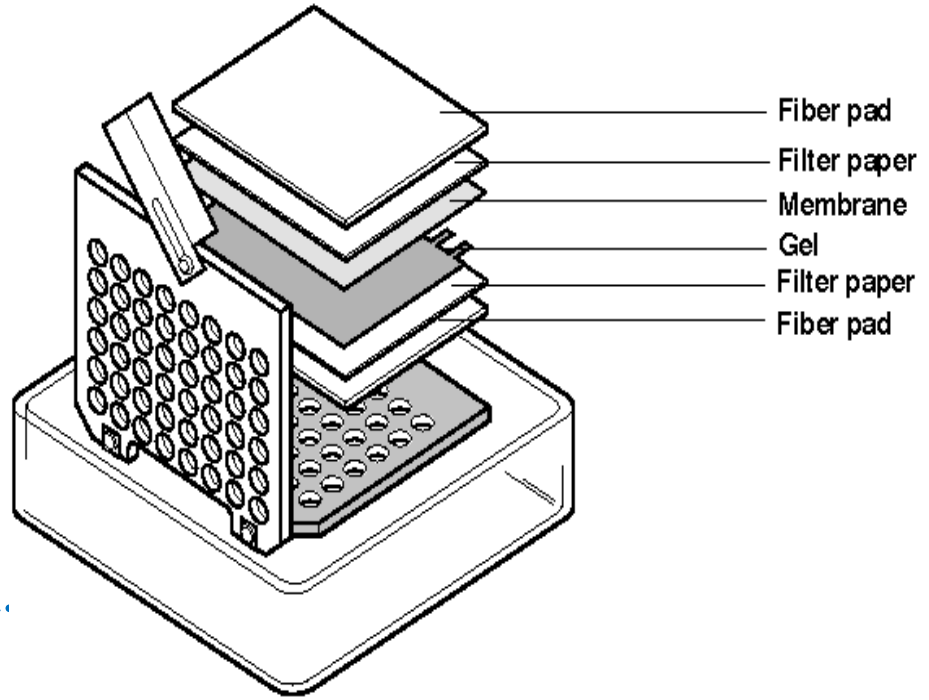
Mini Trans-Blot Transfer Cell





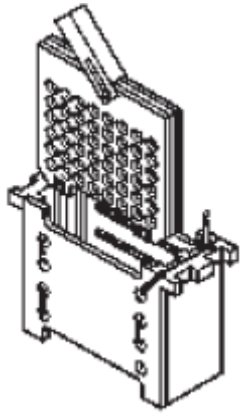
Preparing the Blotting Sandwich

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.



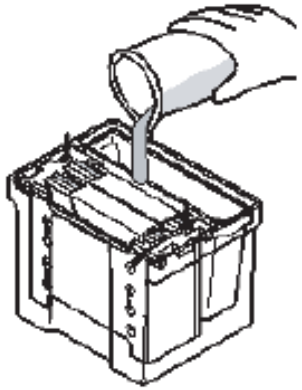
Prepare for Electrophoretic Transfer

1. Place the closed and locked cassette in the electrode module

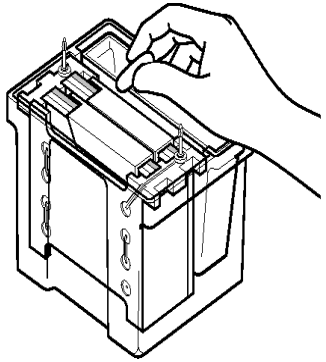


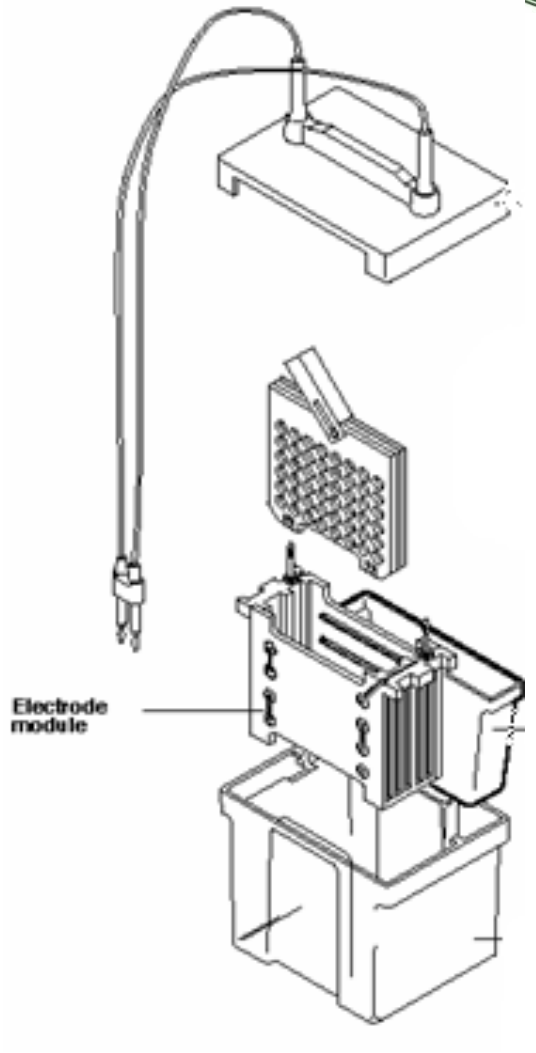
2. Add the frozen Bio-Ice cooling unit and place in tank,

3. Fill the tank with buffer,



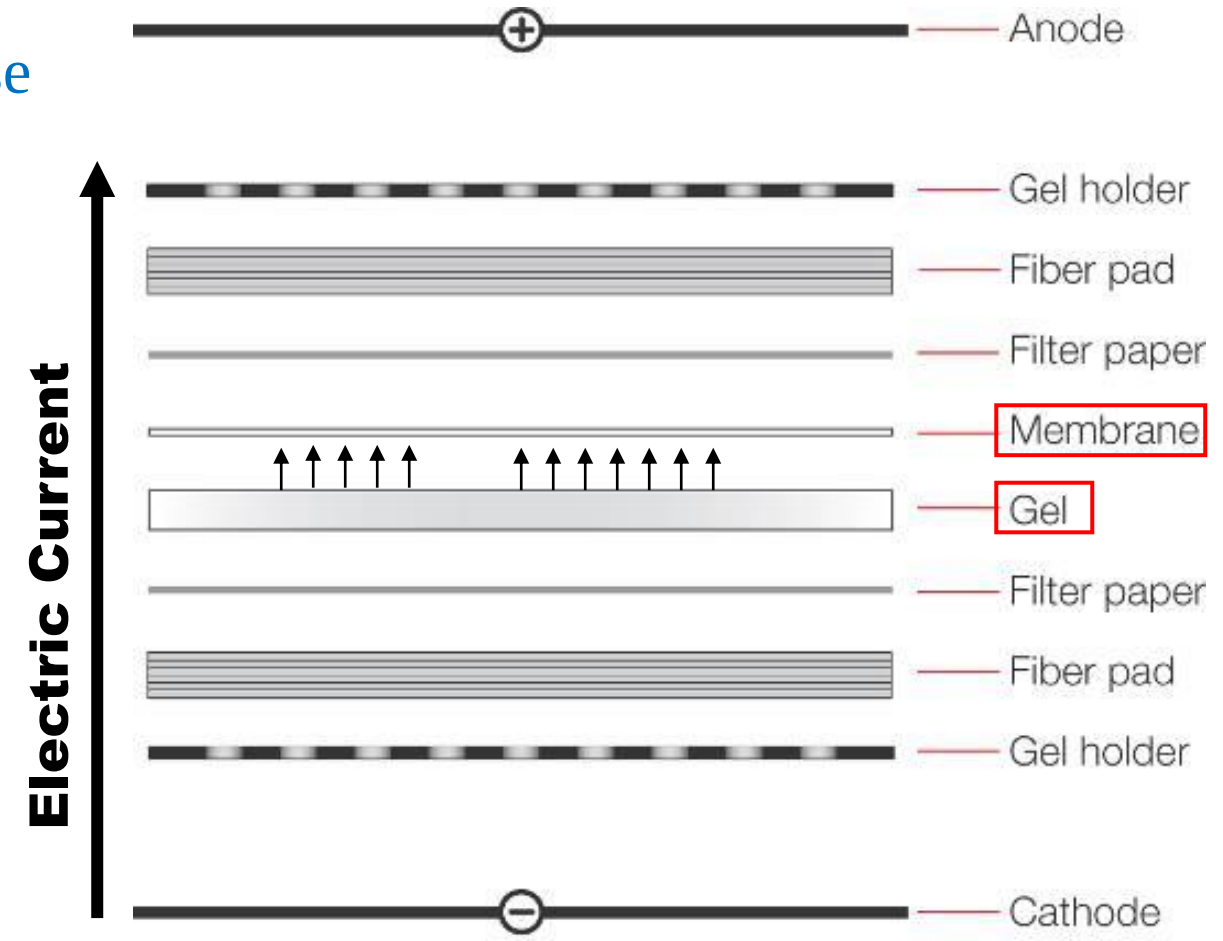
4. A stir bar can be added to help maintain the ion and temperature distribution in the tank even





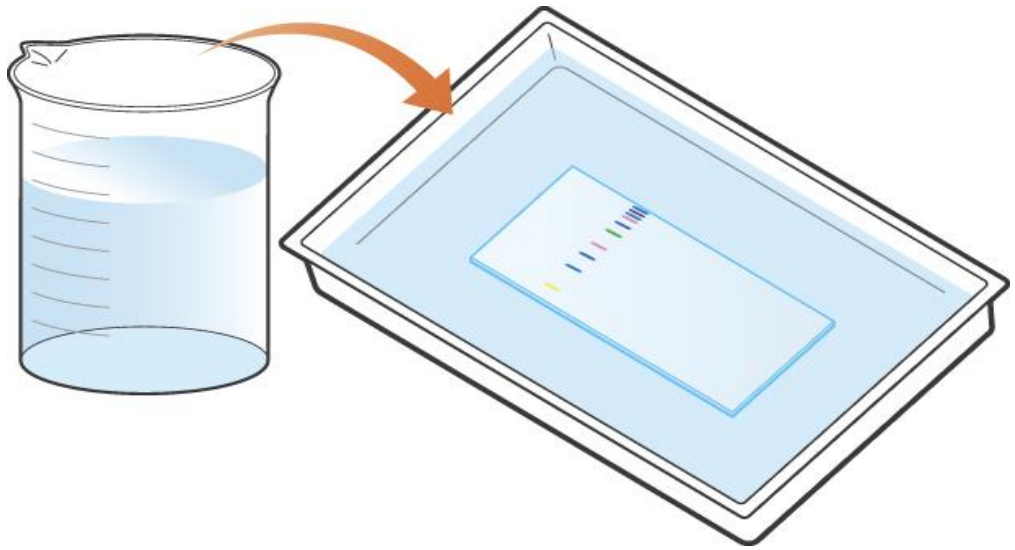
Transfer Proteins from the gel to the nitrocellulose membrane

- Transfer Proteins from the gel to the nitrocellulose membrane,
- 30 minutes
- 100V
- Blotting buffer 1x Tris glycine with 20% ethanol.



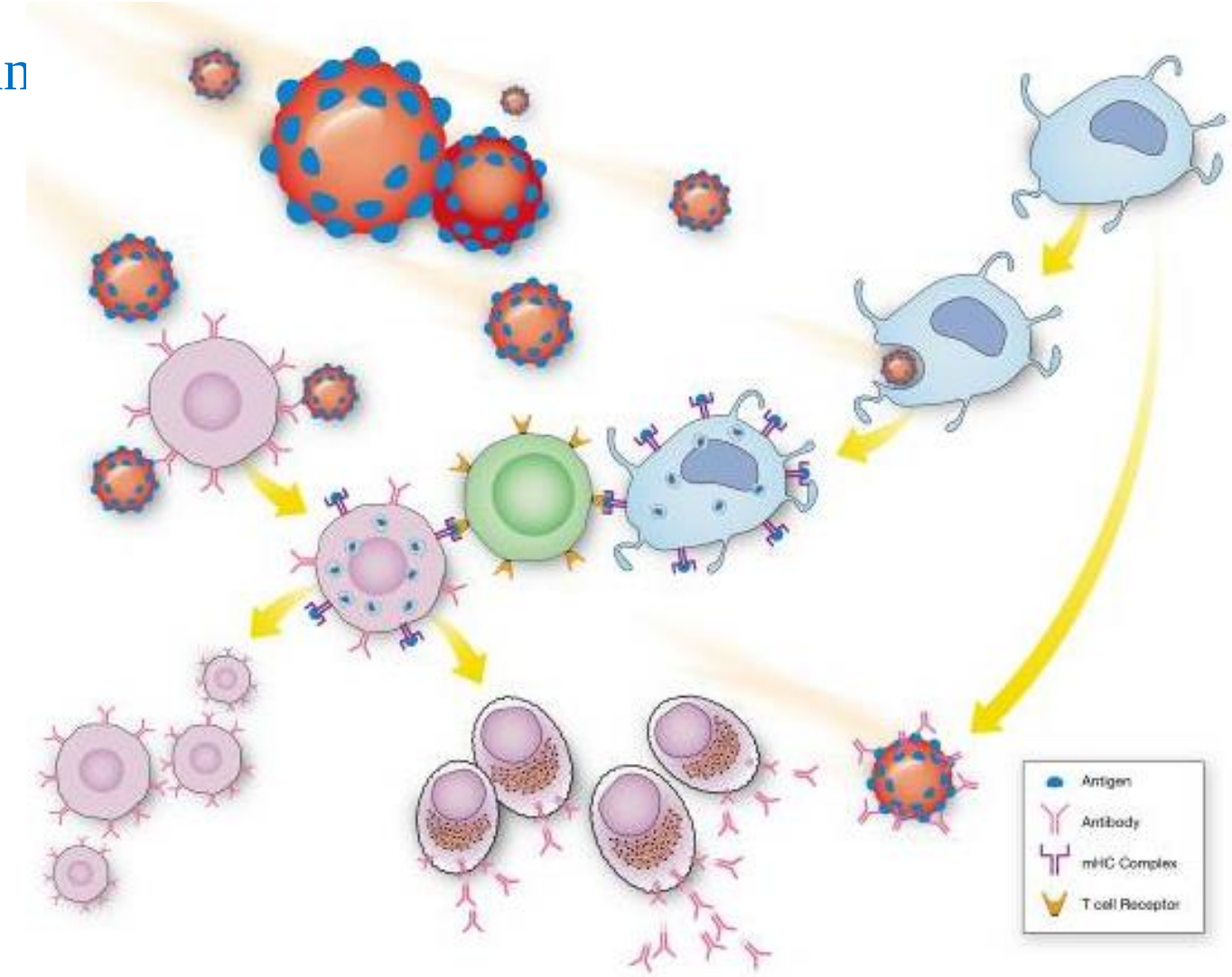
Blocking Buffer

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



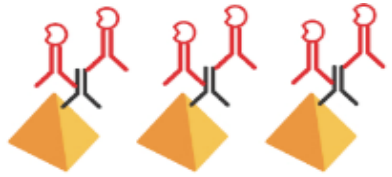
Use of Antibodies as A Diagnostic Tool

- Molecule of interest is injected into primary animal model.

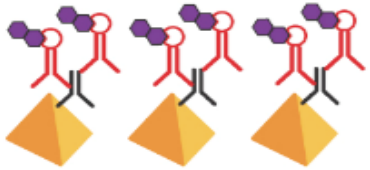
- Animal makes antibodies against the molecule.

- Antibodies are purified (primary antibody).

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

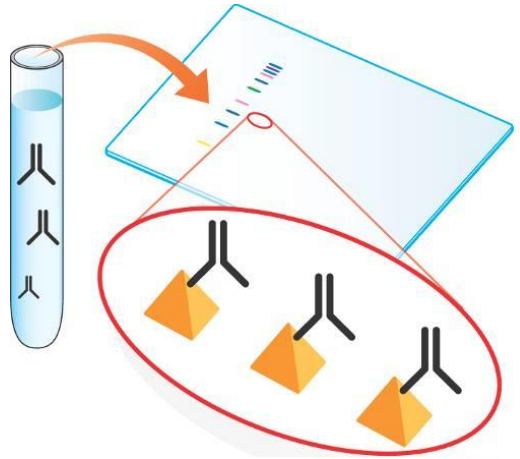


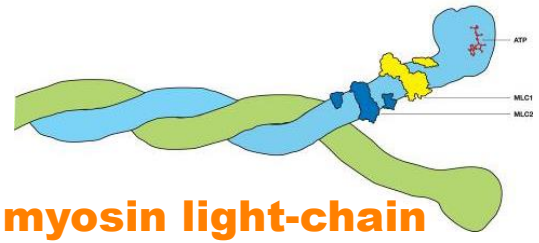
- The secondary antibody is purified and conjugated to a colorimetric substrate or to an enzyme that can cleave a colorimetric compound



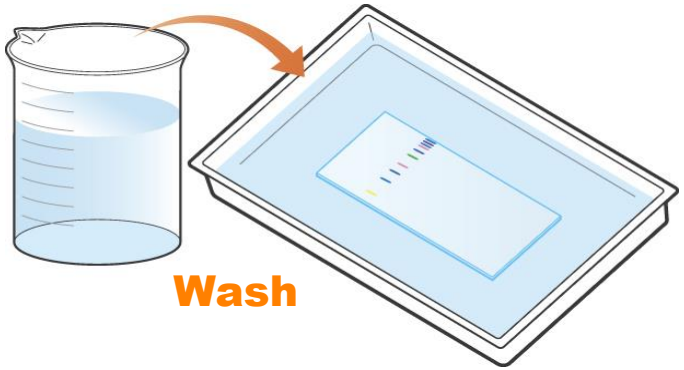
Add the Primary Antibody

- 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.





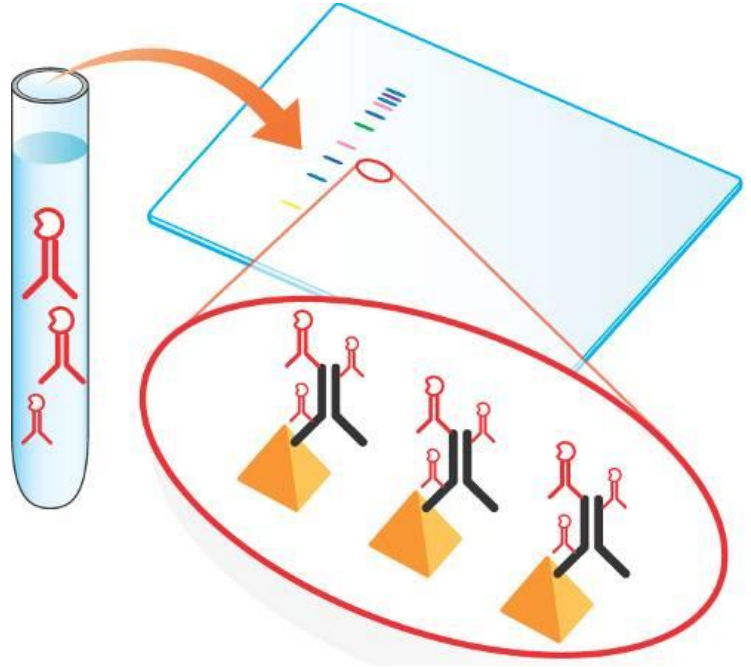
myosin light-chain

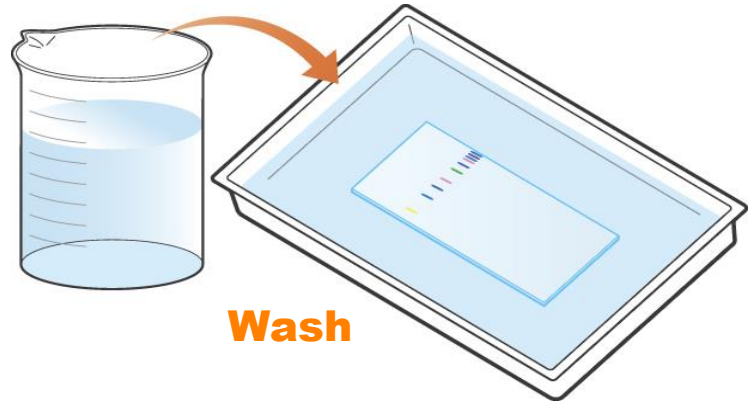


Wash

Add Enzyme-linked Secondary Antibody

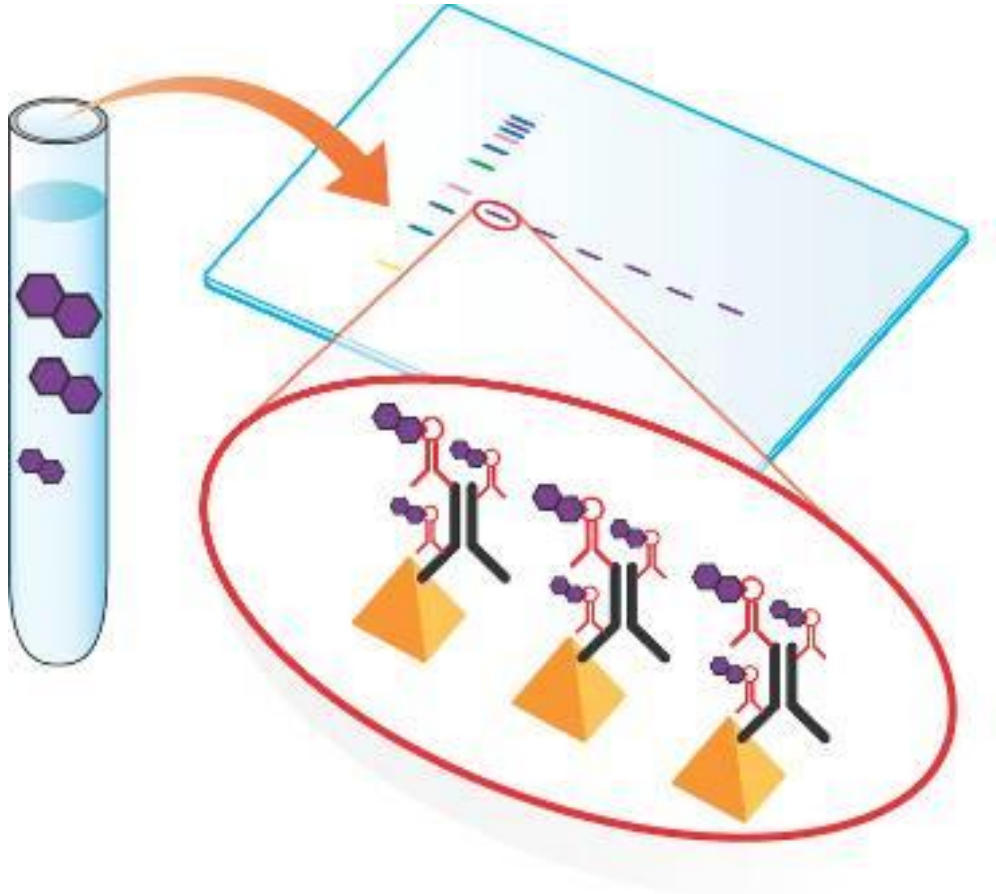
- Discard wash solution,
- Pour 10ml of the secondary antibody onto the membrane and gently rock for 10 minutes,
- Secondary antibody will bind to the primary antibody,
- Quickly rinse membrane in 50ml of wash buffer and discard the wash buffer,
- Add 50ml of wash leave for 3 minutes on the rocking platform





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,

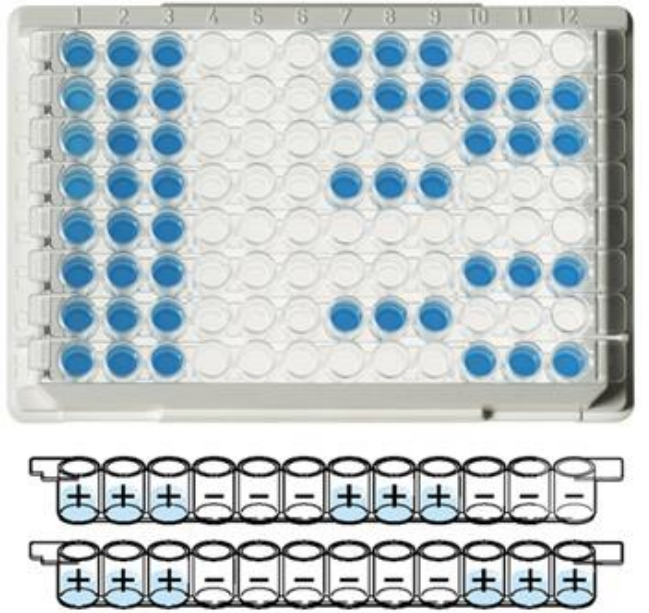


Watch for Color Development

Enzyme-Linked Immunosorbent Assay vs. Western Blot

ELISA

- Quick results,
- Primary screening,
- Identifies proteins by antibody specificity only.



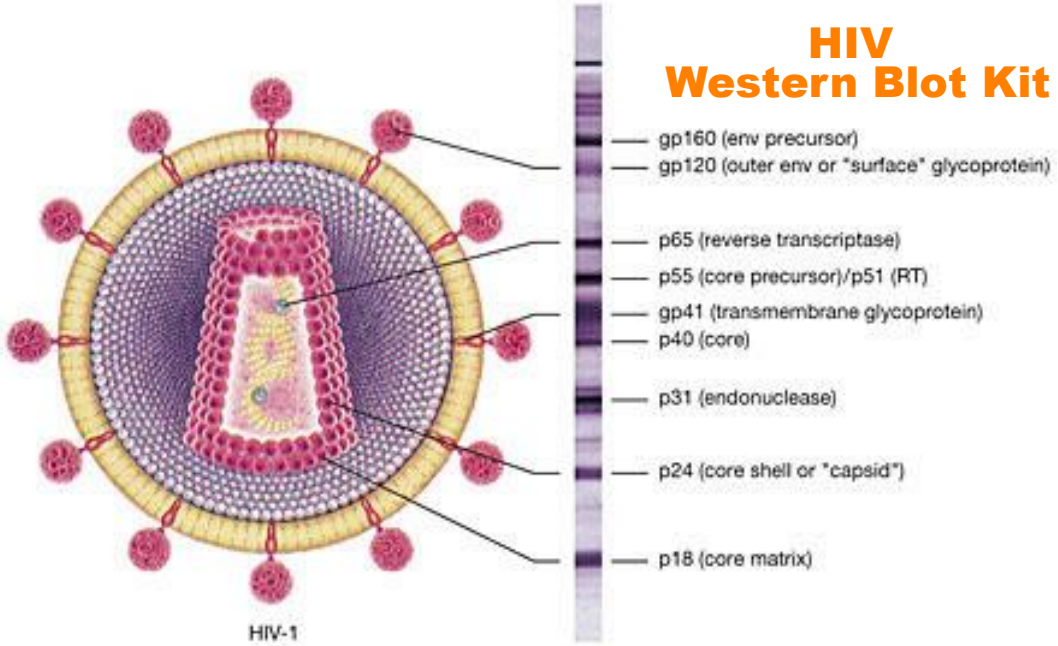
Western Blot

- Confirm ELISA results,
- More specific,
- Identifies proteins by both antibody specificity and size.

Use of Antibodies in a Clinical Diagnostic Kits

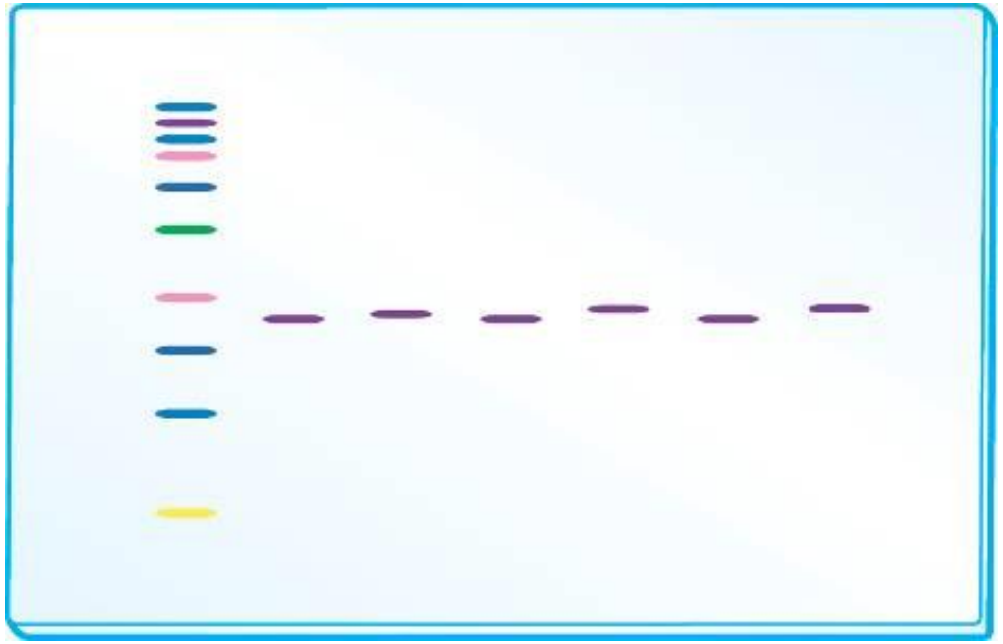
- HIV can be detected by ELISA or western blot technology.
- (Both of which are developed using the basis of the mammalian immune system)
- ELISA tests are very quick.
- Western Blot tests are slower and more expensive and are used for confirmatory tests.

HIV-2
ELISA 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.



References

- Mahmood, T., & Yang, P. C. (2012). Western blot: technique, theory, and trouble shooting. North American journal of medical sciences, 4(9), 429–434. https://doi.org/10.4103/1947-2714.100998.
- Singh, K. K., Gupta, A., Bharti, C., & Sharma, H. (2021). Emerging techniques of western blotting for purification and analysis of protein. Future Journal of Pharmaceutical Sciences, 7(1), 1-14.
- https://www.youtube.com/watch?v=OkH8u84t84M
- http://www.bio rad.com/webroot/web/pdf/lsr/literature/Bulletin_6376.pdf.
- https://microbenotes.com/western-blot/