Cihan University/ Sulaymaniya

College of Health Science

Medical Laboratory Analysis

4th Stage- 1st Semester

Pr. Clinical Immunology

Lab- 8: Western Blotting, or Immunoblotting

2024- 2025

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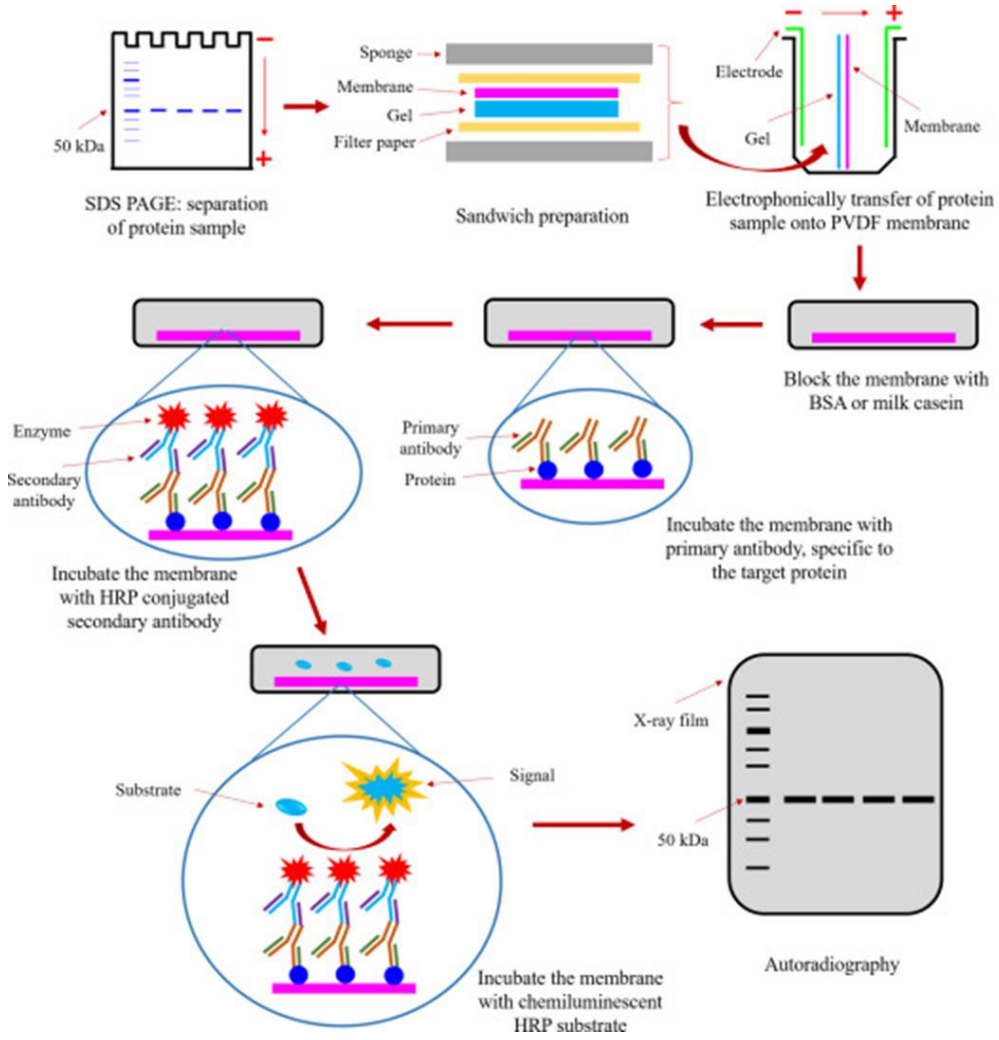
https://www.youtube.com/watch?v=OkH8u84t84M

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:

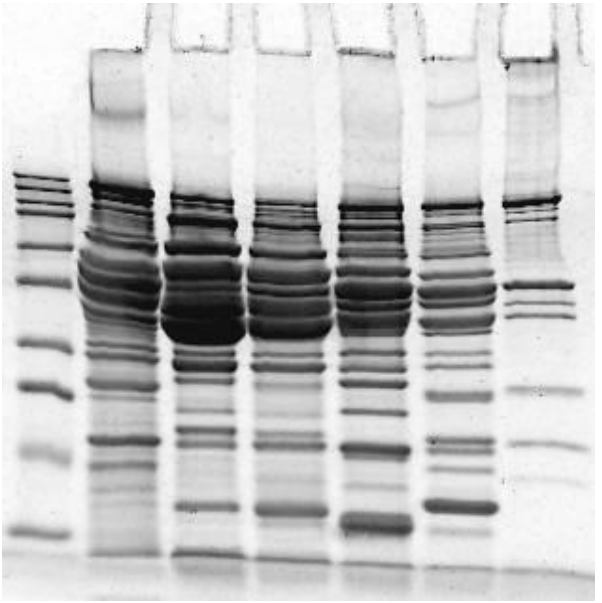
1. Separation by size,
2. Transfer to a solid support, and
3. Marking target protein using a proper primary and secondary antibody to visualize.



From Gel to Blot

Polyacrylamide Gel Electrophoresis:

1. Break protein complexes into individual proteins.
2. Separates protein samples based on size.

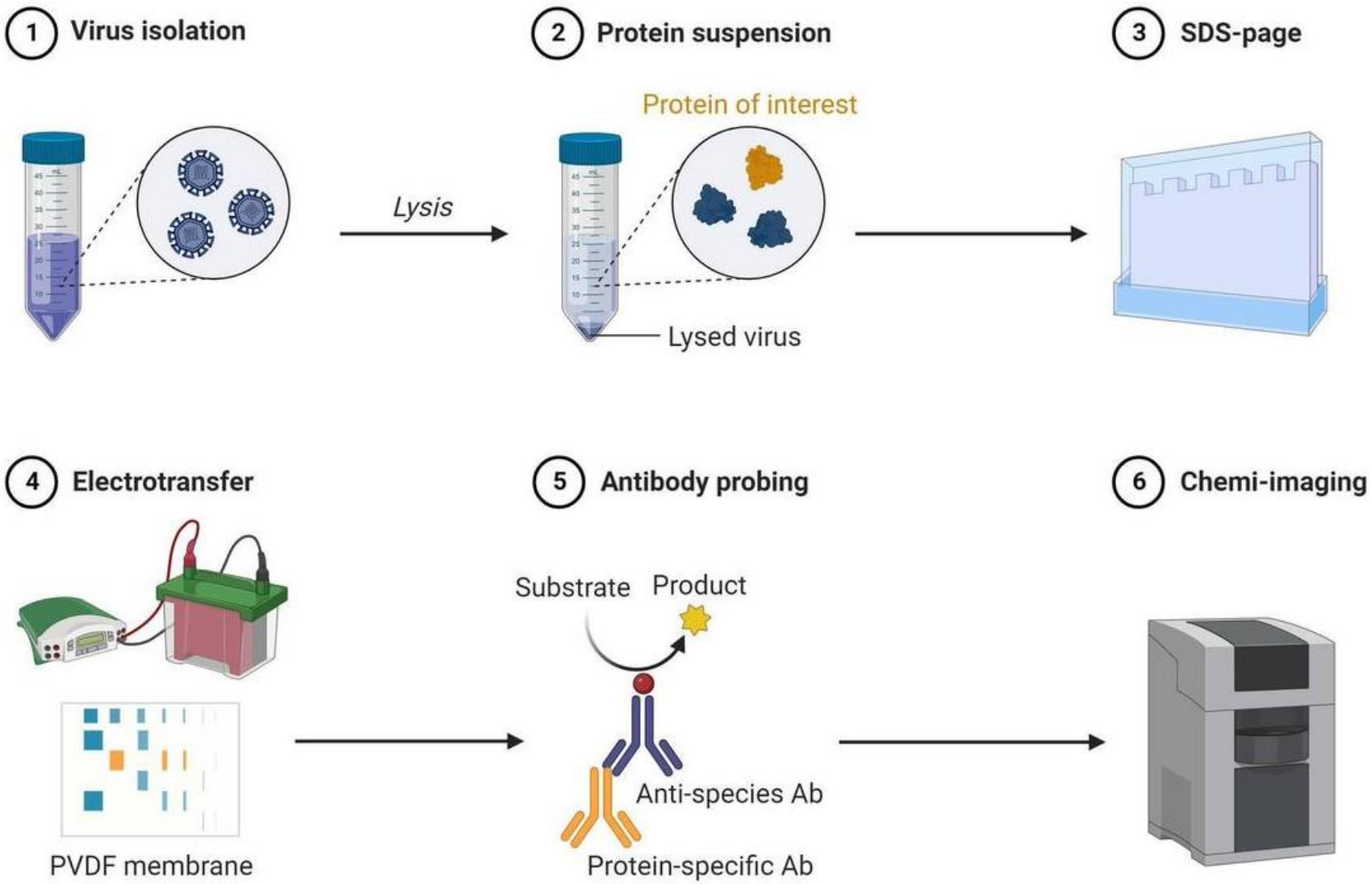


Western Blot Analysis:

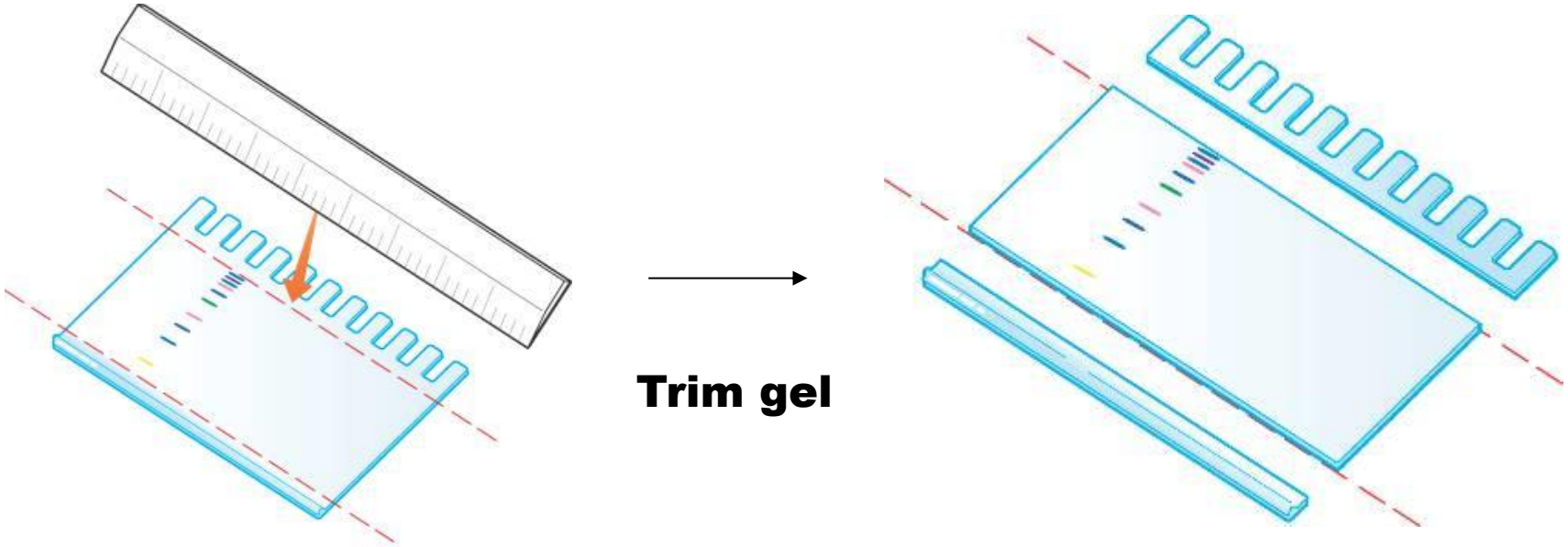
1. Transfer the proteins to a nitrocellulose membrane.
2. More stable and permanent.
3. Identifies proteins by immunodetection: using specific antibodies against the protein of interest

Western Blotting Experiment

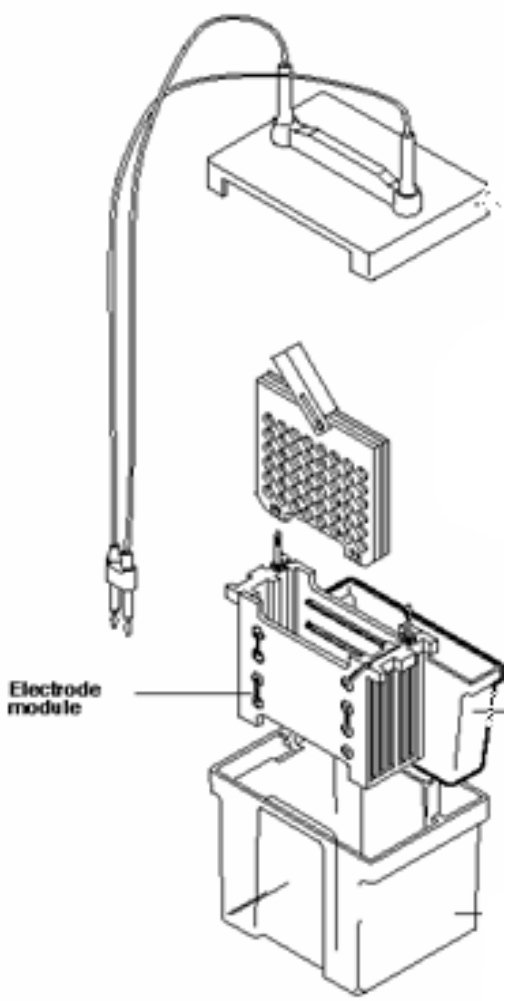
microbenotes.com



Prepare to transfer proteins to a Nitrocellulose membrane



Mini Trans-Blot Transfer Cell



Prepare transfer buffer

Prepare transfer buffer sufficient for the transfer tank and for equilibration of gels and membranes.

Equilibrate gels and membranes

Equilibrate gels and membranes in transfer buffer.

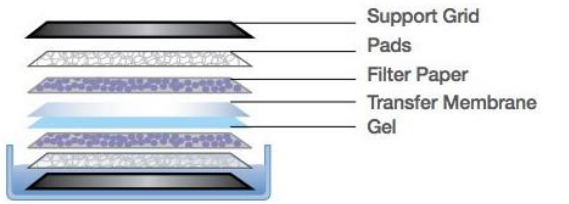
Assemble the gel and membrane sandwich

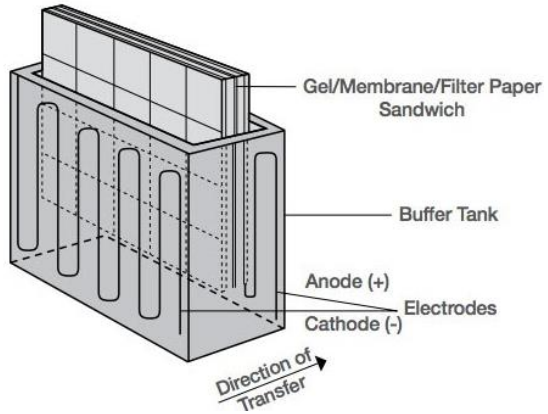
Place the membrane and gel between buffer-soaked filter papers.

Set up the transfer cell

Place the gel, membrane and filter paper sandwich in the transfer tank. Fill the tank with transfer buffer. Connect the tank to the power supply and set the power supply for optimal power and time.

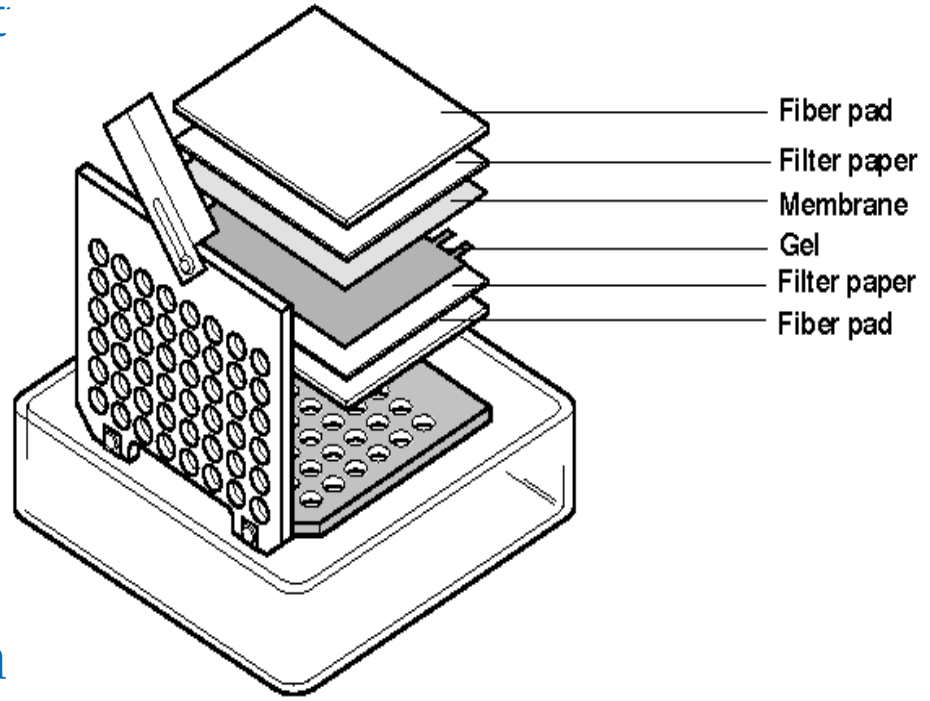
Start the transfer





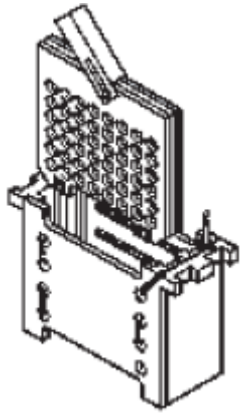
Preparing the Blotting Sandwich

1. Place the cassette with gray side down on clean surface.
2. Place one pre-wetted fiber pad on the gray side of the casset
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.



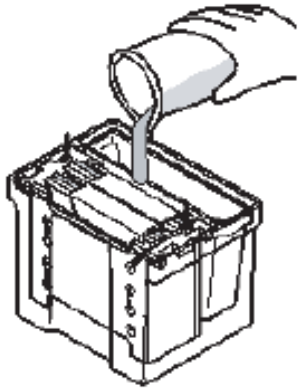
Prepare for Electrophoretic Transfer

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

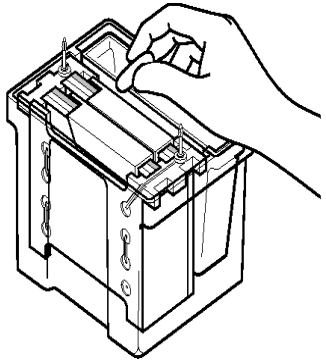


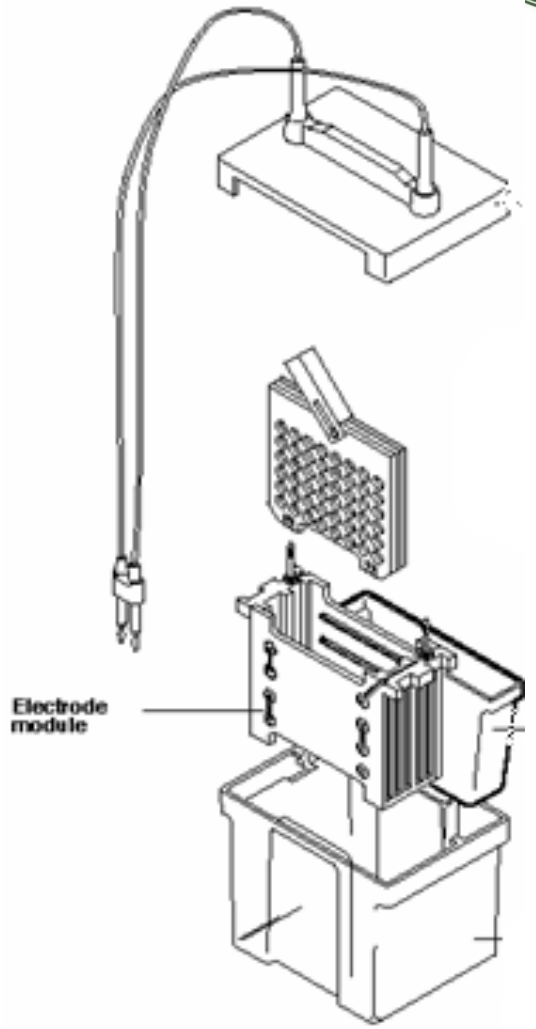
2. Add the frozen Bio-Ice cooling unit and place in tank,

3. Fill the tank with buffer,



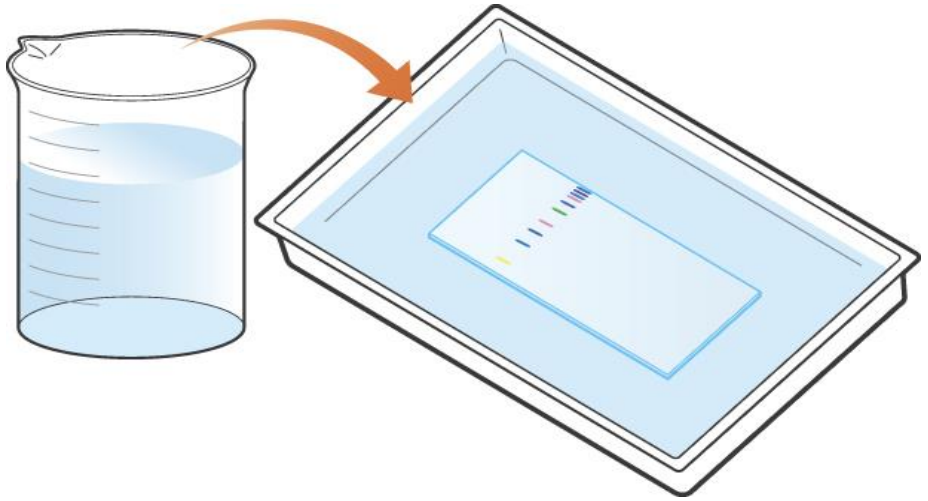
4. A stir bar can be added to help maintain the ion and temperature distribution in the tank even





Blocking Buffer

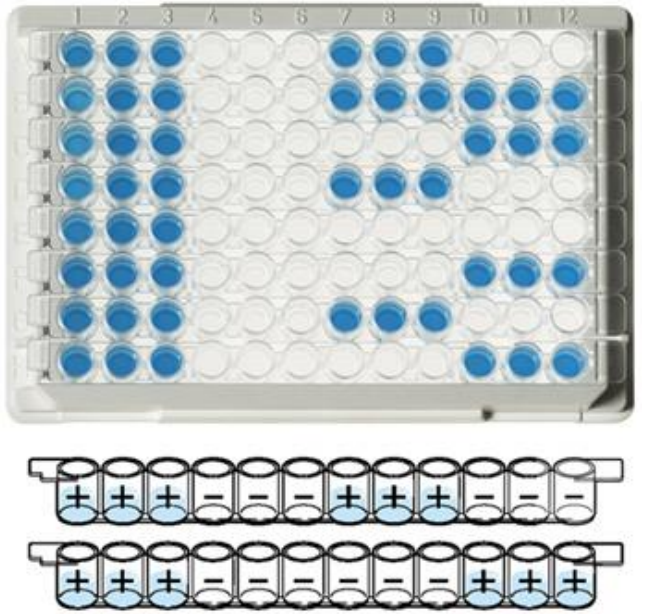
- Remove membrane from the blotting sandwich and immerse in 25ml of blocking solution for 15 minutes.
- **5% non-fat milk:** Prevents the primary antibody from binding randomly to the membrane.
- **Phosphate buffered saline (PBS):** Provides the correct environment (pH, Salt) to maintain protein shape
- **0.025% Tween 20:** non-ionic detergent that prevents non-specific binding of antibodies to the membrane.



Enzyme-Linked Immunosorbent Assay vs. Western Blot

ELISA

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

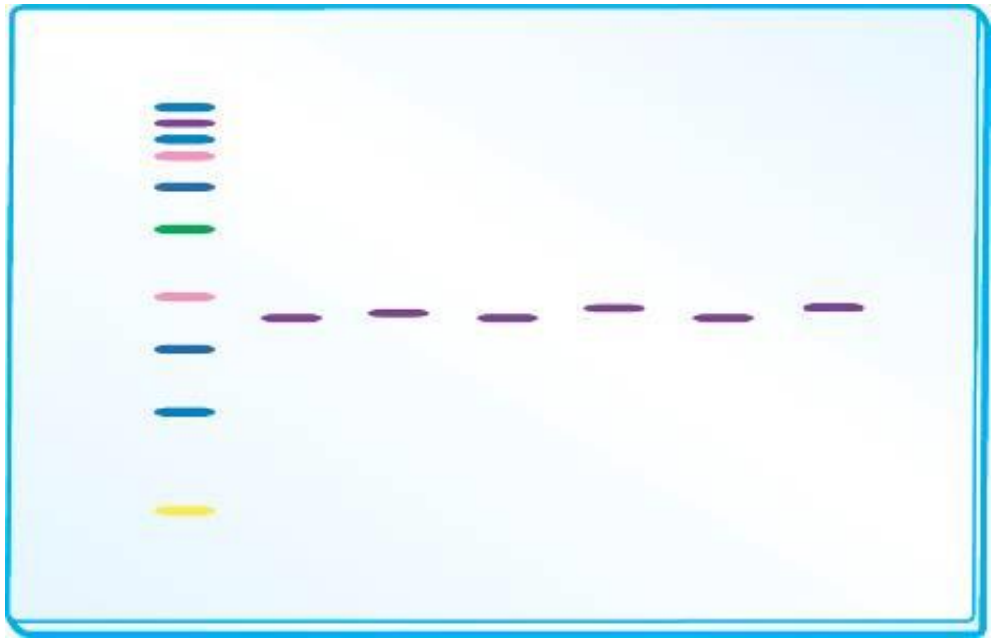


Western Blot

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

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.
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- http://www.bio rad.com/webroot/web/pdf/lsr/literature/Bulletin_6376.pdf.
- https://microbenotes.com/western-blot/