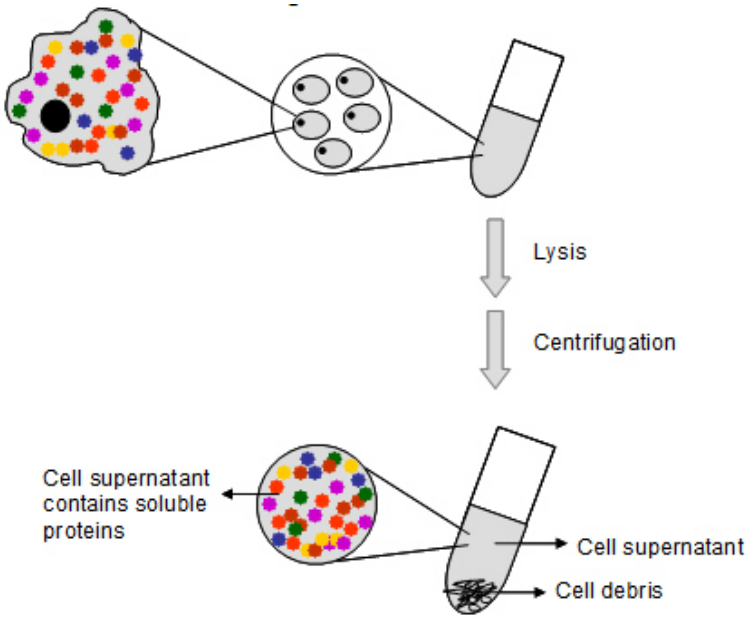
Western Blotting

Western Blot theory overview

Similar to ELISAs, western blot uses antibodies to detect and analyze specific proteins in a given sample of tissue or cell extract. This technique uses gel electrophoresis to separate a mixture of proteins, followed by transfer to a PVDF or nitrocellulose membrane. The membrane then serves as a platform on which antibodies can then be used to probe for target proteins. This guide will illustrate several major steps in western blot, including tissue preparation, gel electrophoresis and transfer, and immunoblotting.

Western Blot procedures

A, Tissue preparation Protein samples could be obtained from various sources, such as whole tissue or cell culture. Bacterial, viral or environmental samples could also be a source of proteins used in western blot. In general, tissues or cells are firstly lysed by sonification or by using a homogenizer to release the proteins of interest. Assorted detergents, salts, and buffers are required for lysis of cells and solubilization of proteins. In addition, protease and phosphatase inhibitors are often added to prevent protein denaturation and degradation.



B, Gel electrophoresis The separation of molecules is dependent on differences in their isoelectric points, molecular weights and/or electric charges. In western blotting, the most commonly used separation methods are denatured gel electrophoresis such as SDS-PAGE and native gel electrophoresis such as PAGE. SDS is a negatively charged anionic detergent which binds proteins, denaturing them and giving them a uniform negative charge. Therefore, separation of proteins in SDS-PAGE only depends on their molecular weights. On the other hand, native PAGE separates proteins by their charge-to-mass ratio. The choice between denatured and native gel electrophoresis depends on which epitope the antibody recognizes. If the epitope resides within the native conformation of the protein, protein denaturation is required to enable access to the antibody. By contrast, if the epitope is made up of non-contiguous amino acids and these amino acids are close to each other in the native structure of the target protein, a native gel electrophoresis should be used.

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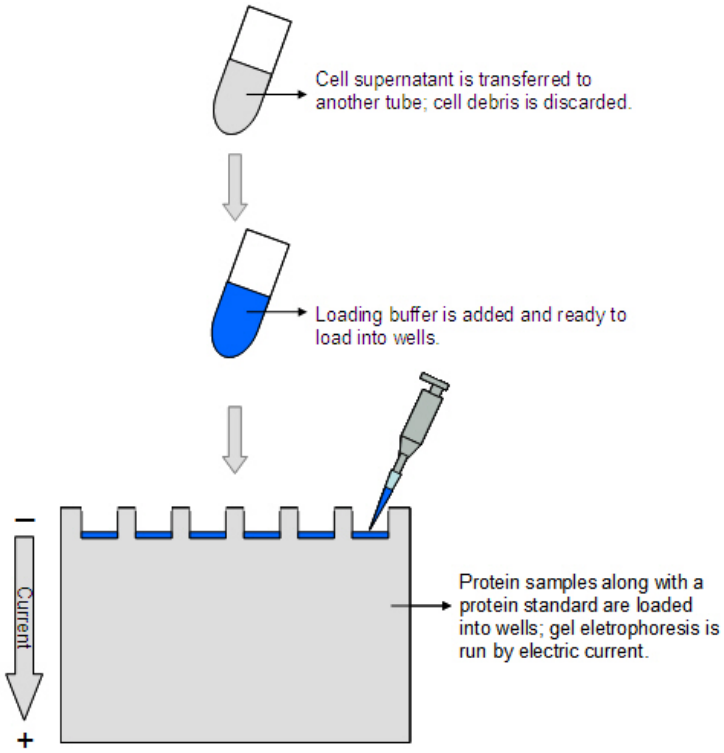
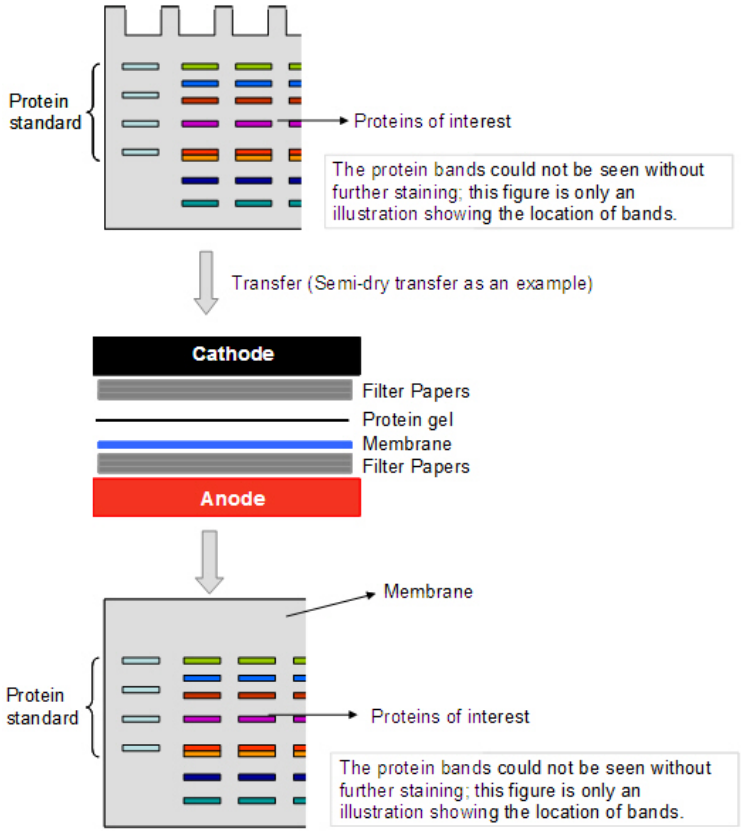


C, Transfer In order to facilitate access of the antibody to the target protein, separated proteins should be transferred from the gel onto a membrane such as nitrocellulose or PVDF (polyvinylidene difluoride). The transfer can be done in wet or semi-dry conditions and by capillary action or applying electric current. .



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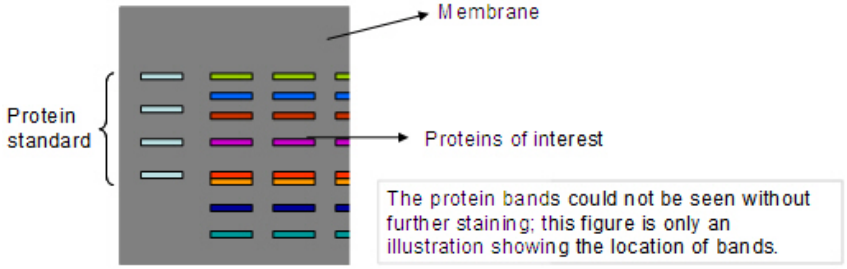
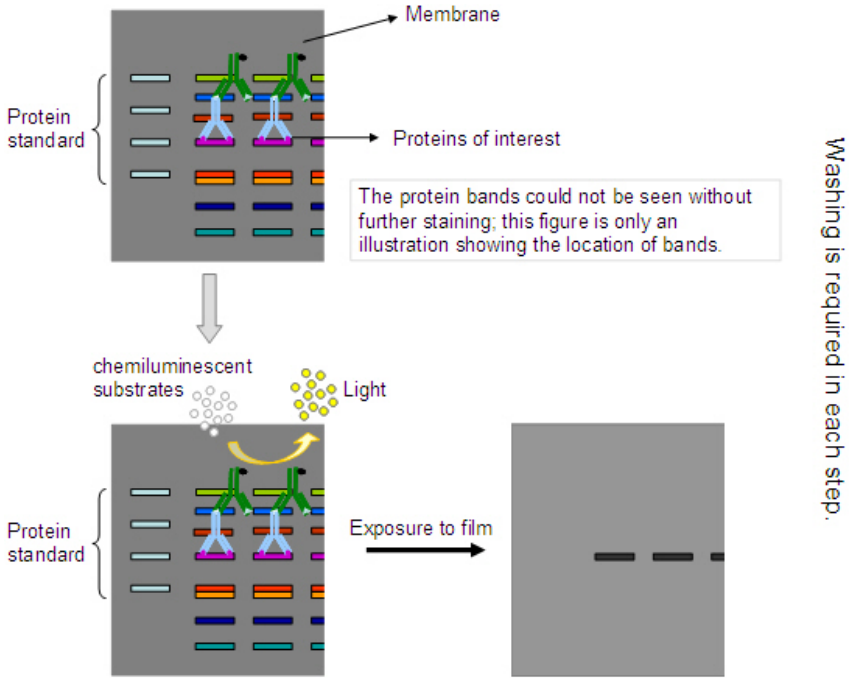
D, Immunoblotting

1. Blocking Blocking of unoccupied sites prevents non-specific binding (high background) of primary and/or secondary antibodies to membrane. Bovine serum albumin (BSA) or non-fat milk solution is commonly used. Addition of a dilute detergent, such as Tween 20, may also help in improving recognition of antigens by antibodies.



2. Detection Normally, secondary antibodies are conjugated to a reporter enzyme and the detection method used is based on the type of enzyme. The most commonly used enzyme for detection in western blotting is horseradish peroxidase (HRP), which reacts with its substrates to produce light. A photographic film is placed against the membrane and an image is created from the reaction. .



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