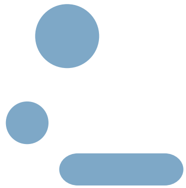
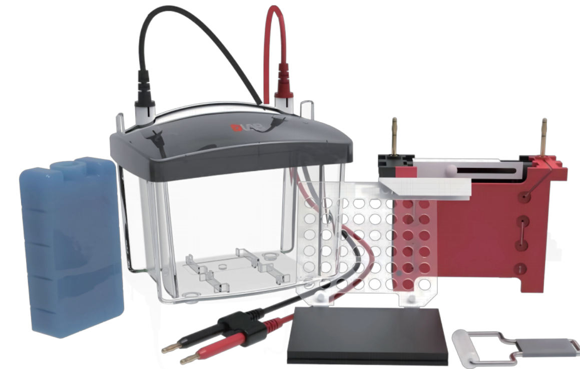


DL–ZY03
Mini transfer electrophoresis tank

operating instruction



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Chapter 1 provides an overview of........P1
1.1 Introduction........P1
1.2 Component........P1
1.3 Specification........P3
1.4 Safety........P4
Chapter 2 Installation and Basic Operations........P4
2.1 Gel Preparation........P4-P6
2.2 Electrophoresis module assembly and sample loading P6-P9
Chapter 3 Separation Theory and Optimization of P10
3.1 Gel Pore size and separation range........P10-P11
3.2 Buffering system and electrophoresis conditions........P11-P12
3.3 Non-denatured polyacrylamide gel electrophoresis (Native PAGE)........P12-P13
Chapter 4 Reagent Preparation and Stockpiling solution P13
4.1 Volume of P13 required per gel P13
4.2 Concentrate the gel to prepare........P13
4.3 Separation gel configuration (Tris-glycine SDS system)........P14
4.4 Commonly Used Buffers and Sample loading buffer P14
Chapter 5 Maintenance of........P15
Chapter 6 Troubleshooting Guide........P15
Chapter 7 References........P16
Chapter 8 Quality Assurance........P16

1. Overview

1.1 Introduction

The core component of the DL-ZY03 electrophoresis tank is its electrode module, which accommodates two gel trays between parallel electrodes spaced only 4 cm apart. The driving force for blotting applications is voltage applied across this narrow electrode gap. This compact 4 cm spacing enables enhanced driving force, resulting in efficient protein transfer. A key feature of the electrode module is its ability to shift sideways to accommodate a blue cooling unit. The cooling system is fully integrated within the DL-ZY03 tank, effectively dissipating Joule heat generated during rapid electrophoretic transfer. This internal cooling design eliminates the need for expensive external cooling tanks and bulky cooling pipes. Additional features include easy-to-assemble gel holder clamps, color-coordinated gel trays, and electrodes that ensure correct gel orientation during transfer.

As well as an efficient design that simplifies the insertion and removal of gel boxes from the electrode assembly. These features create an electrophoretic transport system that is easy to use and produces excellent imprint results.

1.2 Technical specifications

Table 1.DL-ZY03 Components

Assembly	Specifications/quantity	Remarks
Electrolysis tank body (including wires)	1 unit	Core transfer module
Transfer the core	1 unit	Fixed transfer clamp
Sandwich transfer clamp	2 sets	Marked in red and black
Transfer the sponge pad	Four	Removable design
Blue ice boxes	One	Assist cooling
Foam roller	One	Remove bubbles between gel and membrane

Table 2.DL-ZY03 parameters

Project	Specifications
Transferring area	110×90mm (single gel 83×73mm, transferable on two pieces at the same time)
electrode gap	4cm (optimized electric field strength)
Electrode material	99.99% high purity platinum wire, excellent electrical conductivity
outline dimension	16*12*18cm
coolant passage	Built-in blue ice box, rapid heat dissipation
Buffering fluid dosage	1100ml
Power supply adaptation range	Constant current 200-250mA (quick transfer) or constant voltage 25-30V (overnight transfer)

1.3 Safety Notes

The power supply for the DL-ZY03 electrophoresis tank is provided by an external DC voltage source. This power supply must be grounded and isolated to ensure that the DC voltage output remains floating relative to ground. All DL power supplies meet this critical safety requirement. Regardless of the type of power supply used, the maximum specified operating parameters for batteries are:

Maximum voltage limit of 400 VDC direct current

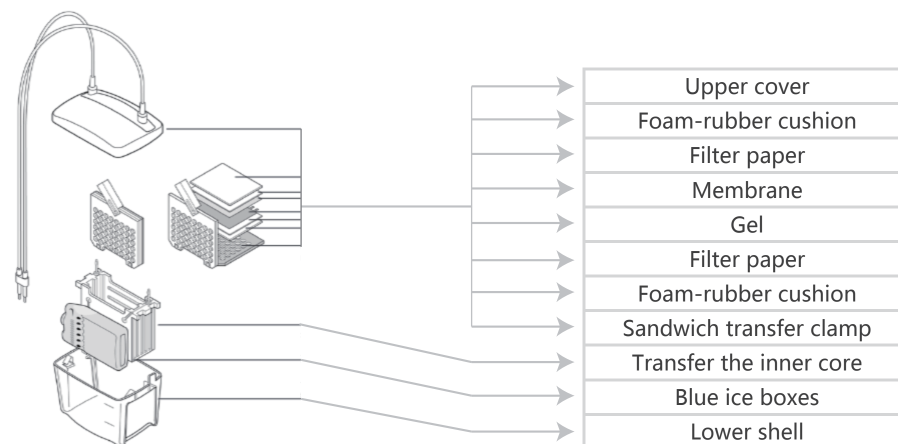
Maximum power limit of 500 W

Maximum ambient temperature limit 40°C

The current supplied to the battery by an external power source enters the device through the cover assembly, providing a safety interlock for users. When removing the cover, the current flowing to the battery is cut off. Do not attempt to bypass this safety interlock, and always turn off the power supply before removing the cover or using the battery in any way.

2. Assembly and transfer preparation

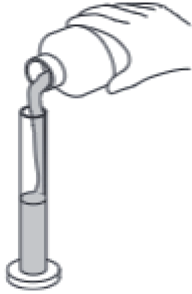
2.1 Transfer tank description and component assembly



2.2 Preparation of imprints

Store the blue cooling unit in a 120°C laboratory refrigerator until ready for use. After use, rinse the external container with water and return the cooling unit to the refrigerator.

1. Prepare the transfer buffer (see section 3.3 for the formulation of the buffer. The use of a buffer cooled to 4°C improves heat dissipation).



2. Cut the membrane and filter paper to the gel size, or use pre-cut membranes and filter paper. Always wear gloves when handling the membrane to prevent contamination. Balance the gel by soaking the membrane, filter paper, and fiber pad in transfer buffer (15-20 minutes, depending on the gel thickness).

3. Prepare the gel sandwich.

a. Place a pre-wet sponge pad on one side of the sandwich.

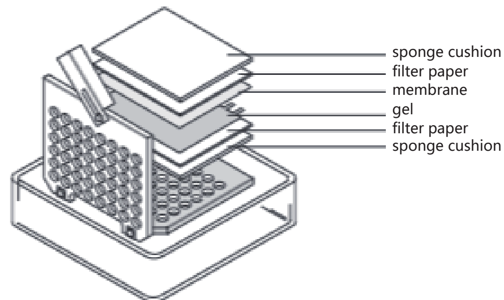
b. Place a filter cloth on the sponge pad.

c. Place the balanced gel on a filter paper.

d. Place the prewetted membrane on the gel.

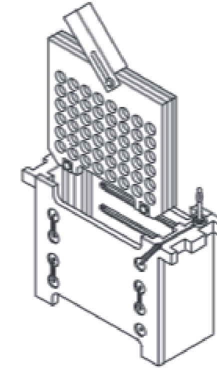
e. Complete the sandwich by placing a filter cloth on the membrane.

f. Cover with a sandwich transfer clip.

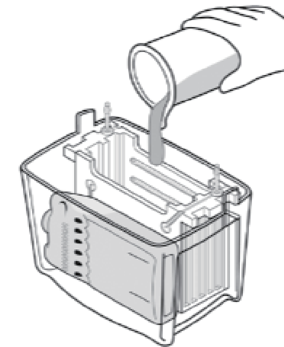


4. Secure the sandwich transfer clamp and be careful not to move the gel and filter paper layer. Lock the sandwich transfer clamp with a white latch

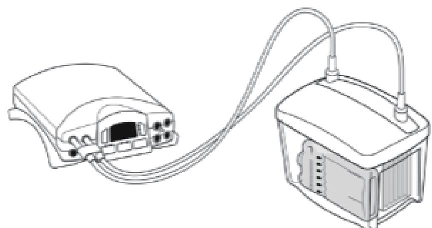
5. Place the sandwich transfer clamp into the module. Repeat the above steps for another sandwich transfer clamp.



6. Add the frozen blue cooling device and fill the tank with an appropriate amount of buffer solution



7. Cover the cover, plug in the cable to the power supply and run it. See section 3 for the running time and electricity of various buffers Pressure setting.



2.3 Acid transfer

If transferring under acidic conditions, replace the gel and membrane according to the instructions. This places the membrane on the cathode side of the gel. Under acidic conditions, proteins will transfer in the opposite direction to the negative cathode.

3.Transfer conditions

3.1 Transfer conditions and operation conditions

Table 3 provides guidelines for power conditions using different buffers. Power conditions are provided for different operating times. When displaying multiple conditions, the higher the voltage, the shorter the time required for operation. Blue cooling devices are always used.

Table 3. Buffer and running conditions guidelines

Buffer solution	Standard magnetic field, high intensity magnetic field buffer overnight transfer	High intensity magnetic field transfer in one hour
SDS-PAGE gel	Buffer A or B or C	Buffer A or B or C
A: 25 mM Tris, pH 8.3, 192 mM glycine, with or without 20% methanol and 0.025%-0.1%SDS	30V constant current 90mA	100V. Constant current 350mA
B: 48 mM Tris, pH9.2, 39mM glycine, with or without 20% methanol and0.025%-0.1%SDS		
C: 10 mM NaHCO3, 3 mM NaCl, pH 9.9. With or without 20% Methanol and 0.025%-0.1% SDS		
DNA and RNA		
TAE: 20 mM Trs, pH 7.8.10 mM		
TBE: 50mM Tris, pH 8.3, 50 mM sodium borate, 1.0mMEDTA		
Native gel		
25 mM Tris, pH 8.3, 92 mM glycine. No methanol	30 volts DC 90mA	100 V constant voltage 350 mA
Electrophoresis, native gel, alkaline protein, acid urea gel		
0.7% acetic acid	30 volts DC 100mA	100V constant voltage 350mA

See section 2.3 before transfer

3.2 Precautions for electrophoretic transfer conditions

These variables will change the total resistance, thereby changing the current reading:

Buffer composition changes, i.e. the addition of SDS, or changes in ion concentration due to the addition of acid or alkali to adjust the pH value of the buffer

- Gel pH value, ionic strength and percentage of acrylamide, especially if the gel is not properly balanced
- Gel quantity; the current increases slightly with the increase of gel quantity
- Buffer liquid volume; when the volume increases, the current increases
- Platinum mass; when the mass increases, the current increases
- Transfer temperature; when the temperature rises, the current increases
- Read the transfer time of the reading; the current usually increases as the buffering capacity decreases

Prebalancing of the gel (15-20 minutes)

Prior to electrophoretic transfer, all electrophoretic gels should be prebalanced in the transfer buffer.

Prebalancing helps remove contaminating electrophoresis buffer salts and neutralizing agents (salts generated during nucleic acid denaturation before transfer). If not removed, these salts would increase the conductivity of the transfer buffer and generate heat during the process. Additionally, a small percentage of gel may shrink in methanol buffer. Balancing allows the gel to reach its final size before electrophoresis transfer.

Current constraints

The gel stand may warp and the transfer buffer may boil and evaporate (further increasing conductivity), which could lead to potential safety hazards.

Transfection buffer pH value

Do not adjust the pH value of the transfer buffer unless specifically stated. When not specified, adjusting the pH of the transfer buffer will increase the conductivity of the buffer. This is manifested by a higher than expected initial current output and reduced resistance.

Recommended transfer buffer

High-quality reagent-grade methanol must be used exclusively. Contaminated methanol may increase the conductivity of transfer buffer and impair the transfer of macromolecules. Never reuse transfer buffer or dilute it below recommended levels. Reusing transfer buffer is not advisable as these solutions may have lost their ability to maintain stable pH during transfer processes. Additionally, diluting transfer buffer below its recommended concentration should be avoided, as this reduces its buffering capacity.

Voltage limits

Do not increase the voltage. Set the voltage higher than that indicated in Table 3.1. If overnight transmission at low voltage is not effective for your application and higher voltage is required, the transmission time must also be shortened. Otherwise, potential safety hazards may occur.

3.3 Buffer solution formulation

1×TAE buffer

Ingredient	Potency	Dosage
Tris alkali	0.40M	48.4g
Acetic acid (85%)	0.20M	11.4 ml
EDTA solution (0.5M,pH8.0)	0.01M	2mL(0.5 M EDTA stock solution)
deionized water		Add 1000ml

1×TBE buffer

Ingredient	Potency	Dosage
Tris alkali	0.89M	10.78g
boric acid	0.89M	5.5g
EDTA solution (0.5M,pH8.0)	0.02M	4mL(0.5 M EDTA stock solution)
deionized water		Add 1000ml

TBST buffer solution

Ingredient	Potency	Dosage
Tris-HCl (pH 7.4-7.6)	10-25 mM	2.42 g (20 mM Tris base)
NaCl	1.5M	8.77g
Tween® 20	0.1% (v/v)	1ml
deionized water		Add 1000ml

Finally PH:7.4-7.6 (adjusted with HCl)

TBST buffer solution

Type of buffer	Fluid	Storage solution preparation
Towbin buffer	1×	25 mM Tris (3.03 g), 192 mM glycine (14.4 g), 20% methanol (200 mL, final concentration), 0.1% SDS (optional, 1 g, for improved transfer efficiency of macromolecules), plus deionized water to 1 L, pH 8.3 (no adjustment required jié)
Low methanol buffer (applicable molecular weight>150KDa)	1×	25 mM Tris (3.03 g), 192 mM glycine (14.4 g), 5-10% methanol (50-100 mL), 0.05% SDS (0.5g) was added to water to 1L
CAPS buffer (under alkaline conditions for transfer)	1×	10 mM CAPS (2.21 g, 3-(cyclohexylamine)-1-propanesulfonic acid), 10% methanol (100 mL), and 0.01% SDS (as available)Selected, 0.1 g), add water to 1 L, pH 11 (adjusted with NaOH)

4. Optimization strategies

4.1 Optimization of protein transfer

Generally, it is difficult to perform quantitative elution of denatured high molecular weight proteins. The following strategies are used individually or in combination,

Will improve efficiency.

Change gel composition

Gradient gels are generally more effective than single concentration gels for the elution of proteins of various molecular weights.

To prepare gels with higher porosity, the total monomer amount was reduced. The percentage of crosslinking agents was increased or decreased. Regardless of acrylamide concentration, the gel with 5.26% C had the smallest pore size among all gels. Reducing the C% enhanced the porosity of the gel while maintaining minimal resolution loss.

% = grams of diacrylamide / (grams of diacrylamide + grams of acrylamide) × 100

Extend the transfer time

Control should be applied first to determine the time required for the transfer. The time required may range from 30 minutes to a full night. Remember that all operations requiring a full night should be performed at 30 volts to minimize heat problems.

Increase the power

Initial controls should be performed to evaluate the efficiency of increasing V/cm and its effect on transfer temperature. Temperature increases may alter buffer resistance and subsequent power transfer, as well as protein denaturation status, thereby affecting transfer efficiency.

Reduce the strength of the buffer

The dilution of the buffer solution will result in a decrease in the current at any given voltage. This will allow higher voltages to be used without excessive heating. However, care should be taken not to dilute the buffer below its buffering capacity.

Change the buffer type and pH value

Maximizing the charge-to-mass ratio. Alcohols in the SDS transfer buffer may strip SDS from proteins. Alkaline proteins in a Tris-glycine-methanol buffer with a pH of 8.3 tend to remain near isoelectric, resulting in poor transfer efficiency. Lysin, for instance, exhibits this characteristic. Buffers with a pH of 9.5-10.0 demonstrate superior elution and binding properties for alkaline proteins.

Under similar V/cm conditions, different buffer types may produce different efficiencies. Generally, tris (3-hydroxymethylaminomethane) buffer is more efficient than acetate or phosphate buffers for transfer.

Add detergent

According to reports, adding 0.1% sodium dodecyl sulfate (SDS) detergent to Tris, glycine, and methanol buffers can enhance transfer efficiency. However, SDS increases relative current, power consumption, and heat generation. Additionally, SDS may precipitate when temperatures are below 10°C, necessitating higher initial buffer temperatures. SDS may also affect the antigenicity of certain proteins. While SDS aids in protein elution from gels, it may reduce binding efficiency between these proteins and the membrane.

Remove alcohols from the membrane transfer buffer

Ethanol in the transfer buffer enhances protein binding to the nitrocellulose membrane. While ethanol removal improves transfer efficiency, it reduces membrane adhesion. The enhanced efficiency occurs because ethanol causes gel pore contraction, trapping high-molecular-weight proteins within the gel matrix.

The use of polyvinylidene fluoride (PVDF) membrane for protein transfer does not require the use of ethanol, which is a reasonable strategy for analyzing high molecular weight or difficult to transfer proteins. PVDF must be first soaked in 100% methanol, and then can be used in a methanol-free buffer.

Change the membrane type

Nitrocellulose and polyvinylidene fluoride can be used for protein transfer.

Change the gel system

If possible, use non-denaturing gradient pore size gel for protein separation. If molecular weight separation is not necessary, consider isoelectric focusing gel or non-denaturing gel.

Enhance gel-membrane contact

Poor contact between the gel and the membrane causes molecules to fail to bind effectively to the membrane, which is often confused with low elution efficiency.

Poor contact is usually caused by excessive moisture at the gel-membrane interface. Proper technique and the use of test tubes or glass pipettes as "wiping rods" should ensure good contact. The correct selection of filter paper pads will help ensure good compression. Before transferring the membrane,

Balancing the gel and membrane in the transfer buffer for 15-20 minutes helps prevent contraction of either component during the transfer process and removes reactants such as urea or sodium dodecyl sulfate (SDS) from the gel.

4.2 Optimization of DNA and RNA transfer

The problem of nucleic acid elution can be solved by changing the gel concentration. Quantitative transfer of large amounts of DNA used in genomic blotting may be a little difficult. In this type of transfer process, the following strategies should be considered to optimize the elution effect.

Change gel composition

Reduce the total percentage of monomer or crosslinker used in polyacrylamide gel.

Reduce the percentage of agarose. This allows better elution of high molecular weight DNA.

Change the DNA denaturant

Studies have found that ethylenediamine is more effective than sodium hydroxide in DNA elution. Boiling polyacrylamide gel to denature DNA also yields good results. Alkaline denaturation often weakens polyacrylamide gel and causes it to stick to the blotting film.

5. Selection of imprint film

5.1 Protein blotting

membrane, nitrocellulose membrane

Nitrocellulose membranes have been widely used for protein binding and detection. They can easily stain total proteins using dye methods (such as amino black,考马斯蓝,丽春红 S,固绿 FCF) or the more sensitive colloidal gold total protein staining method. These membranes are also applicable for radioimmunoassay (RIA), fluorescence immunoassay (FIA), and enzyme immunoassay (EIA). Nitrocellulose exhibits high binding capacity (80-100 $\mu g/cm^2$). Non-specific protein binding sites can be quickly blocked during processing, effectively avoiding background interference. No preactivation is required. However, low molecular weight proteins (especially <15,000 daltons) may be lost during the process. Ethanol added to the transfer buffer may cause loss during post-transfer washing, thereby limiting detection sensitivity. Smaller pore size nitrocellulose membranes (0.2 μm) have proven effective in eliminating such losses. Larger proteins (>100,000 daltons) may experience poor transfer efficiency after SDS denaturation due to ethanol added to the transfer buffer. Ethanol increases SDS-protein binding to the membrane but reduces gel pore size. Removing ethanol during SDS protein transfer significantly decreases binding efficiency. Adding SDS (up to 0.1% concentration) to the transfer buffer enhances protein transfer efficiency but reduces binding quantity. In addition, SDS increases the conductivity of the buffer and the heat generated during the membrane transfer process.

PVDF membrane

Polyvinylidene fluoride (PVDF) membrane is an ideal carrier for amino-terminal sequencing, amino acid analysis and immunodetermination of printed proteins. PVDF can retain proteins even when exposed to extreme conditions such as acidic or alkaline conditions and in the presence of organic solvents.

The enhanced retention rate during sequencing operations improves the likelihood of obtaining information from rare and low-abundance proteins by increasing initial coupling efficiency and higher reproducibility. Additionally, when sodium dodecyl sulfate (SDS) is present in the transfer buffer, polyvinylidene fluoride (PVDF) membranes demonstrate better binding efficiency for blotting materials. PVDF must first be soaked in 100% methanol, but can subsequently be used in methanol-free buffers.

5.2 DNA and RNA

blotting membranes Zeta-Probe ® membrane

Nitrocellulose is not an appropriate medium for nucleic acid electrophoretic transfer due to the requirement of high salt concentration (>10x SSC) for effective binding. Even at high salt concentrations, molecules ≤500 bp cannot bind completely. When current passes through high-salt solutions, the resistance decreases, resulting in potentially harmful currents (and power) even at very low voltages. Since V/cm represents elution force, inefficient transfer occurs under conditions requiring optimal binding. Zeta-Probe membranes can effectively bind single-stranded DNA and RNA of various sizes in low ionic strength buffer solutions. Zeta-Probe membranes serve as an ideal alternative to nitrocellulose in nucleic acid transfer applications. Binding persists during post-transfer washing

It's more stable and can be reprobed up to 10 times.

There are many kinds of blotting films available for immunoblotting, and each has its own specific advantages according to experimental requirements. When selecting appropriate transfer conditions, the physical properties and performance characteristics of the film should be

Table 4. Protein blotting membrane guidelines

Membrane	Aperture	Combining force, capacity (毫克/立方厘米)	Remarks
nitro-cotton	0.45μm	80-100	General protein blotting membrane.
	0.2 μm		
Supporting nitrocellulose	0.45μm	80-100	The pure nitrocellulose is cast on an inert synthetic support; the strength is increased and it is easy to operate
			Creation and re-exploration.
	0.2 μm		
PVDF	0.2 μm	170-200	High mechanical strength and chemical stability for protein sequencing and weslern
			The imprint enhances binding in the presence of SDS. Before equilibrium in the buffer, it must be done
			Must be moistened with alcohol.
nylon	0.2 μm	170	Recommended for nucleic acids.

Note: Nucleic acids cannot be transferred to nitrocellulose membrane by electrophoretic transfer. Use Zeta-Probe membrane instead.

Section 6 Troubleshooting Guide

6.1 Electrophoretic transfer

Poor electrophoretic transfer effect (detected by gel staining) —— protein

1. Too short transfer time.

Extend the transfer time

2. Too low power.

Always check the current at the start of operation. For a particular voltage setting, the current may be too low. If the buffer is not prepared properly, the conductivity may be too low to provide enough power to the battery.

See the power guide for specific applications in Section 3

Reconstitute the buffer or increase the voltage

Try high intensity transfer options

3. The power circuit is not working, or the wrong power supply is used.

Check the fuse. Ensure that the voltage and current output of the power supply meet the requirements of the imager.

4. The transfection device is not assembled correctly and the protein moves in the wrong direction.

The gel/membrane assembly sequence may be incorrect, or the cartridge may be inserted in the wrong direction. Check the polarity of the power supply connection. After transfer, evaluate the transfer efficiency using pre-stained protein standards

5. The charge to mass ratio is incorrect.

Try using a more alkaline or acidic transfer buffer to increase the protein mobility.

6. The protein precipitates in the gel.

Try using sodium dodecyl sulfate (SDS) in the transfer buffer. SDS can improve the efficiency of the transfer, but it also reduces the binding efficiency with nitrocellulose membrane and affects the reactivity of some proteins and antibodies.

Excessive methanol can lead to protein precipitation. Try to reduce the methanol content.

7. Transfer of methanol from the buffer limits elution.

Reducing methanol improves the transfer efficiency of protein from the gel, but also reduces its binding capacity to nitrocellulose.

8. Too high percentage of gel.

Reduce%T (total monomer) or%C (crosslinker). A concentration of 5.26%C (using dibenzacrylamide as crosslinker) will produce a gel with the smallest pore size. Below this concentration, the pore size increases and the transfer efficiency also improves.

Poor transfer —— nucleic acid

1. The gel concentration is too high.

Reduce the T% or C% in acrylamide gel, or reduce the percentage of agarose in agarose gel

DNA was lysed with 0.25 M hydrochloric acid or RNA with dilute sodium hydroxide prior to transfer

2. The transfer time is too short or the power condition is too low.

Extend the transfer time, or try high intensity transfer

3. Because effective binding requires high salt concentration, DNA or RNA cannot be transferred to the nitrocellulose membrane by electrophoresis.

Use Zeta-Probe membrane instead of nitrocellulose membrane

Vortex or strip absence; diffuse metastases

1. Poor contact between the membrane and gel. Bubbles or excess buffer are present between the blot and the gel.

Use a test tube or pipette as a rolling pin and carefully roll the membrane back and forth until the bubbles and excess buffer between the gel and the membrane are squeezed out, ensuring that they are in full contact.

Use thicker filter paper in the gel/membrane sandwich

Replace the sponge pad. The sponge pad will compress over time and will not hold the membrane to the gel.

2. Too high power condition.

Check the current at the start of operation. For specific voltage Settings, the current may be too high. If the buffer is not prepared properly, the conductivity may be too high, resulting in excessive power being delivered to the electrolytic cell. See section 3 for specific application power guidelines.

3. The membrane is not fully wet or has dried.

White spots on the nitrocellulose membrane indicate dryness in the area where proteins cannot bind. If the membrane is not immediately moistened after immersion in transfer buffer, heat distilled water to near boiling point and soak the membrane until fully saturated. Allow it to balance in the transfer buffer until ready for use.

Since polyvinylidene fluoride (PVDF) is hydrophobic, the membrane must be pre-wetted with methanol before being transferred to an aqueous buffer for equilibrium. Do not let the membrane dry after wetting. If necessary, re-wet it with methanol.

4. It may be a problem with gel electrophoresis.

Electrophoresis artifact may be caused by poor polymerization, improper electrophoresis conditions, buffer contamination, excessive amount of sample, etc.

The gel pattern is transferred to the imprint membrane

1. Use of contaminated or too thin sponge pads.

Replace the sponge mat or thoroughly clean the contaminated mat

2. The gel was loaded with an excess of protein, or too much SDS (sodium dodecyl sulfate) was used in the transfer buffer. The protein may pass through the membrane without binding and circulate in the slot blotting system.

Reduce the amount of protein on the gel and SDS in the transfer buffer. Shorten the transfer time or add a second membrane to bind excess protein.

3. The transfer buffer is contaminated.

Prepare fresh solution. Transfer buffer cannot be reused.

Poor bonding with membrane —— nitrocellulose

1. Nitrocellulose requires the addition of 20% methanol in the transfer buffer to achieve optimal protein binding.

Ensure that the buffer contains an appropriate amount of methanol

2. The protein may be passing through the nitrocellulose membrane.

Use polyvinylidene fluoride (which has a stronger binding capacity) or 0.2 micron nitrocellulose (which has a smaller pore size). If using the high strength option, reduce the voltage.

3. The binding capacity of mixed ester cellulose and protein is poor.

Use pure nitrocellulose.

4. When the protein molecular weight is less than 15,000 daltons, the binding capacity with 0.45 micron nitrocellulose may be weakened or washed off from the membrane during detection.

To increase the stability of the bond, the protein can be crosslinked with nitrocellulose using glutaraldehyde.

Use PVDF membrane with higher binding capacity

Tween-20 detergent was used in the washing and antibody incubation steps. Less or no more stringent washing conditions were used

5. Transfer of SDS from the transfer buffer reduces protein binding efficiency. Reducing or removing SDS from the transfer buffer

6. The membrane may not be fully wetted.

The white spots on the membrane indicate dry areas where proteins cannot bind. If the membrane is not immediately moistened after immersion in the transfer buffer, heat distilled water to near boiling point and soak the membrane until fully hydrated. Allow equilibrium in the transfer buffer until ready for use.

Poor adhesion to the membrane —— polyvinylidene fluoride (PVDF) membrane

1. The membrane may not be fully wetted.

Due to the hydrophobicity of polyvinylidene fluoride (PVDF), alcohol must be used before it is transferred to the aqueous buffer for equilibrium

Pre-wet the membrane. Please follow the instructions in the product manual.

2. During operation, the membrane may have been dried.

A completely wet membrane appears gray and translucent. White spots will form on the surface of the membrane, indicating that the membrane has dried. Since the protein does not bind to the dry spots, the membrane needs to be rewetted with methanol and rebalanced in the transfer buffer.

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8. Quality assurance

The product complies with the laboratory electrophoresis equipment standard and provides a 5-year warranty (non-manual damage). Please read the operation manual carefully before use to ensure correct use.

For inquiries or repair service requests, please contact Dalong service personnel.