Mini Tank Transfer (Blotting) Cell

L-VET-TRANS4 Operations Manual

L-VET-TRANS4 — 2024.8 Edition

Contents

Unpacking Instructions **3**
Chapter 1 Product Introduction **3**
1. Overview 3
2. Technical Specifications 4
3. Safety Information 5
Chapter 2 Cell Assembly and Transfer Preparation **6**
Chapter 3 Electrophoretic Transfer Conditions **9**
Chapter 4 Procedures for Optimising Transfer **11**
Chapter 5 Membrane Selection **13**
Chapter 6 Troubleshooting **14**

Unpacking Instructions

The L-VET-TRANS4 mini transfer cell is packed in a high-quality imported corrugated carton with environmentally friendly cushioning, and a clear product label is affixed in a prominent position on the outside. On receiving the product, inspect the outer packaging carefully for any obvious damage. If you find any problem with the outer packaging, please contact us promptly.

Packing list:

- Buffer chamber and upper lid with dedicated cable — 1 each;
- Transfer electrode core — 1;
- Gel sandwich cassettes — 4;
- Transfer pads — 8;
- Cooling modules — 2;
- Operations manual — 1.

Before use, please read this manual carefully and become familiar with the performance characteristics and operation of each component of the electrophoresis system. Operating strictly in accordance with this manual gives the best optimisation of experimental conditions and the best results, while avoiding accidental damage to the system and extending its service life.

Recommendation: Before using the L-VET-TRANS4 mini transfer cell, clean all components with a neutral laboratory detergent and then rinse thoroughly with distilled water.

Chapter 1 Product Introduction

1. Overview

The L-VET-TRANS4 mini transfer cell is part of a mini electrophoresis system that also includes a mini vertical electrophoresis cell capable of running SDS-PAGE and native-PAGE gels.

The L-VET-TRANS4 holds up to four transfer cassettes at once and can be used to transfer (blot) protein and nucleic acid samples from polyacrylamide and agarose gels.

A cooling module is standard with the L-VET-TRANS4; once frozen, it absorbs the heat generated during high-speed transfers. The built-in cooling avoids the cost of an external recirculating-water cooling bath and the inconvenience of tubing connections. Other features of the L-VET-TRANS4 include simple, easy-to-use latches on the gel cassettes and colour-coded cassettes and electrode core for accurate orientation during transfer.

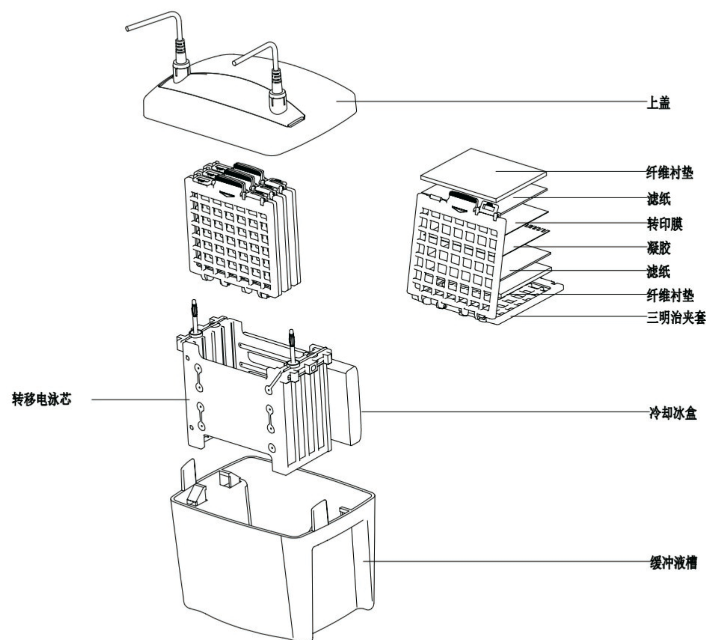

Figure 1: L-VET-TRANS4 cell and component assembly (exploded view)

Callouts (top to bottom): upper lid; fiber pad; filter paper; transfer membrane; gel; filter paper; fiber pad; gel sandwich cassette; transfer electrode core; cooling module; buffer chamber.

2. Technical Specifications

Product parameters:

Parameter	Specification
Model	L-VET-TRANS4
Transfer area	110 × 90 mm
Transfer capacity	1–4 gels/blots simultaneously
Platinum electrode	φ0.25 mm
Dimensions (L × W × H)	180 × 120 × 160 mm

Materials and capacities:

Item	Detail
Electrode core	Polycarbonate
Gel sandwich cassette	Polycarbonate
Electrodes	Platinum wire drawn from a platinum ingot
Buffer chamber and lid	Polycarbonate
Cooling unit	Polyethylene + high-efficiency cold-storage agent
Gel cassette size	10.9 cm × 11.6 cm
Buffer volume	Without cooling unit: 850 ml; with cooling unit: 650 ml

Cleaning: Clean the electrodes, gel cassettes and buffer chamber with a neutral detergent and warm water. Take particular care when cleaning the electrodes to avoid stretching or breaking

the platinum wire. Do not use abrasives or harsh detergents. Rinse the fiber pads with hot water, then rinse clean with distilled, deionised water.

Chemical compatibility: None of the components of the L-VET-TRANS4 may come into contact with chlorinated hydrocarbons (e.g. chloroform), aromatic hydrocarbons (e.g. toluene, benzene) or acetone. Damage caused by the use of organic reagents is not covered by the warranty.

Accessories and spare parts:

Part No.	Name	Description	Pack unit	Std. qty
210119002	Transfer frame (cassette holder)	With platinum electrode	1 pc/pack	1
210119003	Transfer cassette	95 × 80 mm, two perforated plates joined together	1 pc/pack	4
210119004-3	Filter-paper-free sponge for 4 gels (white)	For wrapping the gel and nitrocellulose membrane	5 pcs/pack	10
210119006	Thin sponge (black)	For wrapping the gel and nitrocellulose membrane	5 pcs/pack	10
710104006	Cooling module	For cooling; interchangeable	1 pc/pack	2
210110011	Upper shell (with cord)	Upper lid with power cord	1 pc/pack	1
210110012	Buffer chamber	—	1 pc/pack	1
210119005	Transfer set	—	1 pc/pack	—

Note: “Std. qty” is the quantity included in the standard configuration; “—” indicates an optional accessory or spare part not included as standard.

3. Safety Information

Power for the L-VET-TRANS4 is supplied by an external DC power supply. The output of this supply must be isolated from earth/ground so that the full DC output passes through the cell and does not form a loop with the earth line. All compatible series power supplies meet this safety standard. Regardless of the power supply used, the following are the maximum operating parameters permitted for the L-VET-TRANS4:

- Maximum input voltage: 150 V (DC)
- Maximum input power: 40 W
- Maximum operating temperature: 50 °C

All current through the cell is connected via the upper lid, providing a safety interlock. When the lid is opened, the current to the cell is cut off. Always switch off the power before opening or removing the lid. Never attempt to operate the cell without the upper lid in place.

Note: Our products are designed and manufactured to meet recognised safety standards, and operating them strictly in accordance with the instructions will be safe. The equipment must not be modified or altered in any way. Modification will void the warranty, breach safety standards and create potential hazards. We accept no liability for damage or loss caused by deliberate misuse or unauthorised modification of the product.

Chapter 2 Cell Assembly and Transfer Preparation

1. Cell and Component Assembly

Familiarise yourself with the components and their assembly using the exploded view in Figure 1 above. Assemble the gel sandwich cassette, transfer electrode core, cooling module and buffer chamber as shown.

2. Transfer Preparation

(1) Place the cooling module (ice block) in a −20 °C freezer to freeze before use; after use, return it to the freezer for storage.
(2) Prepare transfer buffer (see the buffer formulations in Section 3.3). Cooling the buffer to 4 °C helps dissipate heat.
(3) Cut filter paper and membrane to the size of the gel. Always wear gloves when handling the membrane to avoid contamination. Equilibrate the gel and soak the membrane, filter paper and transfer pads in transfer buffer (15 minutes to 1 hour, depending on gel thickness).

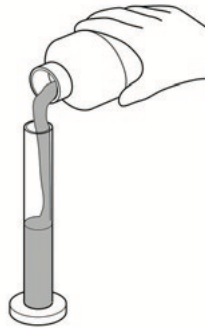

Figure 2: Wetting the membrane and pads in transfer buffer

Assemble the gel sandwich:

(1) Place the sandwich cassette on a clean bench with the black side down.
(2) Place a pre-wetted transfer pad on the black side of the cassette.
(3) Place a soaked filter paper on the transfer pad.
(4) Place the equilibrated gel on the filter paper (expel any air bubbles between gel and filter paper).
(5) Place the soaked membrane on the gel (expel any air bubbles between membrane and gel).
(6) Place a filter paper on the membrane and expel any bubbles, then add a transfer pad.

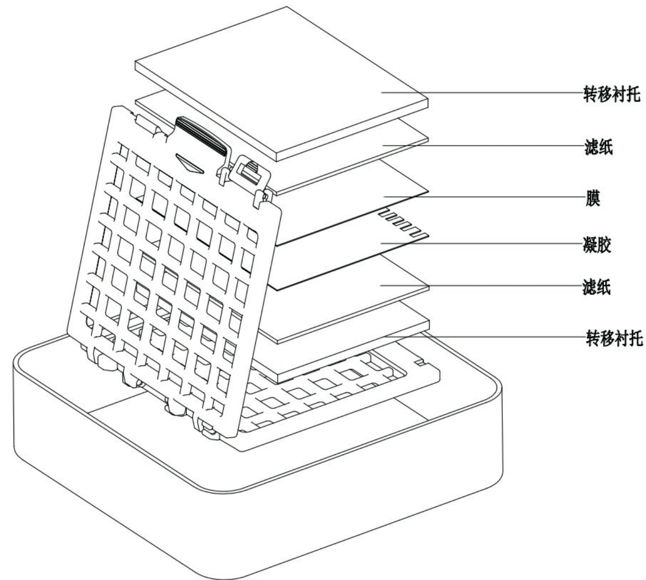

Figure 3: Order of the gel/membrane sandwich (exploded view)

Stack order (top to bottom): fiber pad; filter paper; gel; transfer membrane; filter paper; fiber pad — on the cassette.

Note: Completely expelling air bubbles is the key to a good transfer. Use a glass rod to roll out bubbles gently.

(1) Clamp the cassette closed, taking care not to move the gel-and-filter-paper sandwich, and lock the cassette with the white slider.
(2) Insert the cassette into the transfer electrode core. Repeat the above steps to prepare another gel sandwich cassette.

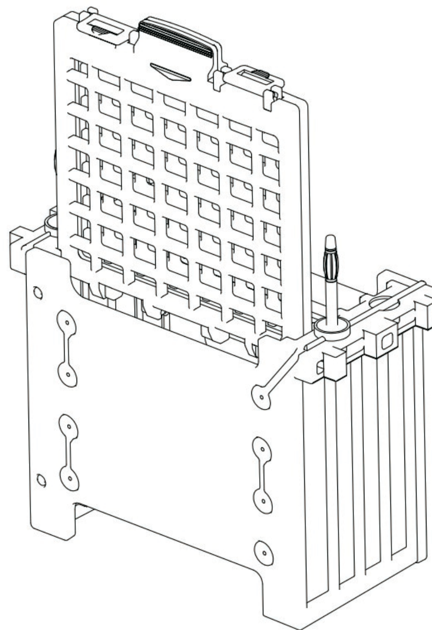

Figure 4: Inserting the cassette into the transfer electrode core

(1) Place the electrode core into the buffer chamber, add the frozen cooling module and fill with buffer.

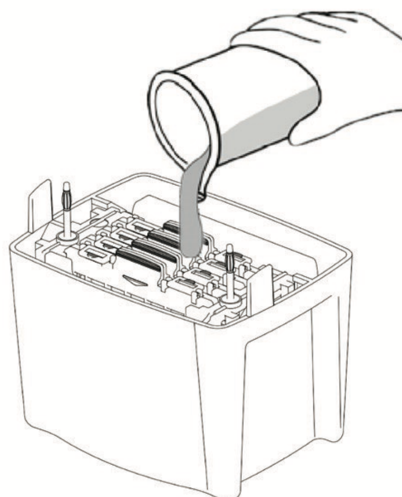

Figure 5: Adding the cooling module and filling with buffer

(1) Add a stir bar to help keep the buffer temperature and ionic strength uniform; set as fast a speed as possible to even out the ion distribution.
(2) Fit the safety lid, plug the power cable into the electrophoresis power supply and begin the transfer. For voltage settings and transfer times with different buffers, see Chapter 3.

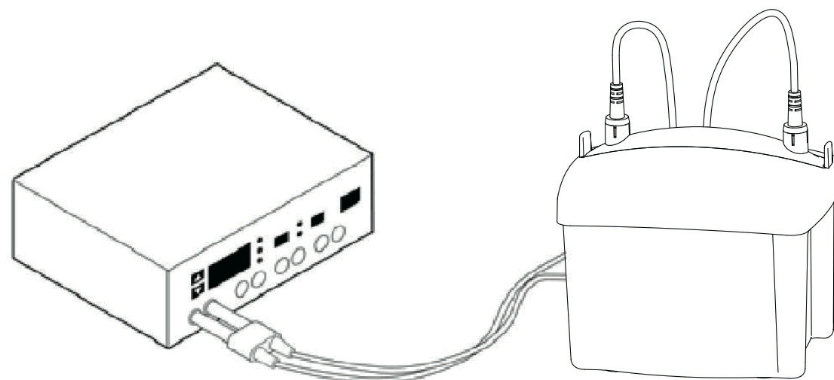

Figure 6: Connecting the power supply and running the transfer

(1) After the transfer is complete, disassemble the sandwich cassette and remove the membrane for the next step. Clean the cell, cassettes and pads with a neutral laboratory detergent, then rinse clean with deionised water.

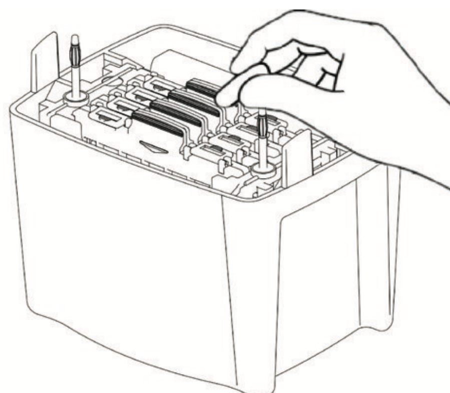

Figure 7: Removing the electrode core after the run

3. Acidic Transfer

If the transfer is carried out under acidic conditions, swap the positions of the gel and membrane, placing the membrane on the negative-electrode side of the gel. Under acidic conditions, proteins transfer in the opposite direction — migrating toward the negative electrode. Do not reverse the electrodes themselves, or the cell will be damaged.

Chapter 3 Electrophoretic Transfer Conditions

1. General Guidelines for Transfer Buffers and Run Conditions

Table 3.1 gives power conditions for different buffer environments and for different transfer times. Across these conditions, a higher voltage gives a shorter run time. A cooling module must be used during the transfer.

Table 3.1 Transfer buffers and recommended power conditions

Buffer	Standard field strength (overnight)	High field strength (4 cm electrode gap, 1 h)
SDS-PAGE gels: A: 25 mM Tris, pH 8.3, 192 mM glycine, ± 20% MeOH and 0.025–0.1% SDS B: 48 mM Tris, pH 9.2, 39 mM glycine, ± 20% MeOH and 0.025–0.1% SDS C: 10 mM $NaHCO_3$, 3 mM Na_2CO_3, pH 9.9, ± 20% MeOH and 0.025–0.1% SDS DNA and RNA: TAE: 20 mM Tris, pH 7.8, 10 mM sodium acetate, 0.5 mM EDTA TBE: 50 mM Tris, pH 8.3, 50 mM sodium borate, 1.0 mM EDTA	Buffer A / B / C: 30 V, 90 mA	Buffer A / B / C: 100 V, 350 mA (or 80 V, 500 mA)
Native gels: 25 mM Tris, pH 8.3, 192 mM glycine. No methanol	30 V, 90 mA	100 V, 350 mA
Isoelectric focusing, native gels, basic proteins, acid-urea gels: 0.7% acetic acid	30 V, 100 mA	100 V, 350 mA

2. Considerations for Transfer Conditions

The following changes alter the resistance and therefore the current:

- Changes in buffer preparation — e.g. adding SDS, or using more acid or base when adjusting pH, which changes ionic strength.
- Gel pH, ionic strength and acrylamide percentage, especially if the gel is not well equilibrated.
- Number of gels: more gels slightly increase the current. Buffer volume: a larger buffer volume increases the current.
- Platinum electrode mass: heavier electrodes increase the current.
- Transfer temperature: higher temperature increases the current.
- Transfer time: as the run proceeds, the buffering capacity weakens and the current rises.

Pre-equilibration of the gel

All gels must be pre-equilibrated in transfer buffer before electrophoretic transfer. Pre-equilibration helps remove salt contamination from the electrophoresis buffer and neutralise salts (from denaturing nucleic acids before transfer). If salt is not removed, it raises the conductivity of the transfer buffer and generates excessive heat during the run. In addition, low-percentage gels (<12%) shrink in methanol-containing solutions; equilibration brings the gel to its final size before transfer.

Use of a stir bar during transfer

In all transfer applications, a stir bar must be placed in the L-VET-TRANS4 so the buffer is stirred throughout the run. This helps maintain uniform conductivity and temperature in the transfer buffer. Failure to control the buffer temperature adversely affects the transfer of macromolecules and creates a potential safety hazard.

Transfer buffer pH

Do not adjust the pH of the transfer buffer unless specifically directed. Adjusting the pH raises the buffer conductivity, as shown by a higher-than-expected initial current and reduced resistance. It is recommended to check the initial current before each transfer.

Transfer buffer recommendations

Use high-quality, reagent-grade methanol. Contaminated methanol raises the buffer conductivity and can cause macromolecule transfer to fail. Do not reuse transfer buffer or dilute it below the recommended concentration. Reuse is not recommended because the buffer cannot maintain a stable pH during the run; diluting below the recommended concentration reduces its buffering capacity.

Maximum voltage

For overnight transfers, do not exceed the voltage settings listed in Table 3.1; the buffer conductivity should be close to the currents listed. A current limit should be set on the power supply. If a low-voltage overnight transfer is ineffective for your application, use a higher-voltage transfer but reduce the transfer time accordingly — otherwise a safety hazard may arise.

3. Buffer Formulations

All formulations are prepared for 1 litre of buffer. The L-VET-TRANS4 requires approximately 500 ml of buffer. Do not adjust the buffer pH by adding acid or base. Use analytical-grade methanol; metal contaminants in low-purity methanol will contaminate the electrodes.

Note: Some pH electrodes are insensitive to Tris. If the measured pH seems off, check whether the electrode is suitable for Tris buffers. If the electrode is correct but the buffer pH is below 8, re-prepare the buffer.

Protein transfer buffers

- Buffer A — 25 mM Tris, 192 mM glycine, 20% v/v methanol, pH 8.3: mix 3.03 g Tris, 14.4 g glycine and 200 ml methanol; add dd H_2O to 1 L.
- Buffer A (no methanol) — 25 mM Tris, 192 mM glycine, pH 8.3: mix 3.03 g Tris and 14.4 g glycine; add dd H_2O to 1 L.
- Buffer B — 48 mM Tris, 39 mM glycine, 20% v/v methanol, pH 9.2: dissolve 5.82 g Tris and 2.93 g glycine in ddH_2O, add 200 ml methanol, make up to 1 L with ddH_2O.
- Buffer B (no methanol) — 48 mM Tris, 39 mM glycine, pH 9.2: mix 5.82 g Tris and 2.93 g glycine; add ddH_2O to 1 L.
- Buffer C — 10 mM $NaHCO_3$, 3 mM Na_2CO_3, 20% methanol, pH 9.9: dissolve 0.84 g $NaHCO_3$ and 0.318 g Na_2CO_3 in ddH_2O, add 200 ml methanol, make up to 1 L with ddH_2O.

Nucleic acid transfer buffers

- 1.0× TBE (Tris-borate-EDTA), pH 8.3 — 90 mM Tris-borate, 1 mM EDTA. 5× stock: 54 g Tris base, 27.5 g boric acid, 20 ml 0.5 M EDTA (pH 8.0). For 1.0× buffer, add 200 ml of 5× stock to 800 ml ddH_2O.
- 1× TAE (Tris-acetate-EDTA) — 40 mM Tris-acetate, 1 mM EDTA. 50× stock: 242 g Tris base, 57.1 ml glacial acetic acid, 100 ml 0.5 M EDTA (pH 8.0). For 1× buffer, add 20 ml of 50× stock to 980 ml ddH_2O.

Chapter 4 Procedures for Optimising Transfer

1. Optimising Protein Transfer

In general, quantitative elution of high-molecular-weight proteins is difficult. The following measures, used alone or in combination, can improve transfer efficiency.

Different gel compositions

Gradient gels are better than single-concentration gels for eluting proteins over a wide molecular-weight range. Lowering the total monomer concentration produces a more porous gel. Regardless of the acrylamide concentration, a gel with a crosslinker (bis-acrylamide) concentration (%C) of 5.26% has the smallest pore size; increasing or decreasing %C enlarges the pore size and reduces resolution.

%C = bis-acrylamide (g) / [bis-acrylamide (g) + acrylamide (g)] × 100%

Increase the transfer time

The initial control conditions determine the time needed for complete transfer. Transfer times range from 30 minutes to overnight, depending on the conditions. Note that the voltage for an overnight transfer should be set to 30 V to reduce heat generation.

Increase the field strength

Initial conditions must balance adequate transfer efficiency (V/cm) against the effect of transfer temperature. A higher temperature changes the protein denaturation state and the buffer resistance, altering the field force and thus the transfer efficiency.

Lower the buffering capacity

Diluting the buffer lowers the current at a given voltage, so a higher voltage can be used without generating excessive heat.

Different buffer types and pH

Changing the buffer type and pH can maximise the charge-to-mass ratio. Ethanol in SDS transfer buffers appears to strip SDS from proteins. In a Tris/glycine/methanol, pH 8.3 buffer, basic proteins may be at their isoelectric (neutral) point and fail to transfer — lysozyme shows this behaviour. A buffer of pH 9.5–10 gives better transfer of basic proteins such as lysozyme and histones. Different buffer types can give different transfer efficiencies even at similar field strength (V/cm); in general, Tris buffers transfer better than acetate and phosphate buffers.

Add detergent

Adding 0.1% SDS to a Tris/glycine/methanol buffer has been reported to improve transfer efficiency. However, SDS increases current, field strength and heat; because SDS precipitates below 10 °C, the initial buffer temperature should be slightly higher. SDS also affects the antigenicity of some proteins. SDS helps elute proteins from the gel but can reduce their binding to nitrocellulose membranes.

Remove alcohol from the transfer buffer

Alcohol in the transfer buffer only promotes binding of SDS-coated proteins to nitrocellulose. Removing alcohol increases transfer efficiency but reduces membrane binding. The efficiency increase occurs because alcohol shrinks the gel pores, retaining large proteins. Using PVDF membrane eliminates the need for alcohol with SDS-coated proteins and allows a sound analysis strategy for large and hard-to-transfer proteins. PVDF must first be wetted with 100% methanol.

Limited protease treatment

Limited digestion of proteins with a protease during transfer has been reported to enhance transfer efficiency without reducing immunological activity.

Change the membrane type

As noted above, using PVDF allows transfer to be performed without methanol.

Change the gel type

Where possible, use a non-denaturing gradient gel to separate proteins of different molecular weight. If separation by molecular weight is not essential, consider an isoelectric-focusing gel or a native gel.

Improve gel-to-membrane contact

Failure of proteins to bind effectively to the membrane due to poor gel-to-membrane contact is often mistaken for ineffective elution. Poor contact is usually caused by excess liquid between gel and membrane. Using a tube or glass rod as a 'roller' ensures good contact. Choosing the correct filter-paper spacers helps ensure firm pressure; equilibrating the gel and membrane in transfer buffer for 30 minutes to 1 hour before transfer helps prevent shrinkage during transfer.

2. Optimising DNA and RNA Transfer

Nucleic acid elution problems can be addressed by varying the gel percentage. Quantitatively transferring large amounts of DNA in genomic bands is somewhat more difficult. The following strategies can be considered for such transfers.

Change the gel composition

Lower the total monomer percentage (%T) or crosslinker percentage (%C) of a polyacrylamide gel. Lower the percentage of an agarose gel; this favours transfer of high-molecular-weight DNA.

Change the DNA denaturant

Glyoxal denaturation favours DNA elution more than sodium hydroxide. Boiling a polyacrylamide gel to denature DNA also gives excellent results. Alkaline denaturation usually softens polyacrylamide gels and makes them stick to the membrane.

Chapter 5 Membrane Selection

1. Protein Blotting Membranes

Nitrocellulose membrane

Nitrocellulose is widely used for protein binding and detection. Total protein can be detected simply with protein dyes (e.g. amido black, Coomassie blue, Ponceau S, fast green FCF) or the more sensitive colloidal gold stain, and it is compatible with radioimmunoassay (RIA), fluorescence immunoassay (FIA) and enzyme immunoassay (EIA). Nitrocellulose has a high binding capacity of 80–100 µg/cm², requires no pre-treatment and has low non-specific binding.

PVDF membrane

PVDF (polyvinylidene difluoride) is an ideal support for N-terminal sequencing, amino-acid analysis and blot-based protein immunoassays. PVDF retains protein under extreme conditions such as exposure to acid or base or organic solvents. In sequencing, its strong retention, enhanced initial binding and high reproducibility improve the chance of obtaining information on precious low-abundance proteins. PVDF also shows better performance in SDS-containing transfer buffers.

2. DNA and RNA Blotting Membranes

Zeta-Probe® nylon membrane

Because a high salt concentration (>10× SSC) is required for binding, nitrocellulose is not suitable as a medium for electrophoretic transfer of nucleic acids. Even at high salt, nucleic acids <500 bp do not bind. High-salt solutions cause low resistance, which produces potentially damaging high current (or power) at low voltage. Because the voltage drop per centimetre (V/cm) is the eluting force, ineffective transfer can occur under the required binding conditions. Zeta-Probe membrane is recommended for nucleic acids.

Several blotting membranes are available for immunoblotting, each with particular advantages depending on the experiment. When selecting transfer conditions, the physical properties and performance of the membrane must be evaluated.

Note: Nucleic acids cannot be electrophoretically blotted onto nitrocellulose; use Zeta-Probe membrane.

Membrane comparison:

Membrane	Pore size	Binding capacity (µg/cm²)	Notes
Nitrocellulose	0.45 µm / 0.2 µm	80–100	General-purpose protein blotting membrane.
Supported nitrocellulose	0.45 µm / 0.2 µm	80–100	Pure nitrocellulose cast on an inert synthetic support for added strength, easier handling and better reproducibility.
PVDF	0.2 µm	170–200	High mechanical strength and chemical stability; for protein sequencing and blot transfer; improves binding in SDS; must be wetted with methanol before equilibrating in buffer.
Nylon	0.2 µm	170	Recommended for nucleic acids.

Chapter 6 Troubleshooting

1. Electrophoretic Transfer

Poor transfer (detected by gel staining) — protein

- Time too short: increase the transfer time.
- Power too low: always check the current at the start of the transfer. A given voltage setting may make the current too low. If the buffer is incorrectly prepared, the conductivity will be too low and there is not enough driving force. Re-prepare the buffer or raise the voltage and try a high-intensity transfer.
- Transfer assembly incorrect, protein moving in the wrong direction: the gel/membrane sandwich is in the wrong order, or the cassette is inserted into the chamber the wrong way round. Check the polarity of the power connections.
- Incorrect charge-to-mass ratio: try a more acidic or more basic transfer buffer to increase protein mobility. A buffer pH near the protein's isoelectric point causes transfer to fail.

Generally, the buffer pH should be at least 2 pH units below or above the isoelectric point of the protein of interest.

- Protein precipitated in the gel: try adding SDS to the buffer. SDS improves transfer efficiency but reduces binding and may affect some protein–antibody reactions.
- Power circuit not working, or unsuitable power supply: check the fuse and confirm that the supply's voltage/current output matches the transfer equipment.
- Methanol in the buffer limits elution: reducing methanol increases transfer of proteins from the gel but lowers their binding to nitrocellulose and PVDF membranes.
- Gel percentage too high: lower the %T (total monomer) or %C (crosslinker). 5% C (Bis as crosslinker) gives the smallest pore size; lowering it enlarges the pores and increases transfer efficiency.

Poor transfer — nucleic acids

- Gel percentage too high: reduce %T or %C in polyacrylamide gels, or the agarose percentage in agarose gels. Before transfer, nick DNA with 0.25 M dilute HCl, or treat RNA with dilute NaOH.
- Transfer time too short or power too low: increase the transfer time or try a high-intensity transfer.
- DNA or RNA cannot be electrophoretically transferred to nitrocellulose because the required salt concentration for binding is too high: replace nitrocellulose with Zeta-Probe membrane.

Distorted or missing bands, diffuse transfer

- Poor membrane-to-gel contact, with bubbles or excess buffer between blot and gel: roll the membrane surface carefully in different directions with a tube or pipette until all bubbles and excess buffer are expelled and full contact is achieved. Use thicker filter paper in the sandwich. Replace the fiber pads — prolonged compression thins the pads so they no longer press the membrane and gel firmly.
- Power too high: always check the current at the start. A given voltage setting may make the current too high. If the buffer is incorrectly prepared, the conductivity will be too high, providing excessive force.
- Membrane not fully wetted or has dried: white spots on nitrocellulose mark dry areas where protein cannot bind. If the membrane does not wet immediately when immersed in buffer, heat distilled water to just below boiling, immerse the membrane fully, then equilibrate in buffer before use. Because of its hydrophobicity, PVDF must be fully wetted with methanol before equilibrating in aqueous transfer buffer — follow the product instructions.
- Gel electrophoresis may have been faulty: abnormal runs can result from poor gel polymerisation, unsuitable run conditions, contaminated buffer, sample overload, etc.

Cassette pattern transferred onto the membrane

- Contaminated or too-thin transfer fiber pads were used: replace the pads or clean contaminated pads thoroughly.
- Too much protein on the gel, or too much SDS in the buffer: protein can pass through the membrane without binding and float free in the cell. Reduce the protein on the gel and the SDS in the buffer. Add a second membrane to bind the excess protein.
- Transfer buffer is contaminated: re-prepare the solution.

Binding failure — nitrocellulose membrane

- Nitrocellulose needs 20% methanol in the buffer to optimise protein binding: confirm the buffer contains the correct amount of methanol.
- Protein may have passed through the nitrocellulose: use PVDF or nylon (high binding capacity), or a smaller-pore (0.2 µm) nitrocellulose. Lower the voltage or switch to a standard transfer if a high-intensity transfer was used.
- Mixed-ester cellulose binds protein poorly: use pure nitrocellulose.
- Proteins <15,000 daltons bind less well to 0.45 µm nitrocellulose or are washed off during analysis: to improve stability, crosslink the protein to the membrane with glutaraldehyde, or use higher-capacity PVDF or nylon. Use Tween-20 as detergent during washing and antibody incubation and reduce or remove harsh wash conditions.
- SDS in the transfer buffer reduces protein binding: reduce or remove the SDS.
- The membrane may not be fully wetted: white spots on nitrocellulose mark dry, non-binding areas. If it will not wet immediately, immerse it in distilled water heated to just below boiling, then equilibrate in buffer.

Binding failure — PVDF membrane

- Membrane not fully wetted: because of its hydrophobicity, PVDF must be fully wetted with methanol before equilibrating in aqueous transfer buffer — follow the product instructions.
- Membrane dried during handling: a fully wetted membrane looks grey and translucent. White spots indicate it is drying. Since protein will not bind to dry spots, re-wet with methanol and re-equilibrate in transfer buffer.

2. Immunodetection

High overall background

- Unsuitable blocking conditions: the blocking agent must match the membrane. PVDF and nylon need more blocking — non-fat dried milk is commonly used. Increase the blocking concentration and time as needed. The blocking agent must be pure protein and free of substances that bind the probe non-specifically.
- Ineffective washing: increase the number, duration or vigour of washes, including progressively stronger detergents (strength: SDS > NP-40 > Tween-20).
- Blot left in substrate too long: remove the blot from the substrate when the signal-to-noise ratio is acceptable; do not over-develop. Immediately immerse the blot in double-distilled water to stop the reaction.
- Contamination in a previous step (electrophoresis or transfer): discard the gel and buffer; replace or thoroughly clean the fiber pads. Excess protein on the gel or too much SDS in the buffer lets protein pass through the membrane and float free — reduce protein or SDS, and add a second membrane to bind excess protein.
- Primary or secondary antibody too concentrated: increase the antibody dilution; use a dot-blot test to optimise the working concentration.
- Incubation tray contaminated: clean the tray or use a disposable one.

Bound protein does not react specifically with the probe

- Primary or secondary antibody contaminated with non-specific or cross-reacting IgG: use purified IgG as the primary and affinity-purified, blot-grade secondary antibody.
- A monoclonal antibody may react non-specifically with SDS-denatured protein: compare other monoclonal or polyclonal antibodies, or blot with non-denatured protein.

- Meaningless interactions from ionic combinations (e.g. avidin, glycoproteins) may bind more acidic proteins on the membrane: increase the ionic strength of the incubation buffer; increase the number, duration and vigour of washes (progressively stronger detergents, SDS > NP-40 > Tween-20); include Tween-20 in the antibody diluent to reduce non-specific binding.

No or weak signal

- Insufficient sample: increase the protein load; concentrate the sample before loading, or use a more sensitive detection method.
- Insufficient antigen bound to the membrane: after transfer, stain the gel or use a pre-stained or kaleidoscope standard to assess transfer efficiency. See the earlier chapters to improve the transfer method.
- Primary or secondary antibody inactivated or unsaturated: store reagents correctly; avoid repeated freeze–thaw, bacterial contamination and heat inactivation. Detergents can affect some antibodies — remove detergent from the system except for the post-blocking wash. If the antibody titre is too low, optimise with a dot-blot test and increase the incubation time.
- Enzyme conjugate inactivated or unsaturated: assay reagent activity (see below). Store reagents correctly and avoid freeze–thaw, contamination and heat. Sodium azide is an effective horseradish-peroxidase inhibitor; use thimerosal as a bacteriostat. Impure water can also inactivate the enzyme — use distilled, deionised water throughout. If the conjugate is too dilute, optimise with a dot-blot test.
- Chromogenic reagent inactivated: test reagent activity (see below) and re-prepare if needed.

Testing detection-reagent activity

- Chromogenic solution: mix 1.0 ml of chromogenic solution with 10 µl of secondary-antibody conjugate; colour should develop immediately. If no colour appears within a few minutes, the reagent is inactive — prepare a fresh solution and repeat.
- Coenzyme solution: mix 1.0 ml of the tested chromogenic solution with 1.0 ml of 1:3000-diluted coenzyme solution; a pale blue glow should appear within 15 minutes. If none appears within 25 minutes, the coenzyme is faulty — repeat with freshly prepared coenzyme.
- Primary-antibody solution: assay the antigen–antibody reaction by ELISA, radioimmunoassay, double immunodiffusion or precipitation. If possible, repeat with several primary-antibody dilutions.

3. Total Protein Detection

Colloidal gold total-protein stain — high background

- Insufficient or omitted blocking: block with TBS containing 0.3% Tween-20 and wash three times for 20 minutes each.
- Membrane unsuitable for this stain: positively charged nylon cannot be used for colloidal gold staining — use biotin-blot total-protein detection instead.
- Membrane contaminated in a previous step (electrophoresis or transfer): discard the gel and buffer; replace or thoroughly clean the fiber pads.
- Excess protein on the gel or too much SDS in the buffer: protein passes through the membrane without binding and floats free — reduce protein and SDS, and add a second membrane to bind excess protein.

- Colloidal gold stain contaminated: the stain can be reused; store used reagent in a separate, clean plastic container in a refrigerator. Discard any reagent with sediment at the bottom. If the solution is bright blue rather than deep wine-red, it has been contaminated by buffer salts (which react with the gold sol and cause non-specific deposition) — discard it.

Colloidal gold stain — low sensitivity

- Increase incubation time for low signals: overnight incubation is possible, but background also increases.
- Transfer incomplete: see the transfer-failure section to improve efficiency.
- Staining ineffective, confirmed by over-long staining time and loss of the deep wine-red colour: discard the reagent.
- Buffer-salt contamination, solution pale blue instead of deep wine-red: discard the reagent.
- Sample amount too low relative to the reagent: use a gold-enhancement kit to detect 10 pg of protein per band.

Biotin-blot total-protein detection — high background

- Insufficient blocking: match the blocking agent to the membrane. Nylon needs added MPO in several solutions — see the biotin-blot manual for details.
- Membrane left in chromogen too long: remove the membrane from the chromogenic solution when signal appears but background has not, then immediately transfer it to distilled water to stop development.
- Excess protein on the gel or too much SDS in the buffer: reduce protein and SDS, and add a second membrane to bind excess protein.

Biotin-blot detection — no or weak colour

- Incomplete transfer: see the transfer-failure section to improve efficiency.
- Sample amount in the gel too low relative to the reagent: increase the protein load.
- NHS-biotin solution inactivated: NHS-biotin hydrolyses in aqueous solution. Equilibrate the vial to room temperature before opening to prevent water-vapour condensation; use a sterile syringe to avoid contamination. Add NHS-biotin reagent to the borate-Tween solution just before use.
- Amines in buffer salts compete with the biotin reagent: rinse the membrane thoroughly in borate-Tween solution to remove residual electrophoresis and transfer salts.
- Avidin-HRP conjugate inactivated: follow the activity-test procedure to check.
- Chromogenic solution inactivated: follow the activity-test procedure to check.

Anionic stain — high background

- Insufficient destaining: increase the number and duration of destain washes.
- Stain concentration too high: re-prepare the buffer.
- Nylon membrane incompatible with anionic stains: use a biotin-blot protein detection kit.

Anionic stain — low sensitivity

Anionic-dye staining cannot detect less than 100 ng of protein per band. Use a more sensitive stain (e.g. colloidal gold total-protein detection or a biotin-blot kit), or increase the sample amount to the level required for anionic-dye staining.

Beijing Labgic Technology Co., Ltd.
Add:No.9 Yumin Street, Area B of the Airport Industrial Zone,
Shunyi District, Beijing 101318 China
Toll Free:400-600-4213
Website:www.labgic.com