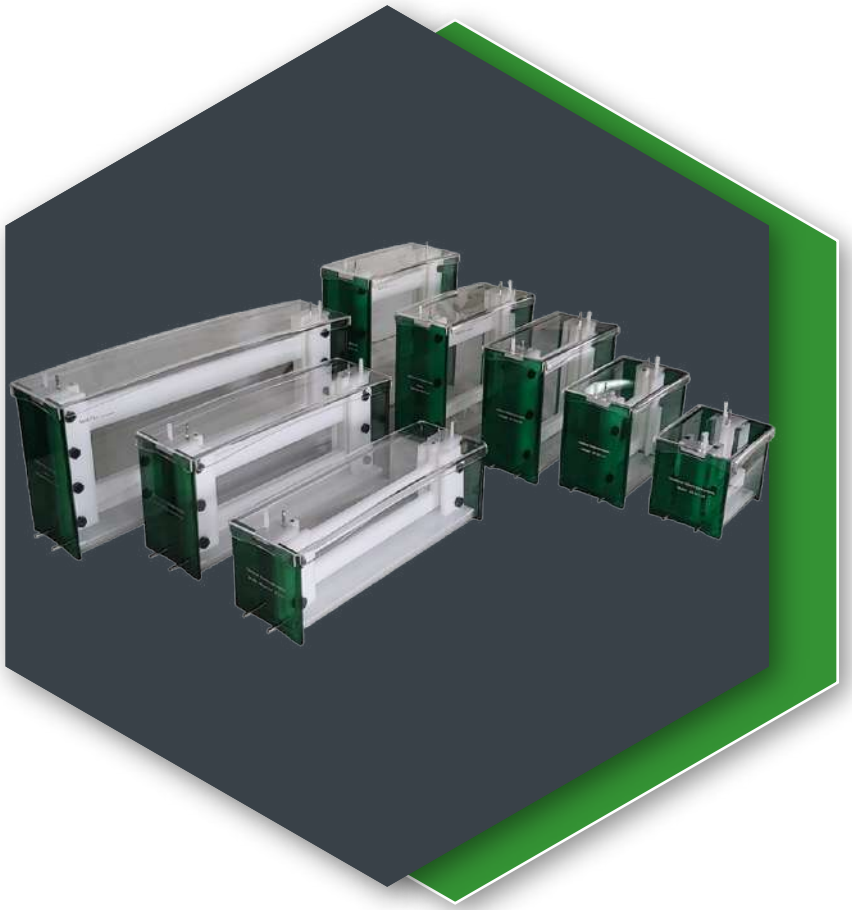
Vertical Electrophoresis User Guide

Denagene Tajhiz Company

Biotechnology Lab Equipment manufacturer and designer



Vertical Electrophoresis

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Thanks for choosing Denagene Tajhiz Company's Vertical Electrophoresis. This opertion manual outlines the function of the instrument. To ensure correct operation، please read the manual carefully before using it. Keep this manual for future reference in case you encounter any difficulties. Upon first opening the packing، please verify the instrument and accessories against the packing list. If anything does not match، please contact us.

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

Within these pages، you will find detailed instructions، diagrams، and troubleshooting guides to assist you in maximizing the potential of our products. We have organized the content logically، making 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 this manual to ensure you always have the most current information at your fingertips.

Content

Introduction

Denagene Tajhiz Company is a designer and manufacturer of various models of vertical electrophoresis tanks for the separation of proteins and nucleic acids.

The vertical electrophoresis tanks produced by Denagene Tajhiz are available in models such as Mini VD 10x10، Midi VD 15x15، Maxi VD 20x20، Maxi VD 22x23، and the Mega Gel family، catering to researchers and specialists.

The company also designs and manufactures custom and specialized models. All these models are designed to eliminate the need for clamps and outdated systems for gel preparation.

The gel is prepared within the device itself، after which it is placed in the tank compartment، and the device is connected to a power supply for molecule separation.

Device Information

The vertical electrophoresis tank from Denagene Tajhiz is provided as a complete package، including the tank chamber، gel casting module، tank cover، glass plates for gel casting، combs، connecting wires، spacers، gel board، and gel separator spatula.

The gel casting module is designed for direct gel casting، and the device is placed in the tank for molecule separation. For ease of gel preparation، the spacers are pre-attached to the square glass plates and have a default thickness of 1 millimeter. If customers require spacers of different thicknesses، they can request them before the device is shipped.

The connecting wires for the vertical electrophoresis device are designed to be compatible with all standard laboratory power supplies. However، for optimal results، it is recommended to use the DGT Universal power supply model.

The circulator system in the device maintains the chamber temperature and prevents overheating، eliminating the need for a chiller system.

Each device comes with two standard combs for vertical electrophoresis tanks. If additional or custom combs are required، they must be requested before the device is shipped.

Safety Precautions

- When the device is used correctly، it poses no significant risks. However، if operated by unqualified individuals، the device can pose a risk of electric shock.
- Anyone intending to use the device must thoroughly read the user manual before beginning any operation.
- Never use the device without its cover in place.
- Do not use the device if there are any issues with the tank body or cover.
- Acrylamide in its monomer form is a highly dangerous neurotoxin. Even polymerized gels contain some amount of acrylamide monomer. Therefore، it is essential to follow safety measures such as wearing gloves and masks when handling this material.
- Do not fill the tank with running buffer beyond the maximum buffer capacity lines.
- Avoid moving the tank while running samples.
- During electrophoresis، small amounts of various gases are generated near the electrodes. The type of gas produced depends on the buffer composition used. Ensure the device is run in a well-ventilated area to disperse these gases.

Technical Specification

Model	Mini VD 10×10	Midi VD 15×15	MaxiVD 20×20	MaxiVD 22×23
Plate Dimensions (cm)	10×11	15×15	20×20	22×23
Gel Dimensions (cm)	8×8.5	13×13.5	18×18	20×21
Gel Quantity	2	2	2	2
Gel Volume (ml)	2×6.5	2×16.9	2×32.4	2×44.1
Tank Dimensions (cm)	17×12.5×14	21×12.5×18	26×12.5×23	30×12.5×25
Internal Buffer Volume (ml)	80	230	500	750
External Buffer Volume (ml)	1000	2000	3600	4000
Maximum Sample	2×11	2×15	2×21	2×24

Table 1. Technical Specifications of Various Vertical Electrophoresis Models

Maintenance

- Use warm water and a mild detergent to clean the device. Water above 60°C can damage the device. The gel casting module should be carefully rinsed with warm water to prevent salt buildup، but take care not to damage the electrodes.

- Aliphatic hydrocarbons، hexane، and dishwashing liquid are ideal for cleaning the device. Do not immerse the device in detergents for more than 30 minutes.

- Never expose the device to the following cleaning agents as they can cause irreversible damage: acetone، phenol، chloroform، carbon tetrachloride، methanol، ethanol، isopropyl alcohol، and alkaline.

To eliminate RNase، follow this protocol:

1. First، clean the device with a standard detergent as previously described.
2. Then، wash the device with 3% hydrogen peroxide (H2O2) for 10 minutes.
3. Rinse the device with 0.1% DEPC-treated water.
4. Note that DEPC is a carcinogen، so always follow all safety precautions when using it.

- Check the device monthly for any leaks at the connected joints. To do this، wrap the device in a paper sheet and fill it with distilled water up to the maximum capacity. Any leaks will show on the paper. If any leakage is detected، do not attempt to repair it yourself; contact Denagene Tajhiz immediately.

- The platinum electrodes are typically partially covered for protection. Avoid using cleaning brushes on the electrode area; rinsing with distilled water is usually sufficient.

Detailed Explanation of SDS-PAGE Technique

SDS-PAGE is a cost-effective، fast، and reproducible method for studying proteins.

This technique is commonly used to examine purification steps، calculate relative amounts، and determine the molecular weight of proteins. The excellent resolving power of SDS-PAGE is due to the use of sodium dodecyl sulfate (SDS) and the superior properties of polyacrylamide gel.

SDS is an anionic detergent that denatures proteins by binding to their hydrophobic regions. SDS masks the natural charge of the proteins and creates a uniform distribution of negative charges. As a result، protein separation is based solely on molecular weight.

Proteins are linearized by treating them with sufficient SDS and the reducing agent Mercaptoethanol، which breaks disulfide bonds، and then heating for a few minutes. The necessary amount of SDS for binding to proteins is 1.4 grams of SDS per gram of protein.

During electrophoresis، the proteins are separated based only on their molecular weight، meaning that larger molecules move more slowly due to greater friction with the surrounding medium.

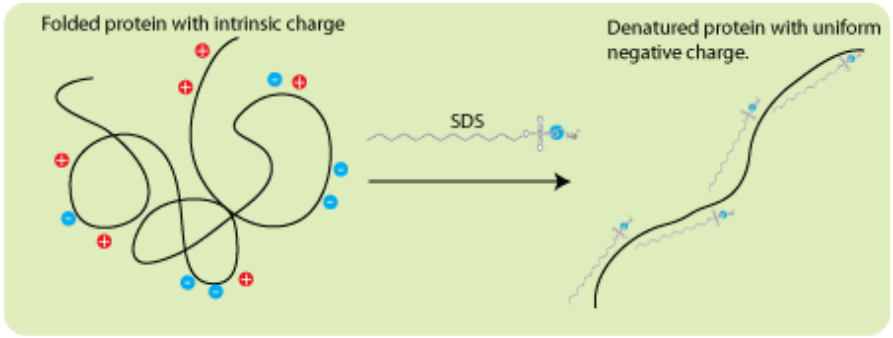


Figure 1. SDS Mechanism of Action

Usually، SDS molecules do not bind to sugars، so proteins with large sugar moieties take up less SDS relative to their molecular weight.

This reduced binding of SDS leads to slower migration on the gel، resulting in an overestimation of their molecular weight.

To address this issue، SDS-PAGE can be performed in gradient gels or Tris-borate-SDTA buffer systems instead of the conventional Tris-glycine buffer.

Borate binds to the sugars، increasing the negative charge of glycoproteins and compensating for the reduced SDS binding.

1. The Role of Polyacrylamide Gel

Polyacrylamide gel plays a crucial role in the separation of proteins in SDS-PAGE.

The pore size of the polyacrylamide gel، which depends on the concentration of its two components (C% and T%)، determines the range of molecular weights that can be separated in SDS-PAGE.

For example، in a gel with 5%، 10%، or 15% concentration (assuming C% is 2.6%)، proteins in the range of 20-300، 15-200، and 12-100 kDa، respectively، can be separated.

It should be noted that the relationship between the migration distance and the logarithm of molecular weight is linear only within a limited range.

To increase the extent of this linear relationship، electrophoresis should be conducted in a gradient of polyacrylamide gel concentration.

2. SDS-PAGE in Reducing and Non-Reducing Conditions

Disulfide bonds، whether intrachain or interchain، play a significant role in forming the tertiary and quaternary structures of proteins. Reducing these bonds with thiol-containing reagents (such as 2-mercaptoethanol) leads to the disruption of the tertiary and quaternary structures of proteins.

Proteins with disulfide bonds exhibit different migration patterns under reducing and non-reducing electrophoresis conditions. Reducing these disulfide bonds results in the separation of subunits in multimeric proteins and the complete linearization of all proteins، leading to uniform SDS binding.

Therefore، comparing the electrophoresis patterns of a protein under reducing and non-reducing conditions can provide valuable information about its tertiary structure. DTT and 2-mercaptoethanol are the most commonly used reducing agents for disulfide bonds.

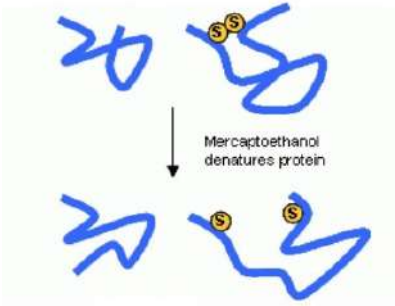


Figure 2. Mechanism of Action of Mercaptoethanol

Buffer System

The polyacrylamide gel is composed of two parts: the stacking gel and the resolving gel. The stacking gel، positioned at the top، has a different composition from the resolving gel. In the stacking gel، proteins are compressed into a thin layer due to uniform charge density، and they move at the same speed. When the protein mixture reaches the beginning of the resolving gel، separation based on molecular weight begins. The net charge of protein-SDS does not change significantly within the pH range of 7-10، and protein migration in this range remains relatively constant.

Tris-glycine buffer is the most commonly used discontinuous buffer system in SDS-PAGE. In a discontinuous buffer system، the ionic composition، buffer pH، and gel composition in the sample، gel، and reservoirs differ from each other. Typically، the discontinuous buffer system includes two types of gel (top and bottom gels). In a discontinuous buffer system، electrophoresis efficiency is less dependent on sample volume.

In a continuous buffer system، the concentration، ionic composition، and pH are uniform throughout the electrophoresis path.

The Laemmli buffer system is the most common discontinuous buffer system used in electrophoresis. In this system، the protein sample and the top gel contain Tris-HCl buffer at pH 6.8، while the bottom gel contains Tris-HCl buffer at pH 8.8، and the electrode buffer (reservoir buffer) is Tris-glycine at pH 8.3

Sample Preparation

To prepare samples for SDS-PAGE، add sample buffer to the protein sample in a specific ratio، then heat for a few minutes in boiling water. This process denatures proteins completely due to the effects of SDS and the reducing agent (under reducing conditions). The amount of SDS in the sample buffer should be several times greater than the amount of protein (3:1 ratio) to ensure complete saturation of the protein with SDS.

The presence of glycerol or sucrose in the sample buffer increases the density of the sample، ensuring it stays at the bottom of the well، which is particularly important if sample loading takes a long time.

Typically، after adding sample buffer to the protein and before loading، the mixture is heated in boiling water for 15-20 minutes (depending on the type of protein). Heating helps dissociate subunits in multimeric proteins and facilitates the saturation of polypeptide chains with SDS. Additionally، this process inactivates many proteases، preventing protein degradation. However، some proteases remain active under these conditions، so protease inhibitors should be added to the samples.

Some proteins behave similarly under SDS treatment alone and with heat، but others may show different behaviors.

Materials Required for SDS-PAGE

Stock Acrylamide Solution (30.8%): Dissolve 30 grams of acrylamide and 0.8 grams of bis-acrylamide in distilled water to a final volume of 100 milliliters. Filter the solution through Whatman #1 paper and store it in a dark container. This solution is usable for up to 3 months in the refrigerator.

Note: Avoid inhaling acrylamide and bis-acrylamide powders during weighing and avoid contact with the solution.

- Lower Gel Buffer: Dissolve 18.2 grams of Tris base and 0.4 grams of SDS in 70 milliliters of distilled water. Adjust the pH to 8.8 with 2M hydrochloric acid. Add distilled water to a final volume of 100 milliliters. The Tris concentration in this buffer is 1.5M.
- Upper Gel Buffer: Dissolve 6.1 grams of Tris base and 0.4 grams of SDS in 50 milliliters of distilled water. Adjust the pH to 6.8 with 2M hydrochloric acid. Add distilled water to a final volume of 100 milliliters. The Tris concentration in this buffer is 0.5M.
- Electrode Buffer (Reservoir Buffer): Dissolve 3 grams of Tris base، 14.4 grams of glycine، and 1 gram of SDS in 1 liter of distilled water. The pH of this buffer is approximately 8.3 and does not require adjustment.
- Sample Buffer (5X): Mix 10 milliliters of upper gel buffer، 5 milliliters of glycerol، 1 gram of SDS، 0.2 milliliters of bromophenol blue solution (0.5% in ethanol)، and 1 milliliter of 2-mercaptoethanol. Adjust the final volume to 20 milliliters with distilled water.
- Ammonium Persulfate 10%: Dissolve 0.1 gram of ammonium persulfate in 1 milliliter of distilled water. This solution should be prepared fresh.

TEMED 10%: Dissolve 0.1 milliliters of TEMED in 0.9 milliliters of distilled water. This solution should be prepared fresh.

- Molecular Weight Markers: Ensure molecular weight markers are ready for use.

Performing the Experiment

Before starting any experiment، ensure that the plates، spacers، and combs are washed with a laboratory detergent.

Avoid using corrosive materials for cleaning. If the gel is required for subsequent steps، such as silver staining، it is advisable to incubate the glass plates overnight in chromic acid.

Afterward، wash them with distilled water and finally clean with ethanol، acetone، and ethanol sequentially.

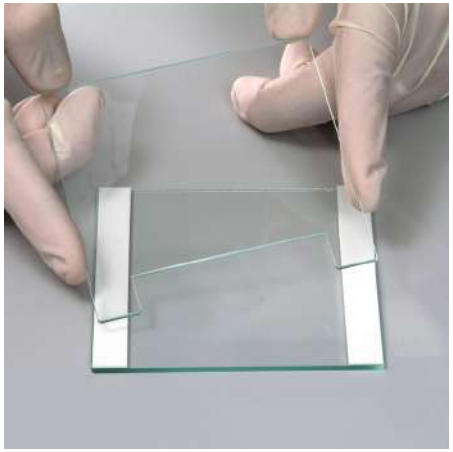
Never allow chromic acid or organic solvents to come into contact with plastic components. Handle the glass plates with clean gloves.

Assembling the Plates

First، clean the spacers and combs with a standard laboratory detergent.

For specialized cleaning، the glass plates can be left overnight in chromic acid، then washed with water، and subsequently cleaned with ethanol، acetone، and ethanol again.

Avoid letting organic solvents or chromic acid come into contact with plastic parts.



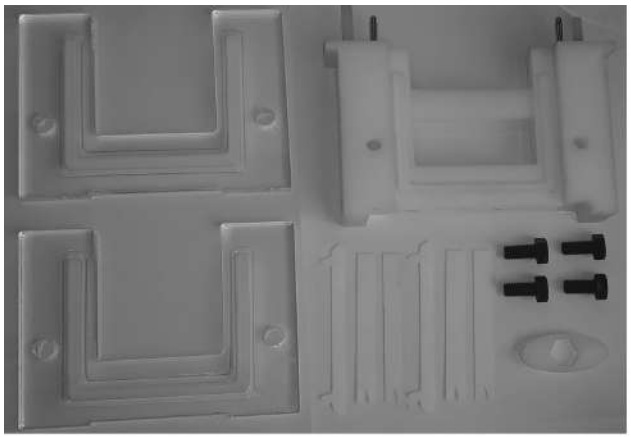
Handle the clean plates with clean، gloved hands (also، clean any marks with acetone). By placing the concave part of the spacer at the bottom of the glass and applying a small amount of petroleum jelly to the designated area as shown in the image below، leakage from the tank can be prevented.

Preparation of the Inner Chamber

First، place the inner chamber on a clean surface، then position the U-shaped glass in the designated spot، and place two spacers on the sides of the glass. Next، place the flat glass on top of them. Be cautious not to tighten the screws in this position; instead، stand the chamber vertically on the table and tighten the screws by pressing down on the glass and spacers with your fingers.

If done correctly، the glasses and spacers will align with the base of the tank. At this point، slightly tighten the screws، lay the chamber horizontally، and then fully tighten the screws using the wrench provided.

Repeat this process for the other side of the tank. Then، pour the gel and place the combs between the two glass spaces to create wells.



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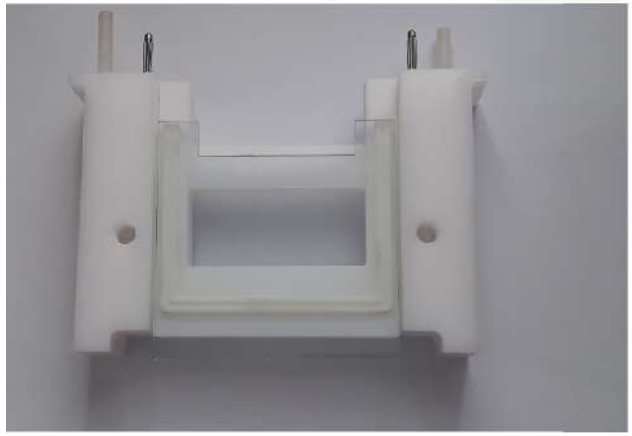

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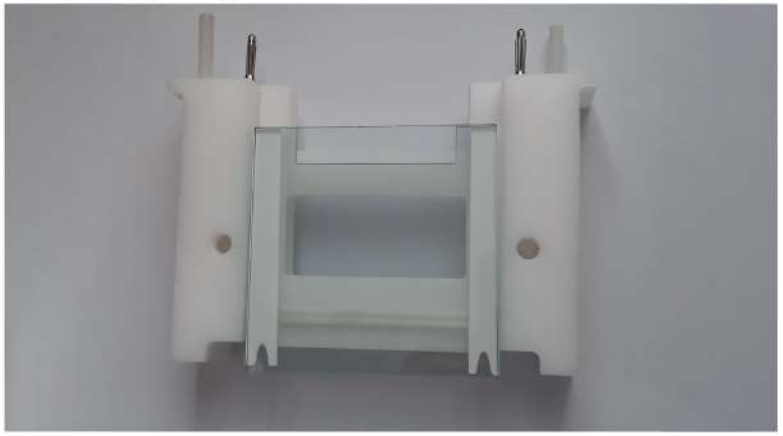

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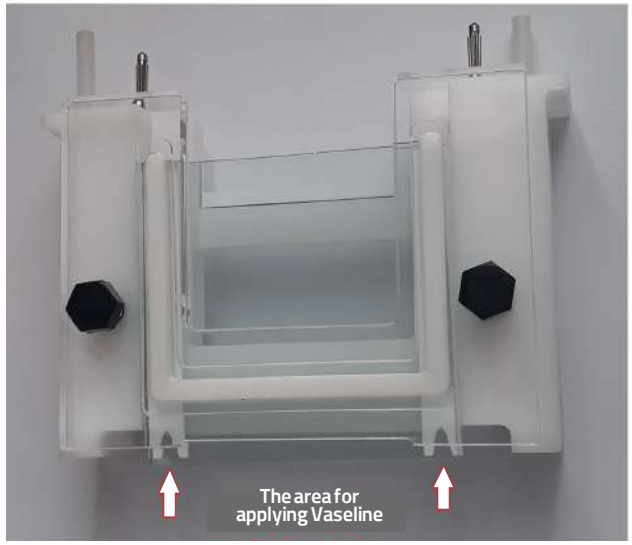

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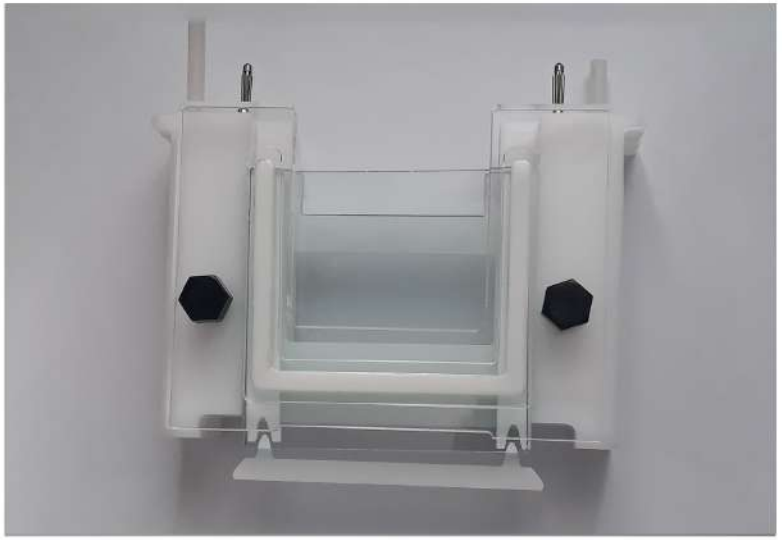

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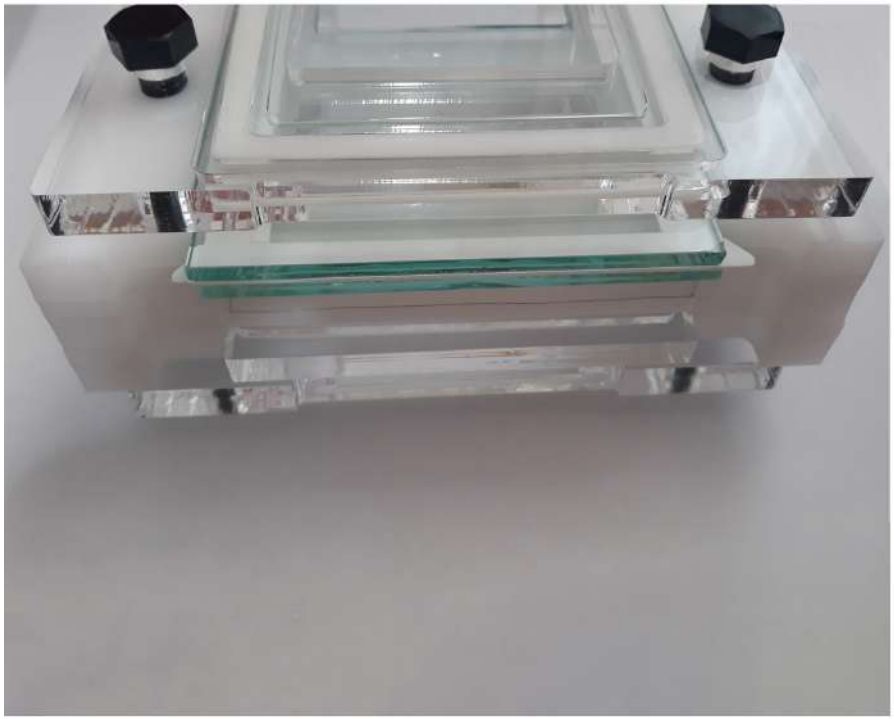

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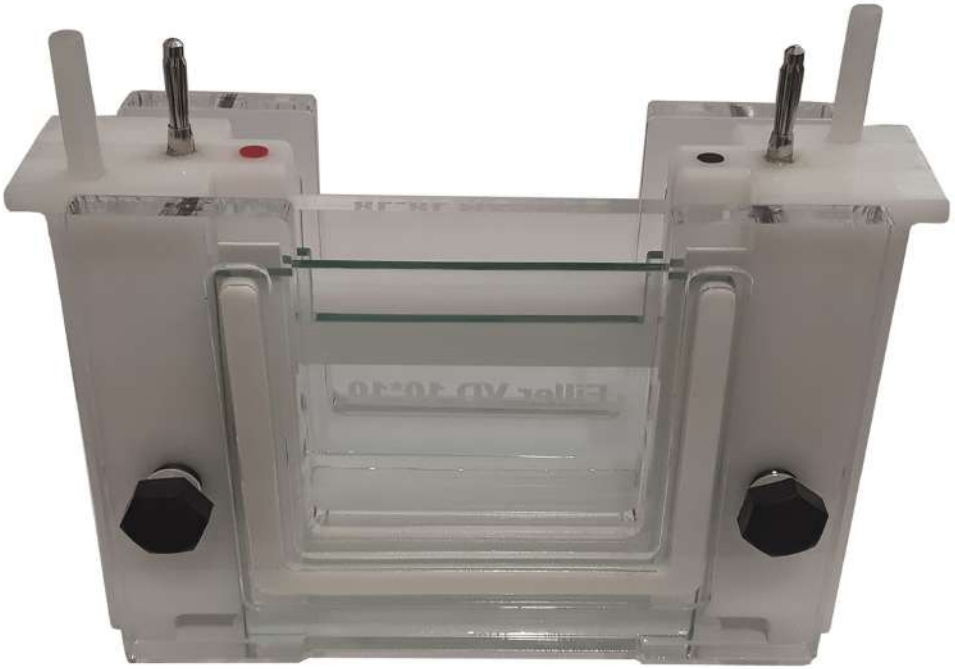

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Place the lid on the device and properly connect the power cables.

First، ensure that the power supply unit is turned off; now، the system is ready to run.

Pouring the Lower Gel (Separating Gel)

Prepare the lower gel solution from its components according to the required percentage. The preparation method for 12 milliliters of the lower gel solution is provided in the table below.

Components of the Lower Gel	Percentage of T					
	20%	17.5%	15%	12.5%	% 10	% 7.5
Lower Gel Buffer	3 ml	3 ml	3 ml	3 ml	3 ml	3 ml
Acrylamide Stock Solution	% 7.8	% 6.8	% 5.9	% 4.9	3.9%	2.9%
Distilled Water	1.2%	2.2%	3.2%	4.1%	5.1%	6.1%
٪۱. Ammonium Persulfat	0.05%	0.05%	0.05%	0.05%	0.05%	0.05%
٪ ۱. TEMED	0.05%	0.05%	0.05%	0.05%	0.05%	0.05%

Table 2. Preparation of 12 Milliliters of Lower Gel Solution with Different Concentrations

Mix the components of the lower gel، excluding TEMED، in a suitable container. Degas the solution under a vacuum for about 30 seconds. Then، add TEMED.

After quickly mixing، pour the solution between the glasses to the appropriate height. Ensure that about 3 centimeters of space is left for the upper gel.

Gently add about 0.5 milliliters of distilled water using a pipette along the side of the glass so that it does not mix with the gel.

The lower gel typically takes 15-45 minutes to polymerize. The polymerized gel can be clearly distinguished from the distilled water on top due to the difference in refractive index.

Upper Gel (Stacking Gel)

After the lower gel has polymerized، prepare the upper gel according to the table below.

Components of the Upper Gel	Percentage of T		
	5٪	4٪	3٪
Upper Gel Buffer	1.25 ml	1.25 ml	1.25 ml
Acrylamide Stock Solution	0.81 ml	0.65 ml	0.5 ml
Distilled Water	2.9 ml	3.05 ml	3.2 ml
Ammonium Persulfate	0.05 ml	0.05 ml	0.05 ml
٪1. TEMED	0.015 ml	0.015 ml	0.015 ml

Table 3. Preparation of 5 Milliliters of Upper Gel Solution with 3%، 4%، and 5% Concentrations

The concentration of this gel is typically 3%، 4%، or 5%. Mix the components of the upper gel، except for TEMED، in a suitable container. Completely drain the water from the surface of the lower gel. To remove any remaining water droplets، swirl about 1 milliliter of the upper gel solution along the inner wall of the glass، then drain it again.

Add TEMED to the remaining upper gel solution and، after quickly mixing، pour it on top of the lower gel to the appropriate height. Carefully insert the comb into the upper gel، ensuring that the teeth are about 1.5 centimeters above the gel surface. The upper gel usually polymerizes in less than 15 minutes (the edges of the comb's teeth can be seen in the solidified gel).

It is advisable to mark the ends of the comb's teeth on the glass with a marker before removing the comb to facilitate sample loading and well identification.

Preparation for Running

- After the upper gel has solidified، remove the combs and place the apparatus inside the tank.
- Fill the tank reservoirs and the apparatus with electrode buffer and sample buffer، respectively، up to the appropriate level.
- Remove any air bubbles at the bottom of the gel by injecting buffer with a syringe.

Adding Samples

Mix one volume of sample buffer (5X) with four volumes of protein sample. If the protein is in powder form، dissolve the required amount in sample buffer that has been diluted five times with distilled water (1X buffer). Place the sample and sample buffer in a small sealed container and heat it in boiling water for 5 minutes. If the sample is cloudy or contains insoluble particles، centrifuge it at 10،000 g for 10 minutes. Then، carefully load 10-20 microliters of each sample into the wells using a Hamilton syringe or suitable pipette. Due to the presence of glycerol، the sample will settle at the bottom of the well. The amount of protein in each well depends on the purity of the sample and the staining method used.
Now، connect the wires to the electrophoresis unit and set the current to a constant flow of 20-30 milliamperes. In this condition، the dye marker (Bromophenol Blue) will reach the end of the gel within 1.5 to 2 hours.

After electrophoresis is complete، turn off the power، remove the apparatus from the tank، and then loosen the screws. Carefully remove the glass plates containing the gel. Using the spatula provided with the device، gently separate the glass plates and remove the gel. If necessary، stain the gel.

Staining proteins in polyacrylamide gel can be done using various methods. Coomassie Blue (types R and G) and silver are among the most commonly used stains for proteins. Here، the staining process using Coomassie Blue، which is very popular، is explained.

Staining with Coomassie Blue R-250

Coomassie Blue R-250 is the most common stain for protein visualization. Its advantages include ease of staining، low cost، long-lasting color stability، and relatively high sensitivity. The sensitivity of this method is 0.5-0.2 micrograms of protein per band. In this method، the fixation and staining of proteins occur simultaneously.

Figure 3. Staining Bath for Protein Staining in Acrylamide Gel

Materials

- Staining Solution:

Dissolve 0.25 grams of Coomassie Blue R-250 in 125 milliliters of methanol. Then، add 25 milliliters of glacial acetic acid and 100 milliliters of distilled water.

The concentration of the dye in this solution is about 0.1% w/v. Before use، filter the staining solution through Whatman No. 1 filter paper. This solution can also act as a protein fixative.

- Destaining Solution:

Mix 200 milliliters of methanol، 100 milliliters of glacial acetic acid، and 700 milliliters of distilled water.

Staining Procedure

- Place the gel in a sealed container. Add a sufficient volume of staining solution (e.g.، 100 milliliters for a small gel). Close the lid and place it on a shaker for 1-2 hours. This duration is sufficient for staining a gel with 10% concentration and 1 millimeter thickness.

- Drain the staining solution. Thoroughly wash the gel with tap water، then add the destaining solution. Close the lid and place it on a shaker. After the destaining solution darkens، replace it with a fresh solution. Repeat this process several times until the gel background is clear and the protein bands are visible.

- Place the gel in a 7% acetic acid solution and close the lid. In this state، the gel can be stored for a long period.

Molecular Weight Determination

In the SDS-PAGE technique، protein molecules are linearized using sodium dodecyl sulfate، and their movement is based on molecular weight. As mentioned earlier، the distance traveled by proteins has a linear relationship with the logarithm of their molecular weight. Therefore، the larger the protein، the shorter the distance it will travel.

Typically، when loading samples، a protein marker is also added to one of the wells. Protein markers consist of several peptides with known molecular weights.

By comparing the migration distance of the target protein on the gel with the protein markers and plotting a relative mobility graph، the molecular weight of the target protein can be estimated.

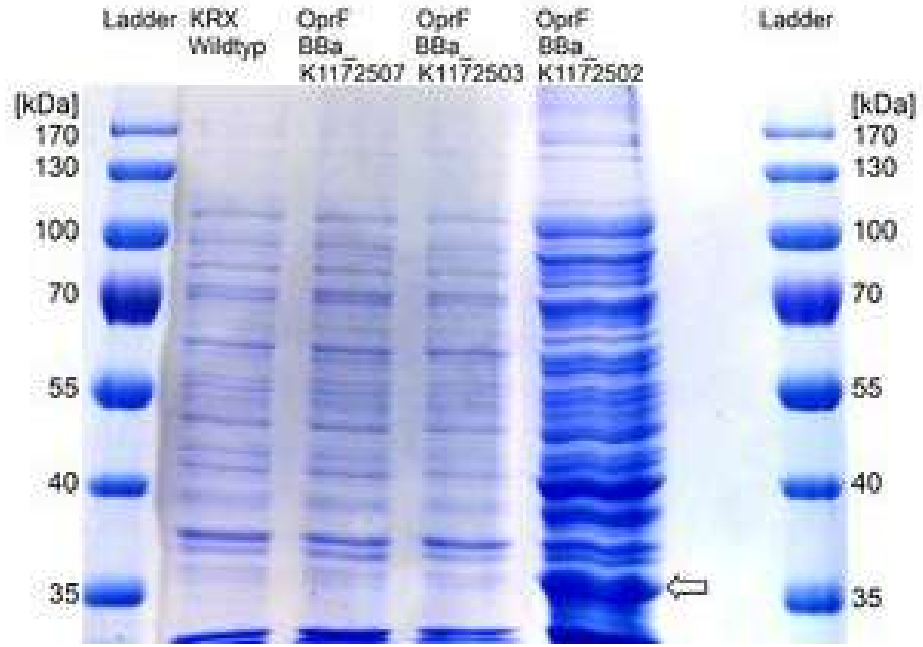

Figure 4. Protein Marker Used in SDS-PAGE Technique Along with the Analyzed Proteins

Documentation and Support

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