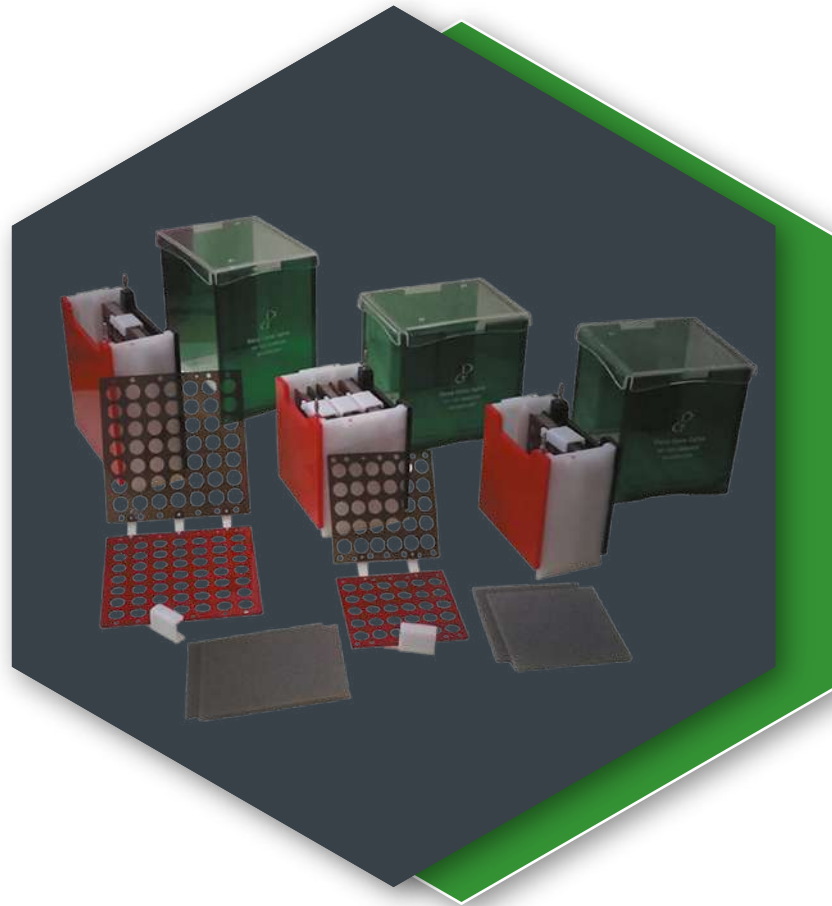


Western Blotting User Guide

Denagene Tajhiz Company
Biotechnology Lab Equipment manufacturer and designer





Western Blotting

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Thank you for choosing Denagene Tajhiz Company's Real-Time Electrophoresis. This operation manual provides a comprehensive overview of the instrument's functions. To ensure correct operation, please read this manual carefully before use. Keep it for future reference in case you encounter any difficulties. Upon first opening the packaging, please verify the instrument and accessories against the packing list. If anything does not match, do not hesitate to contact us.

This manual is a valuable resource for all users of our products, whether you are a seasoned professional or just beginning your scientific journey. It has been meticulously crafted to help you clearly understand the features, functionality, and proper usage of our laboratory equipment.

Within these pages, you will find detailed instructions, diagrams, and troubleshooting guides that will assist you in maximizing the potential of our products. We have organized the content logically to make it easy for you to navigate through the manual and quickly locate the information you need.

Moreover, this manual is a living document that reflects our ongoing commitment to excellence. As we continue to develop and improve our product offerings, we will provide updates and revisions to ensure that you always have the most current information at your fingertips.

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Content

Introduction	1
Safety Instructions	2
Maintenance	3
Technical Specifications	4
Western Blotting Features	5
Blotting Technique	6
Protein Transfer Overview	6
Membranes Used for Western Blots	7
Transfer Method in Tanks	8
Materials Required for Immunoblotting Technique	9
Testing Procedure	10
Electrophoresis Buffers-Choosing the Right Buffer	11
General Protein Staining on Membranes	12
Specific Protein Detection on Membranes	15
Western Blotting Technique	15
Blocking Stage	16
Detection with Antibodies	17
Materials	18
Experiment Procedure	18
Key Points in Western Blot Technique	20
Possible Problems in Western Blotting and Solutions	21

Introduction

With the remarkable advancements in cellular and molecular sciences, the demand for this knowledge and its products is increasing daily in our country.

Denagene Tajhiz Company has been able to meet the needs of our homeland's researchers for foreign equipment and products, albeit to a small extent, by producing essential products in this field.

We would like to express our gratitude for choosing Western Blotting tanks from Denagene Tajhiz Company. It is worth noting that Denagene Tajhiz's Western Blotting tanks, through an in-depth study of the best reputable global manufacturers and considering the needs of researchers and specialists in the country, have been able to provide the best products to serve our country's researchers and experts, using the highest quality raw materials.

All products of DNA Gene Equipment undergo thorough quality control and health tests before being delivered to customers. We hope that, by producing high-quality products, DNA Gene can gain the satisfaction of its customers.







Western Blotting

Western Blotting (Protein Blotting or Immunoblotting) is a laboratory technique used to detect specific protein molecules from a mixture of proteins. This method is widely employed in molecular biology and immunology.

The protein mixture can include all proteins related to a specific tissue or cell type. Western Blotting can be utilized to assess the size of the target protein and measure the level of protein expression.

Denagene Tajhiz's Western Blotting tanks, through an in-depth study of the best reputable global manufacturers and considering the needs of esteemed researchers and specialists, have been able to produce the best product using the finest raw materials.

Safety Instructions

-  Before use, anyone operating this equipment must read and understand these safety instructions.
-  Acrylamide is a potentially hazardous and carcinogenic substance. Use appropriate protective clothing when working with it.
-  Do not exceed the maximum operating voltage of the Western Blotting device.
-  Do not use an inappropriate power source to supply current and voltage. It is recommended to use the DGT-UNIVERSAL power supply model from Denagene Tajhiz Company.
-  Do not overfill the tank with buffer beyond its maximum capacity.
-  Do not move the tank during sample running.

Maintenance

- To remove the safety lid, push your thumbs down on the plastic lugs and lift the lid vertically with your fingers.
- Clean the apparatus with distilled water only before use and ensure it is dry.
- Acrylic plastic is not resistant to aromatic compounds, halogenated hydrocarbons, ketones, esters, alcohols (above 25%), and acids (above 25%). These substances can corrode the tank, so it is recommended not to use them for cleaning the apparatus.
- Before operating with the apparatus and monthly, check it for any leaks in connected areas. Place the apparatus on a sheet of paper, fill it with distilled water up to its maximum capacity, and inspect for any visible leaks on the paper. If there is any leakage, avoid attempting to repair it and promptly inform the Denagene Tajhiz Technical Team.
- The platinum electrode areas are usually relatively protected. When cleaning the tank, do not use abrasive cleaning brushes in the electrode region. Typically, cleaning with distilled water is enough.

Technical Specification

Model	WD15x15	WD10x10
Gel Quantity	2 Gel (15×15 cm)	2 Gel (10×10 cm)
Buffer volume	2000 ml	1000 ml
Power settings for routine mode	30 V/100 mA overnight	30 V/100 mA overnight
Maximum temperature	45 °C	45 °C
Maximum humidity	Up to 80 %	Up to 80 %
Circulation system	Yes	Yes

Table 1. Technical Specifications of Various Models of Western Blotting Apparatus

The Western Blotting apparatus manufactured by Denagene Tajhiz Company is meticulously designed and produced, taking into consideration the examination of the best foreign models and the utilization of cutting-edge technology worldwide. Given the comprehensive expertise of the company in designing and producing various models, custom orders are also accepted.

- Uniform transfer of bands to the membrane
- Quick and non-distorted transfer of bands
- Competitive pricing
- Excellent after-sales services



Features

Blotting Technique

In this technique, protein bands from the gel are transferred to a membrane-like material such as nitrocellulose, which can bind and immobilize proteins.

In the blotting process, protein molecules exit the gel matrix and are positioned on the surface of the membrane at the same locations. Therefore, their study can be easily conducted with fewer materials, or they can be separated and utilized.

To detect transferred proteins or enzymes on the membrane, specific ligands or relevant substrates can be used. Antibodies are among the most common agents employed for the specific detection of proteins on the membrane. Hence, such methods are known as immunoblotting techniques.

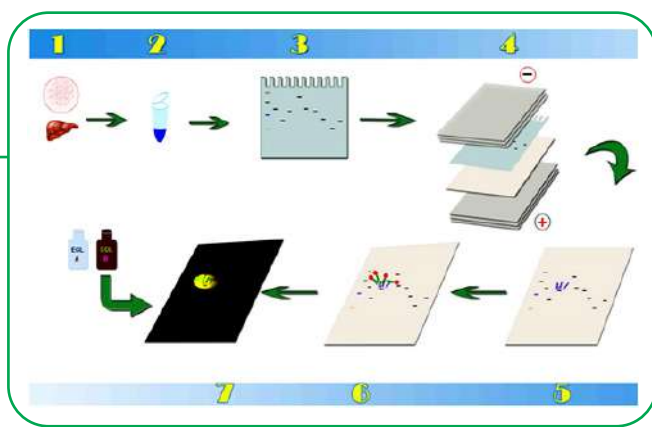


Figure 1. Complete Steps for Protein Identification Using the Blotting Technique

Protein Transfer Overview

In most cases, the transfer of proteins from the gel to the membrane is accomplished using electrical force.

This type of transfer is known as electrophoretic transfer. Electrophoretic transfer occurs in two main ways: tank transfer, also known as wet transfer and semi-dry transfer.

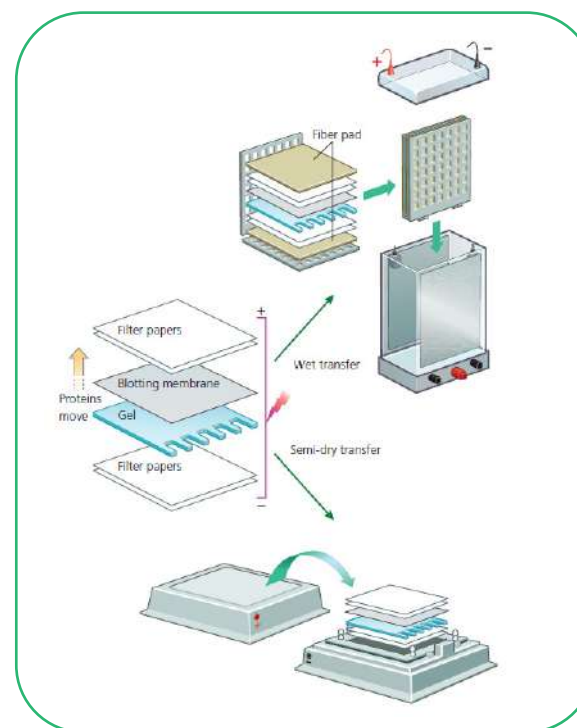


Figure 2. Main Methods of Protein Transfer from Gel to Membrane. Additionally, proteins can be transferred using capillary action or a vacuum pump with a porous membrane

Membranes Used for Western Blots

In Western blotting, membranes are commonly made of nitrocellulose or polyvinylidene difluoride (PVDF). Both membranes have a good capacity for binding to proteins. Nitrocellulose is more cost-effective but has lower mechanical strength and is brittle.

The pore size of nitrocellulose membranes varies between 0.1 to 0.45 microns. Smaller pore sizes result in a higher specific surface area and binding capacity. Other membranes, such as Di-Azo Benzyl Methyl (DBM), Di-Azo Phenyl Thioether (DPT), and nylon, are used in specific cases of blotting.

Nylon membranes have high capacity and mechanical strength but carry a positive charge compared to other membranes. Therefore, often anionic dyes used for protein staining can bind to them. In cases where the separation of a specific protein from the membrane is required, ion exchange membranes are suitable for blotting.

For this purpose, a portion of the membrane containing the desired protein is separated. Then, by exposing the isolated portion to a buffer with a different pH or ionic strength, the protein is released.

Transfer method in tank

The transfer method in the tank is the basic form of transfer using electrophoresis. Therefore, this method is often considered equivalent to electroblotting. An overview of this method is shown in the figure below.

Usually, the transfer in the tank is performed in a Tris-glycine buffer. This buffer is suitable for separating various proteins that have been separated by the SDS-PAGE method.

The presence of methanol in this buffer prevents gel swelling, separates SDS from proteins, and increases the membrane binding capacity. However, methanol, especially at high concentrations, is considered an inhibitory factor for protein transfer. In some cases, a small amount of detergent (e.g., SDS at a concentration of 0.2%) is added to this buffer to increase the solubility of proteins.

However, it should be noted that detergents, especially at high concentrations, can prevent proteins from binding to the membrane.

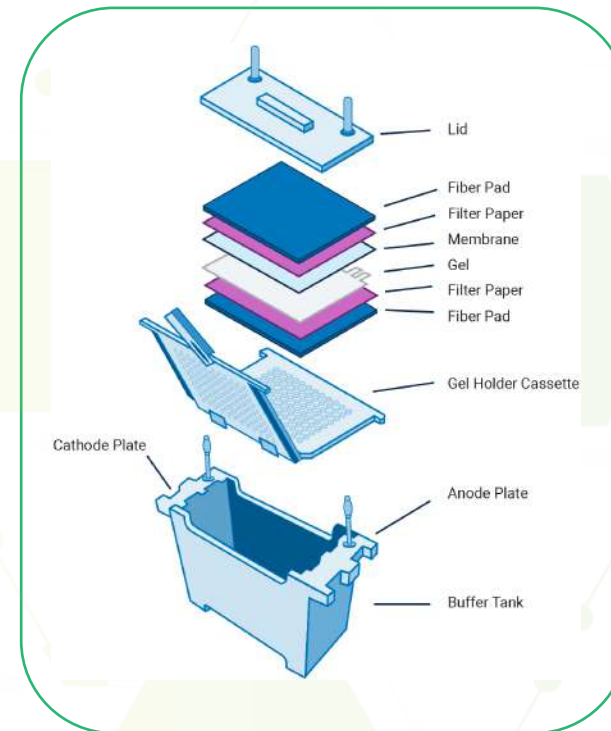


Figure 3. General schematic of the transfer method in the Western Blot tank. The gel cassette holds the gel and membrane together. Smooth papers and their pads make complete contact with both sides of the gel. Finally, the gel cassette is placed vertically in the tank.

Materials Required for Immunoblotting Technique

1. Nitrocellulose membrane with pore size of 0.45 micrometers or Immobilon (PVDF) membrane.
2. Transfer buffer containing 25 mM Tris, 192 mM glycine, and 15% methanol. To prepare, dissolve 6 grams of Tris base and 8.28 grams of glycine in approximately one liter of distilled water. Then add 200 mL of methanol, and bring the final volume to 2 liters with distilled water (the pH of this solution is approximately 8.3 and does not need adjustment).
Store the buffer in the refrigerator until it cools down before use, as methanol dissolves slowly in cold water.
3. Whatman paper or electrophoresis paper.

Testing Procedure

1. Pour a portion of the tank buffer into a clean plastic or glass container. Place the gel in the buffer for at least 10 minutes after cutting the stacking gel portion.
2. Using clean forceps and scissors, cut a membrane sheet to the size of the gel. Avoid direct contact with the membrane without gloves. Wet the membrane with distilled water (for nitrocellulose membrane) or methanol (for PVDF membrane) and transfer it to a container containing a transfer buffer. Prepare several layers of filter paper corresponding to the dimensions of the gel and wet them in the buffer along with sponges. Then arrange the components as shown in the figure. This assembly from bottom to top includes a sponge, several layers of filter paper, the gel, the membrane, several layers of filter paper, and a sponge layer. After placing each layer, remove any potential air bubbles with a glass rod or a test tube from the space between the layers.

Figure 4. Wet-Method Transfer



3. Securely assemble the blotting sandwich in the appropriate plastic frame and place it in the blotting tank filled to the appropriate height with the transfer buffer. Then, run the electrophoresis for 1-4 hours at a current intensity of 200-400 milliamps.

Electrophoresis Buffers-Choosing the Right Buffer

The choice of buffer and the inclusion of methanol in the buffer depend on the type of proteins under study, gel conditions, electrophoresis type, and membrane type. As mentioned before, Tris-glycine buffer is generally suitable for the transfer of most proteins.

Proteins in this buffer typically carry a negative charge and move toward the anode. The addition of SDS to proteins (e.g., in SDS-PAGE) also enhances their negative charge and movement. However, in some cases, altering the conditions of the Tris-glycine buffer or choosing a different buffer may facilitate the transfer.

For example, experience shows that removing methanol from Tris-glycine buffer facilitates the transfer of large glycoproteins.

Methanol removes SDS from proteins and compresses the gel, reducing the transfer of these molecules. SDS is an anionic detergent and has an impact on the transfer of proteins, especially positively charged proteins.

Transfer in 50 mM CAPS buffer occurs more quickly and is associated with less heat generation. Therefore, if necessary, this buffer can be used instead of Tris-glycine.

In situations where blotting is aimed at determining the amino acid sequence in proteins, CAPS buffer is more suitable, as the presence of glycine in the buffer disrupts the determination of protein sequences.

A 10 mM Sodium Borate buffer with a pH of 9.2 is recommended for the transfer of glycoproteins, polysaccharides, and lipopolysaccharides.

In this buffer, borate binds to the sugar units of the above substances, imparting a negative charge to them.

Proteins that undergo electrophoresis under acidic conditions or proteins separated by isoelectric focusing can be transferred to the membrane in a 7.0% acetic acid solution. Under these conditions, proteins carry a positive charge and move toward the negative pole.

General Protein Staining on Membranes

Membrane Type	Sensitivity ($\mu\text{g}/\text{band}$)	Materials Staining
Nitrocellulose ,PVDF	> 1.5	Fast Green FG
Nitrocellulose ,PVDF	> 1.5	Ponceau S
PVDF	1.5	Coomassie blue R-250
Nitrocellulose ,PVDF	1.5	Amido black10B
Nitrocellulose ,PVDF	0.1	Indian Ink
Nitrocellulose ,PVDF , Nylon	0.03	Colloidal iron
Nitrocellulose ,PVDF , Nylon	0.03	Avidin-Biotin-Peroxidase
Nitrocellulose,PVDF	0.004	Colloidal gold

Table2. Materials Used for General Protein Staining on the Membrane

After protein blotting, it is necessary to obtain information about the quality and quantity of the transferred bands or identify specific proteins for subsequent studies (such as sequence determination).

For this purpose, the nitrocellulose or PVDF membrane can be Stained with various Staining agents.

Protein Staining may be reversible or irreversible, depending on the experimental goals. Common materials for general protein staining on the membrane include Ponceau-S, India ink, Amido Black, and Coomassie Blue.

Staining the gel can also indicate the completeness or incompleteness of protein transfer. The positions of protein bands on the gel and membrane may not match exactly due to slight shrinkage of the gel during equilibration in the transfer buffer, which contains methanol.

Reversible Staining with Ponceau-S

The Staining solution consists of 1.0% (w/v) or (v/v) Ponceau S in 5% (v/v) acetic acid. It is commercially available from various companies. To stain the membrane, immerse it in a sufficient amount of the Staining solution for 5-10 minutes. Then rinse with distilled water until the membrane background becomes colorless.

Protein bands appeared red after Staining. If washing continues with water, the bands will also become colorless. The Ponceau S Staining does not interfere with band detection in specific methods (e.g., immunoblotting) or in determining amino acid sequences. The sensitivity of this method is relatively low.

Reversible Staining with Amido Black

The Staining solution consists of 0.1% (w/v) or (v/v) Amido Black in distilled water.

For Staining, place the membrane in a sufficient amount of the Staining solution for 20-30 minutes.

Then rinse with water until the membrane background becomes colorless.

The protein bands gradually fade and eventually disappear.

Irreversible Staining with Coomassie Blue

The Staining solution consists of 1.0% (w/v) Coomassie Blue R-250 in 7% (v/v) acetic acid - methanol (50% v/v) in distilled water. The staining solution contains 7% acetic acid and 50% methanol in distilled water. Gel Staining with Coomassie Blue is also performed using the same solutions.

Place the membrane in a sufficient amount of the Staining solution for 15 minutes. Discard the Staining solution and rinse the membrane in the Staining solution to destain the blue background. Then rinse the membrane with distilled water.

Coomassie Blue is suitable for protein Staining on PVDF membranes. Do not use this type of Stain for nitrocellulose, as the background becomes intensely colored.

Irreversible Staining with Amido Black

The staining solution consists of 0.5% (w/v) or (v/v) Amido Black, 5% (v/v) acetic acid, and methanol (50% v/v) in distilled water. The staining solution contains 5% acetic acid and 50% methanol in distilled water.

Place the membrane in the Staining solution for 5 minutes. Discard the Staining solution and rinse the membrane with the Staining solution to develop a blue background.

Protein Marker Staining

The determination of certain protein properties, such as size or Isoelectric Point (PI), is carried out using protein markers. The following methods can be used to detect markers on membranes:

1. Separate the section containing the markers from the rest of the membrane and stain it using one of the irreversible methods mentioned earlier (stain PVDF with Coomassie and Nitrocellulose with Amido Black). Then, dry this portion of the membrane and place it alongside the other results.
2. The position and number of marker bands vary depending on the brand.
3. Biotinylated markers can be prepared, or markers can be linked to biotin in the laboratory. Under these conditions, the position of the markers can be determined using Avidin Peroxidase Conjugate or Avidin and Anti Avidin Peroxidase Conjugate.
4. Chicken albumin and bovine albumin are proteins found in many types of markers. By having antibodies against these two proteins as a Conjugate with an enzyme (or antibodies against them and a secondary antibody Conjugate), their positions can be determined in the membrane. This method can be executed when these proteins are not used as blocking agents in the membrane.

Specific Protein Detection on the Membrane

The specific detection or staining of protein bands on the membrane is based on their reaction with specific ligands. To accomplish this, ligand molecules are directly (primary) or indirectly (secondary) labeled with radioactive substances, fluorescence, colorants, or enzymes, and they are used to specifically detect proteins.

Antibodies are among the most commonly used specific ligands for the specific detection of proteins, serving various diagnostic purposes.

The foundation of the immunoblotting method, which is of significant importance to biological scientists, supports such a claim.

Western Blotting Technique

This method, also known as immunoblotting, is based on the primary antibody-antigen reaction. In this method, after protein blotting, the free areas of the membrane are blocked (blocking step).

Then, the conditions for antibody reaction (e.g., patient serum) with protein bands are provided, followed by the use of labeled macromolecules (e.g., enzyme-conjugated anti-human immunoglobulin antibody) to visualize the primary reaction.

Western blotting is widely used in medical diagnostics and research. Although the implementation of this method is governed by a set of general principles, the conditions and details depend on the experimental objectives.

Blocking Step

The membranes used in protein blotting have a high capacity for capturing proteins (e.g., 100 micrograms per square centimeter of nitrocellulose membrane). While this property is desirable, it can lead to non-specific binding of labeled proteins in subsequent steps of the experiment, causing interference in detection. Therefore, the free areas of the membrane need to be blocked before the detection steps. The best way to block these free areas is by using proteins that do not react with labeled molecules (such as antibodies).

Cost-effectiveness and ease of procurement are the main criteria for selecting a blocking protein. The table below provides the names and working concentrations of some blocking proteins. Detergents can also prevent the binding of proteins to the membrane, with Tween 20 being the most commonly used detergent in this regard. It should be noted that at high concentrations (more than half a percent), Tween 20 can also interfere with ligand-protein binding in some cases.

Blocking Substances	Sensitivity ($\mu\text{g}/\text{band}$)
Bovine Serum Albumin	In a concentration of 2-5% in a buffer (usually PBS), it is used for 1-2 hours. For blocking nylon membranes, a concentration of 10% is required for 12-16 hours.
Fat-Free Dry Milk	It is used in a concentration of 3%. It is very cost-effective. Due to its high sugar content, it prevents the binding of lectins or anti-carbohydrate antibodies (as labeled molecules).
Chicken Serum Albumin	It is used in a concentration of 1%.
Gelatin	It is used in a concentration of 3.0-3.0%. Fish gelatin has more suitable properties as a blocking substance compared to mammalian gelatin.
Detergent (Tween-20)	A concentration between 0.1-0.5% is sufficient. It is easy and inexpensive. It also prevents non-specific interactions between proteins.

Table3. Types of blocking substances used in blotting

Detection with Antibodies

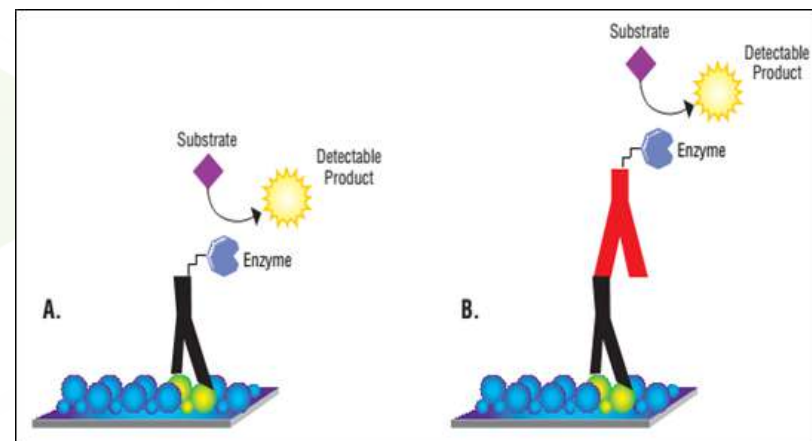
After protein blotting and membrane blocking, specific detection can be performed. As previously mentioned, immunoblotting is often used for diagnostic purposes.

In these cases, the transferred bands include all or part of the protein profiles of microorganisms (bacteria, viruses, or fungi), and the presence or absence of their corresponding serum antibodies in human or animal sera is examined. Identification using antibodies, such as the enzyme-linked immunosorbent assay (ELISA) technique, can be carried out through two methods: direct and indirect.

In the direct method, the primary antibody used for antigen detection is labeled with an enzyme or a fluorescent dye. However, in the indirect method, a primary antibody is first added to bind to the antigen, and then a secondary antibody, which is labeled, is added.

Biotin molecules are used for binding, and fluorescent probes such as fluorescein or rhodamine, or enzyme conjugates such as HRP or alkaline phosphatase, are used for identification and signal generation. The figure below illustrates the general principles of these two types of immunoblotting techniques.

Figure 5. Various methods of identification using antibodies. In part A, the target protein is directly detected. In part B, the target molecule is indirectly identified.



Materials

1. Tris-buffered saline with pH 7.5 (TBS). This buffer contains 20 mM Tris and 0.15 M Sodium Chloride.
2. TBS-Tween buffer with 0.05% Tween (TBS-T). Add 0.5 mL of Tween-20 to each liter of TBS buffer.
3. Primary antibody (patient serum or immunized animal serum). Dilute, if necessary, with TBS-T.
4. Secondary antibody. This antibody is specific to the constant region of the primary antibody and is conjugated with an enzyme (peroxidase). Prepare the required concentration of this antibody in TBS-T.
5. Peroxidase substrate solution consisting of 0.5 mg/mL diaminobenzidine in milliliters and 0.1% hydrogen peroxide in TBS.

Experiment Procedure

1. After blocking, wash the membrane three times for 5 minutes each in TBS-T. Then incubate for 1-2 hours in the primary antibody.
2. Wash the membrane four times for 5 minutes each in TBS-T. Then incubate for 1-2 hours in the secondary antibody (peroxidase-conjugated antibody).
3. Wash the membrane four times for 5 minutes each in TBS-T. Then expose it to a sufficient amount of substrate solution. Band development usually takes 5-15 minutes. After the bands appear, wash the membrane thoroughly with distilled water. Dry the membrane and place it in a dark location.

Note: Diaminobenzidine is a highly toxic substance. Avoid contact and handle it with caution.

General steps of the Western blotting process

Transfer

Blocking of unbound regions on the membrane

Incubation with primary antibody

Washing

Incubation with conjugated secondary antibody or ligand

Washing

Signal generation based on color or luminescence properties

Visualization and imaging using a gel documentation system and analysis of results

Important points in Western blotting technique

1. If you want to examine a large number of serum samples, you can save time and materials by cutting Nitrocellulose or PVDF membranes into 5.0 cm-wide strips and placing each strip in a separate test tube for the analysis of a serum sample. To do this, sample loading should occur across the entire width of the stacking gel. This means that the stacking gel is cast without inserting a comb, and sample loading occurs across the entire surface. The result of electrophoresis in this configuration is the presence of continuous protein bands throughout the width of the gel.
2. The Titer of Conjugated Antibodies depends on factors such as antibody purity, enzyme conjugation method, purification, and conjugate concentration. Conjugated antibodies are usually diluted 1000-10000 times before use. The appropriate titer is one that clearly shows positive control and has a minimal reaction with the membrane background.
3. Peroxidase is the most commonly used enzyme for preparing enzyme conjugates. Alkaline phosphatase, glucose oxidase, urease, and penicillinase are other enzymes used for this purpose.

Poor protein transfer onto the membrane

- Reason: High percentage of gel in electrophoresis.
Solution: Perform electrophoresis with a thinner gel.
- Reason: Trapped air bubbles between the gel and membrane.
Solution: Ensure complete immersion of the blot components in the transfer buffer. Remove air bubbles with a pipette tip after adding each layer of the blot assembly.
- Reason: Insufficient transfer time.
Solution: Perform transfer at a lower voltage for a longer duration overnight.
- Reason: High percentage of methanol in transfer buffer.
Solution: Reduce the percentage of methanol to 5-10%.
- Reason: High concentration of detergents (e.g., SDS).
Solution: Decrease the concentration of SDS in the transfer buffer.
- Reason: Decreased negative charge density on proteins.
Solution: Select a transfer buffer with a higher pH. Add a small amount of SDS to the buffer.
- Reason: Weak membrane binding capacity.
Solution: Use ion-exchange membranes or nylon membranes.

Staining of the membrane background in Western blot

- Reason: Absence of Tween-20 in dilution and washing buffers.
Solution: Add 0.05-0.5% Tween-20 to the buffers.
- Reason: Incomplete blocking (low concentration of blocking reagent or insufficient blocking time).
Solution: Increase the concentration of the blocking reagent or prolong the blocking time.
- Reason: Inappropriate choice of blocking protein (enzyme-contaminated or vaccine-containing antibodies).
Solution: Change the blocking protein or use detergents.
- Reason: High initial concentration of primary or secondary antibodies (conjugate).
Solution: Use higher dilutions of antibodies.
- Reason: Impure or degraded antibodies or conjugates.
Solution: Use purer antibodies and prepare fresh conjugates.
- Reason: Insufficient washing steps or inadequate incubation time of the membrane in enzyme substrate.
Solution: Perform thorough and precise washing steps. Incubate the membrane in enzyme substrate for 5-20 minutes.

Spots or white areas in the blotting

- Reason: Trapped bubbles or white areas on the membrane where proteins won't bind. Both issues can be resolved with careful attention.

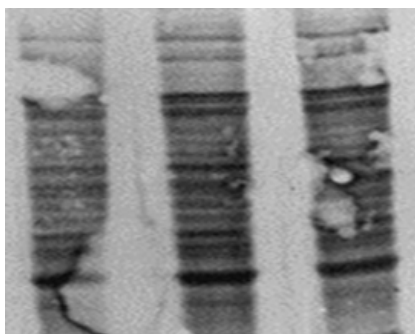


Figure 6. White spots in blotting

Solution: Improper stacking of the Western sandwich or trapped air bubbles in it.

Presence of a very dark background in the blot

- Reason: Incomplete blocking of the membrane.
- Solution:
- Increase the blocking agent concentration.
 - Extend the blocking time.
 - Increase the blocking temperature.
 - Use a different blocking agent.

Contamination of the blocking solution

- Solution:
 - Use a pure protein such as BSA or casein as a blocking agent.
 - Avoid using blocking buffers.

Long-term incubation with the substrate

- Solution:
 - Reduce the incubation time with the detection substrate.
- If a colored substrate is used, remove the blot from the substrate solution when the noise level is acceptable and place it in deionized water.

Excessive antibody quantity

- Solution:
 - Reduce or adjust the concentration of primary or secondary antibodies.
 - Use a dot blot test to optimize the antibody concentrations.
 - Reduce the incubation time.

Specific problems with blotting tanks

Overloading of proteins

- Solutions:
 - Reduce the amount of protein loaded onto the gel.
 - Reduce the concentration of SDS in the transfer buffer.
 - Add an additional membrane to capture the excess protein.

Excessive incubation temperature

- Solution:
 - Use fresh buffer.
 - Filter all buffers with a 0.2-micron filter before use.

Antibody binding to proteins in the blocking buffer

- Solution:
 - Use other blocking agents such as albumin, gelatin, BSA, casein, or fat-free dry milk.
- Do not use milk as a blocking agent when using a biotin-avidin system because milk contains biotin.

Loss of antibody activity due to long-term storage

- Solution:
 - Use freshly prepared antibodies stored at 20°C.
 - If long-term use of antibodies is anticipated, store them at -80°C and divide them into very small aliquots.
 - Avoid freeze-thaw cycles of the antibodies.

Insufficient time between incubation steps

- Solution:
 - Increase the duration and number of washing steps (at least 5x5 minutes).
 - Use larger volumes of wash buffer.

Membrane drying during incubation steps in blotting

- Solution:
 - Ensure that the membrane is fully wetted at the beginning of the process and make sure it does not dry out at any step.
 - Ensure that the membrane is fully immersed during incubation and washing with buffers.

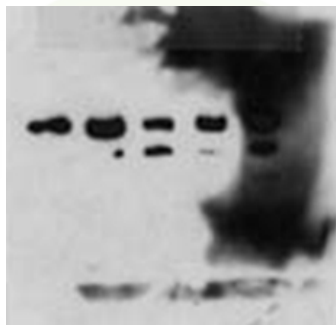
Contaminated buffer

- Solution:
 - Ensure that the membrane is fully wetted at the beginning of the process and make sure it does not dry out at any step.
 - Ensure that the membrane is fully immersed during incubation and washing with buffers.

Background staining

- Solution:

This problem can be caused by dry membranes or insufficient washing. Both issues can be easily resolved by fully immersing the blot and keeping it submerged for the entire duration. Extra care should be taken during handling to prevent such contamination in the laboratories.



Uneven spots on the blot or a stained background

- Cause:

The stained background and spots can be due to the attachment of foreign objects to the membrane, accumulation of antibodies, a dirty scanner, or dirty surfaces of Western blotting components

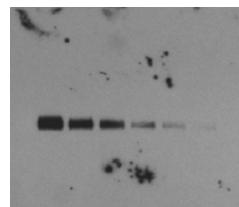


Figure 7. Uneven spots on the blot

Transfer of gel artifacts onto the blot

- Cause:
 - Use of thin or contaminated fiber pads.
 - Excessive amounts of protein were loaded onto the gel and/or too much SDS was used in the loading buffer.
 - The transfer buffer is contaminated.

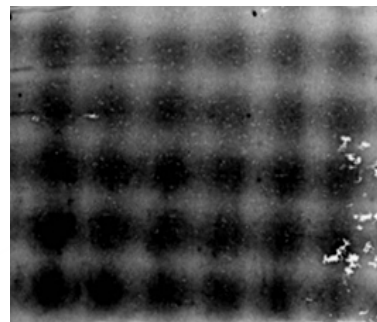


Figure 8. Transfer of gel artifacts onto the blot



Documentation and Support

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