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The Application of Western Blot in Research

1. INTRODUCTION

Western blotting (also known as Protein immunoblotting) is the gold standard technique for detecting and quantifying specific proteins in a heterogeneous mixture isolated from cells or tissue lysate.[1] This technique utilizes gel electrophoresis to separate a mixture of proteins by molecular weight. The results are then transferred to a membrane, which generates a band for every protein. After, the membrane is treated with specific antibodies labelled to target a particular protein.[2]

Western blot (WB) techniques are often employed in various areas of biochemical research, and their application in studying the physiology of skeletal muscle and exercise is on the rise.[3] This can be attributed to the fact that it offers a better knowledge of the molecular processes that exercise and nutrition use to control transcription and translation in health and during ageing.[3] Other application of WB includes the regulatory molecular processes underlying protein turnover, chronic physiological adaptations, and energy metabolism. For instance, protein-protein interactions, protein abundance, cellular localization kinase activity, or post-translational changes such as cleavage, phosphorylation, ubiquitinylation, glycosylation, methylation, and SUMOylation can all be investigated using the WB.[4]

The basic steps that make up the WB's principle are as follows: (a) separation of specific proteins from a complex mixture (from tissue or cell); (b) measurement of protein molecular weight via SDS-PAGE (c) transfer to a nitrocellulose membrane; (d) "blocking" the nitrocellulose membrane to reduce non-targeted binding; (e) detection of antigen by antibodies specific for the

protein(s) of interest; and (f) quantification of the resulting bands via a Chemiluminescence Imager.[3]

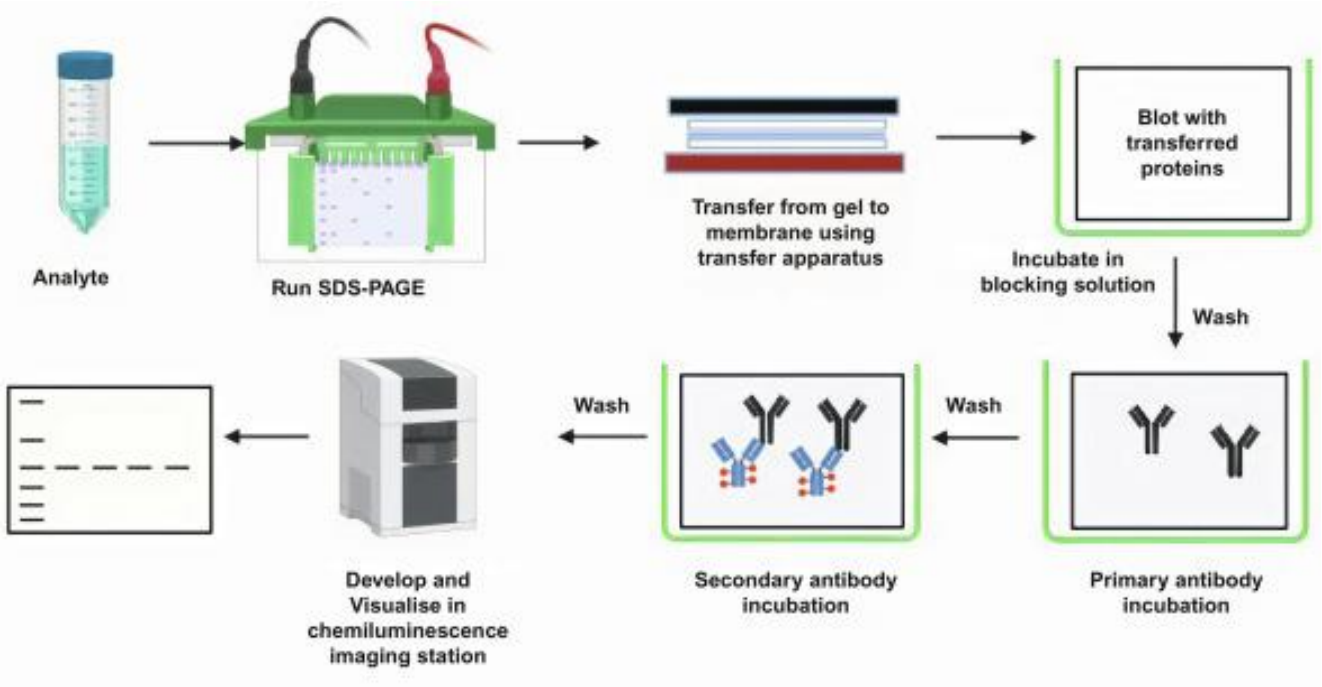


FIG 1: The Western blot assay process. Using gel electrophoresis, isolated proteins are first sorted by size, transported to a nitrocellulose blotting membrane, and then incubated with primary and conjugated secondary antibodies. Finally, depending on the secondary antibody employed, colorimetric, fluorescent, or luminescent techniques are utilized to detect the target protein.[5]

2. WESTERN BLOT APPLICATIONS IN RESEARCH

Although measuring the quantity and size of proteins in a sample is the most typical application of a Western blot, other aspects of protein studies have also shown to benefit from the use of WB.[4]

2.1. Identification of protein isoforms

A single coding gene can create numerous protein isoforms through a post-transcriptional process called alternative splicing.[6] Protein isoforms can be employed as biomarkers and therapeutic targets because they perform variously in diverse physiologic and pathological

processes.[7] Hence, the identification and measurement of particular protein isoforms plays a significant role in the field of protein analysis. Recent efforts to analyze selected protein isoforms has become more accurate and sensitive owing to sophisticated Mass spectrometry (MS)-based methods.[8] However, Protein isoforms data must be verified and validated using a conventional antibody-based method, such as Western blotting, regardless of the high-throughput methodology employed.[4] With only minor size differences, distinct isoforms of the same proteins can be detected using Western blotting. Therefore, identifying various protein isoforms, using monoclonal antibodies to find isoform-specific epitopes may be a beneficial strategy. [9]

2.2.Detection of protein–protein interactions

More than 80% of proteins, which are structural and functional biomolecules, work in complexes rather than alone. Many essential biological activities, such as signal transduction, molecular transport, and various metabolic pathways, are based on protein-protein interactions (PPIs).[10] To examine PPIs, a number of in vivo and in vitro methods have been developed. A molecular biological technique called Far-Western blotting was developed to identify direct PPIs in vitro. It was adapted from the conventional WB technique. In Far WB, the protein(s) of interest on the blot are probed with non-antibody proteins. Spots in the membrane can be seen if the probe interacts with the blotted protein.[11]

2.3.Detection of protein–DNA interactions

The proper control of gene expression is crucial for both disease and human development. In order to regulate gene expression, DNA-binding proteins and transcription factors (TFs) are essential.[12] Hence, identifying TFs may be essential to understanding how genes are regulated.

SouthWestern Blot (SWB) is a method used for quick characterization of DNA-binding proteins and/or transcription factors. The SWB assay is comparable to traditional WB except for the probing phase, which involves probing the blotted membrane with radiolabeled predesigned DNA oligonucleotides.[12]

2.4.Post-translational modification detection

Protein post-translational modifications (PTM) are responsible for the diversification and growth of the proteome. PTMs are essential for the folding, targeting, and subcellular localization of proteins.[4] They have an impact on a variety of physiological processes, including protein breakdown, differentiation, signaling pathways, and protein-protein interactions. The importance of studying PTMs in both healthy and pathological states is due to the fact that abnormal PTMs have been associated with many human diseases. The main techniques used to study PTMs include Immunoprecipitation, Western blot, Mass spectrometry, Biochemical assay, and Proximity ligation assay. The use of multiple assays is advised for the discovery, validation, and mechanistic characterization of a PTM. Western blot is a good approach for low throughput PTM analysis because it doesn't require specific knowledge or equipment. This technique enables researchers to combine immunoprecipitation with low-abundance, endogenous, and new PTMs.[4] However, it should be observed that choosing a PTM-specific antibody is a challenge for PTM detection using this Western Blot.[4]

2.5. Identification of subcellular location of proteins

Protein localization and function are interrelated. Without knowing a protein's subcellular location, it is impossible to fully comprehend its physiological function.[13] In cell biology, determining the subcellular localization of a protein and studying multicompartmental proteins provide significant challenges.[13] Even though flow cytometry and immunocytochemistry (ICC)

using fluorescence microscopy are regarded as standard single cell immunoassays for subcellular localization research, false positive signals brought on by antibody cross reactivity continue to be a setback.[4] Western blotting is a typical technique to assess protein localization; it can be used alone or in tandem with a high-throughput technique for additional confirmation.[3] The main barriers to this WB application are the need for pre-sub-cellular fractionation and the validation of antibody specificity.[4] In addition to traditional WB, single-cell western blotting (scWestern) is regarded as a revolution in both single-cell protein analysis and protein subcellular localization research. With scWestern, single-cell protein studies can now benefit from the precision and selectivity of western blotting.[4]

2.6. Determination of the binding epitope of an antibody

In biomedical research and immunotherapy, high-quality antibodies are the targeted molecules. It is therefore important to confirm, validate, and describe newly produced antibodies.[14] The main method used to characterize and validate antibodies is epitope mapping. Finding an antibody's specific binding sites (or epitopes) on a target protein is referred to as epitope mapping. Understanding an antibody's binding epitope is critical for developing and discovering new vaccines, treatments, and diagnostics.[15] The most effective method for direct detection of an antibody-antigen complex has traditionally been X-ray crystallography. Despite offering high-resolution maps of the antibody-antigen complex, the method is not always feasible due to its time and cost requirements as well as the fact that it is not appropriate for all complexes. One of WB's key strengths compared to other low-throughput approaches for epitope mapping is its high level of specificity.[15] The desired antigen is usually blotted onto the membrane in similar proportions as

variously sized pieces, and then a particular antibody is added. Secondary antibody that has been HRP-conjugated is used to identify the specific epitope(s).[4]

2.7.Disease diagnosis

Western blot is regarded as a potent diagnostic method that is frequently applied in the clinic since it enables direct protein detection. WB is not a quantitative technique; hence the clinical application helps with confirming disease diagnosis.[16] Human Immunodeficiency Virus infection, Bovine Spongiform Encephalopathy, Feline Immunodeficiency Virus, and Hepatitis B Virus are among the disorders it is used to examine.[4]

CONCLUSION

Since the Western blot method was created, biology labs have been able to embrace it, making it one of the most significant methods for both research and clinical applications. Despite several performance and sensitivity-related technical limitations, new developments could enhance the sensitivity and reproducibility of the WB technique. This technique has proven to be currently more effective for research than in clinical practice. However, owing to emergence of new tools and technologies, it is expected to increase in clinical practice in future. [4]

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