# Experiment (5): Western Blot

## Aims:

- To understand how proteins (antigens) can be analysed using antibodies raised against these proteins by Immunoblotting technique.
- To understand the steps in the development of western and antigen-antibody interaction and detection.
- Electroblotting the pre-stained maker.

## Introduction:

Immunoassay is a test that uses the highly specific and selective antigen-antibody reactions forming antibody and antigen complexes [immuno-complexes] as a means of generating measurable results. Western blot (also called protein immunoblot) is a widely used immunoassay technique, used to identify proteins specific proteins [antigens] in a sample of tissue homogenate or extract, based on their ability [the antigens] to bind to antibodies resulting in colour indicate the presence of this specific protein. [1,2]

Western blot has various applications for research use such as protein expression level, epitope mapping and to detect the phosphorylation signal and structure domain analysis.[3]

- PAUSE AND THINK ➔ Is there other reactions with high specificity than antigen-antibody reactions?

## Principle:

The mixture of proteins is separated based on molecular weight, and thus by type, through SDS-PAGE. These results are then electro-transferred to a nitrocellulose polyvinylidenedifluoride (PVDF), or nylon membrane producing a band for each protein. The transferred protein is detected by incubating the gel with specific primary antibody to the protein of interest, secondary antibody labelled with an enzyme which target the primary antibody, and substrate which in the end you will get coloured product. Alkaline phosphatase (AP) and horseradish peroxidase (HRP) are the two enzymes used most extensively as labels for protein detection. The colour indicates the presence of the protein of interest. The thickness of the band corresponds to the amount of protein present; thus doing a standard can indicate the amount of protein present. [4]

Thus, the molecular weight and amount of the desired protein can be characterized from a complex mixture of proteins by western blotting.

## Western blot performing steps:

The technique uses three elements to accomplish this task: (1) separation by size using SDS-PAGE, (2) transfer to a solid support (electro-blotting), and (3) marking target protein using a proper primary and secondary antibody to visualize.

- **1st phase (SDS-PAGE):** A protein sample is subjected to polyacrylamide gel electrophoresis. The separation of the sample can be confirmed by: 1.Replica of the gel and stain it as usual [with Coomassie brilliant blue R-250] , 2.prestained marker and 3.Ponceau S.

- **2nd phase (Electro-blotting):** After that the gel is placed over a sheet of nitrocellulose, the protein in the gel is electrophoretically transferred to the nitrocellulose membrane. The transfer can be done by wet method or semi-wet method. This done by Creating a transfer sandwich: filter papers-gel-nitrocellulose membrane-filter papers. The filter papers, gel and nitrocellulose membrane will soaked in transfer buffer. Because the samples in the gel are negatively charged, the applied electric current will facilitate their transferring to nitrocellulose membrane, the samples will move toward the anode. Also, the capillary action has its effect in the movement of the samples from the gel to the nitrocellulose membrane.

- **3rd phase (Marking target protein to visualize):** The nitrocellulose is then soaked in blocking buffer to block the nonspecific binding of the proteins. The nitrocellulose is then incubated with the specific primary antibody for the protein of interest. After that the nitrocellulose will washed and then incubated with a second antibody, which is specific for the primary antibody. The second antibody will typically have a covalently attached enzyme which, when provided with a chromogenic substrate, will cause a color reaction (detection step) by converting a colorless substrate to a colored product. Several substrates can be converted to colored precipitate "product" by (AP) and (HRP) enzymes. As the precipitate accumulate on the membrane, a visible band develops.

## Buffer preparations

- **1X Transfer buffer:**
  28.8 g of glycine + 6.04 g of tris-base + 200 ml ethanol and complete the volume to 1L with d.$H_2O$.

- **Blocking buffer:**
  0.1%v/v Tween 20 in tris-buffer saline (TBS)**.**

- **Tris-buffer saline (TBS):**
  100 mM Tric-HCl, pH 7.8 +0.9 w/v NaCl.

## Supporting materials:

- Performing western blot: http://www.youtube.com/watch?v=VgAuZ6dBOfs
- Ponceau S staining: http://www.youtube.com/watch?v=Jj_37cDsO7o

## References:


1. Sosnik A, Biomedical Applications of Functionalized Nanomaterials, 2018.
2. https://www.thermofisher.com/sa/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/overview-western-blotting.html
3. http://www.sinobiological.com/western-blot-applications-for-research.html
4. Mahmood, T., & Yang, P.-C. (2012). *North American Journal of Medical Sciences*, *4*(9), 429–34.