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FACULTY OF ENGINEERING & TECHNOLOGY  
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# Protein blotting

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- a Protein blotting is an analytical method that involves the immobilization of proteins on membranes before detection using monoclonal or polyclonal antibodies. There are different blotting protocols (dot blot, 2D blot); one of the most powerful is western blotting.



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# Principle of Western Blotting

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- a Western blotting is an Immunoblotting technique which rely on the specificity of binding between a molecule of interest and a probe to allow detection of the molecule of interest in a mixture of many other similar molecules.
  - a In Western blotting, the molecule of interest is a protein and the probe is typically an antibody raised against that particular protein.
  - a The SDS PAGE technique is a prerequisite for Western blotting .

# Western blot (Immunoblotting)

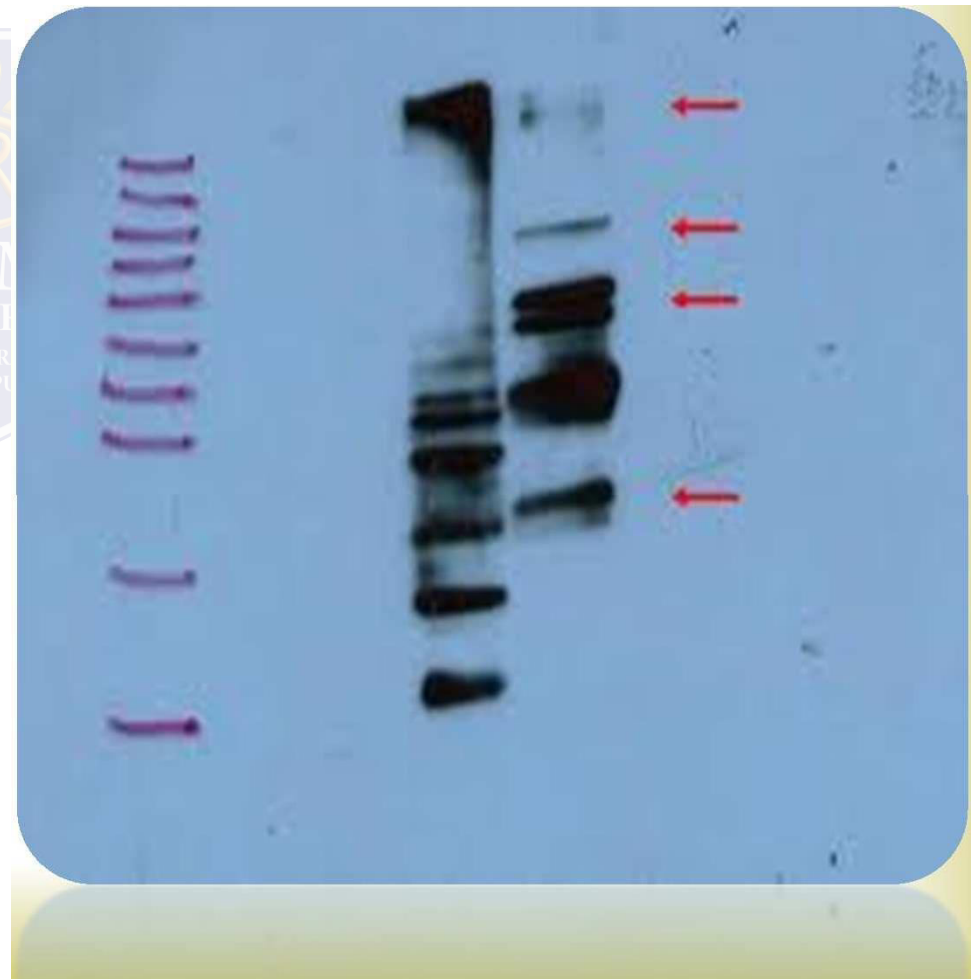
- a A technique for detecting specific proteins separated by electrophoresis by use of labeled antibodies. So called since it has some similarity to a Southern blot.



# Definition

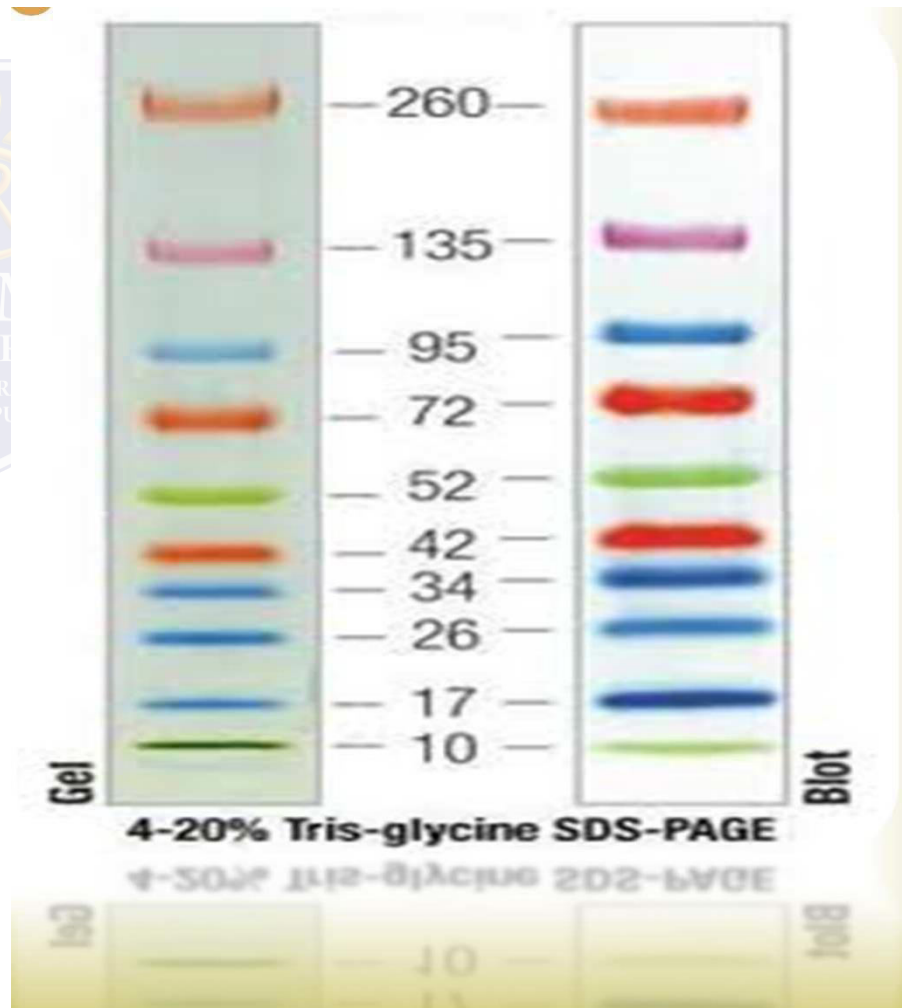
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a The Western Blot is an analytical technique used to detect specific proteins in a given sample of tissue homogenate or extract.



# Advantages of Western Blot

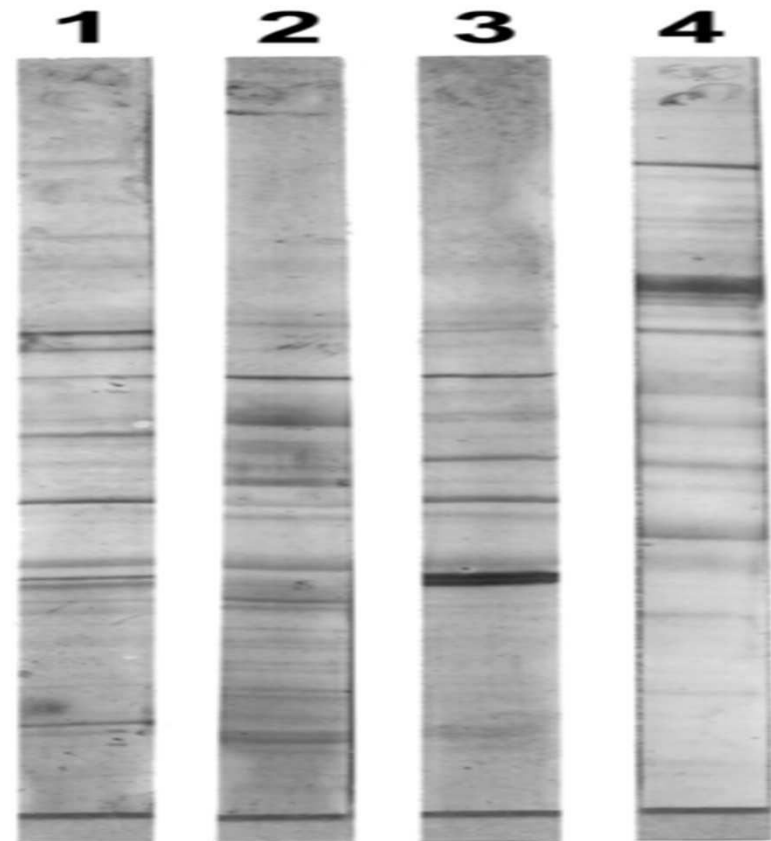
- a Western blot analysis can analyze any protein sample whether from cells or tissues, but also can analyze recombinant proteins synthesized in vitro. Western blot is dependent on the quality of antibody you use to probe for your protein of interest, and how specific it is for this protein



# Gel electrophoresis

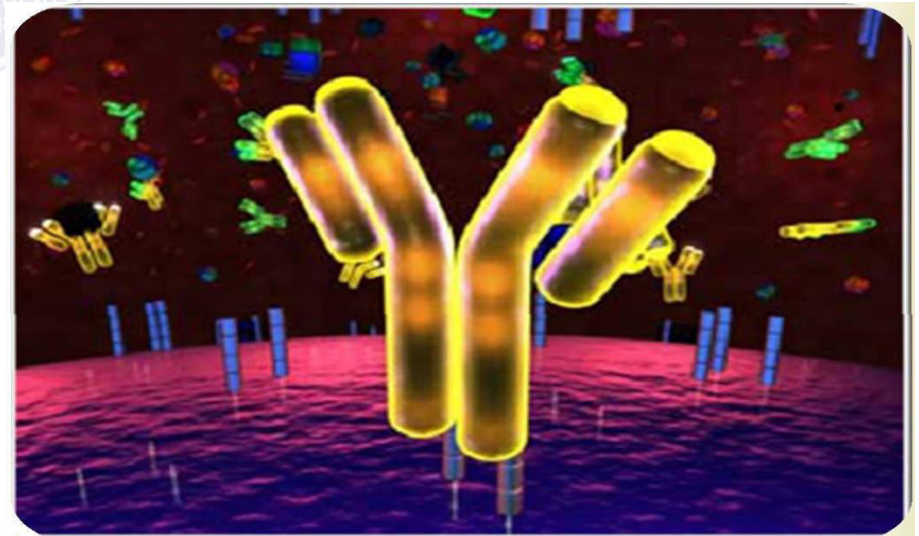


- **a** Uses gel electrophoresis to separate proteins by the length of the polypeptide (denaturing structure of the protein (native/ non-denatured) are then transferred to a membrane (typically nitrocellulose) where they are probed (detected) using an antibody against the protein.



# Monoclonal and Polyclonal Antibodies are used

- a Here are now many reagent companies that specialize in providing antibodies (both monoclonal and polyclonal antibodies) against tens of thousands of different proteins.



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# The procedure includes..

- a Tissue preparation
- a Gel electrophoresis
- a Transfer
- a Blocking
- a Detection
- a Analysis



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