miniBLOT Units

Instruction Manual

Catalogue Numbers

618250 (CVS8-SBU)

Record the following for your records:

Model _____

Catalogue No. _____

Date of Delivery _____

Warranty Period _____

Serial No. _____

Invoice No. _____

Purchase Order No. _____

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Safety Information

When used correctly, these units pose no health risk. However, these units can deliver dangerous levels of electricity and are to be operated only by qualified personnel following the guidelines laid out in this instruction manual. Anyone intending to use this equipment should read the complete manual thoroughly. The unit must never be used without the safety lid correctly in position. The unit should not be used if there is any sign of damage to the external tank, lid or cables.

These units comply with the following European directives:

2014/35/EU Low Voltage Directive

2014/30/UE (official Title 2004/108/EC) EMC Electromagnetic Compatibility

By virtue of the following harmonised standards:

BS EN IEC 61010-1: 2010 Safety Testing of Lab Equipment

BS EN IEC 61326-1:2013 EMC Electro Magnetic Compatibility

ROHS DIRECTIVE 2011/65/EU

BS EN 50581:2012 Restriction of Hazardous Substances

Packing List

Each miniBLOT unit includes a tank, lid, cables and the following items:

SKU	Inner module	Cassettes	Fibre pads	Cooling pack
618250 CVS8-SBU	618251 – miniBLOT blotting module, pk/1	618252- miniBLOT Blotting Cassette, pk/2	618254 - miniBLOT Porous fibre pads pk/5	2 of 618253 - miniBLOT Cooling Block, pk/1

Packing List Checked by: _____

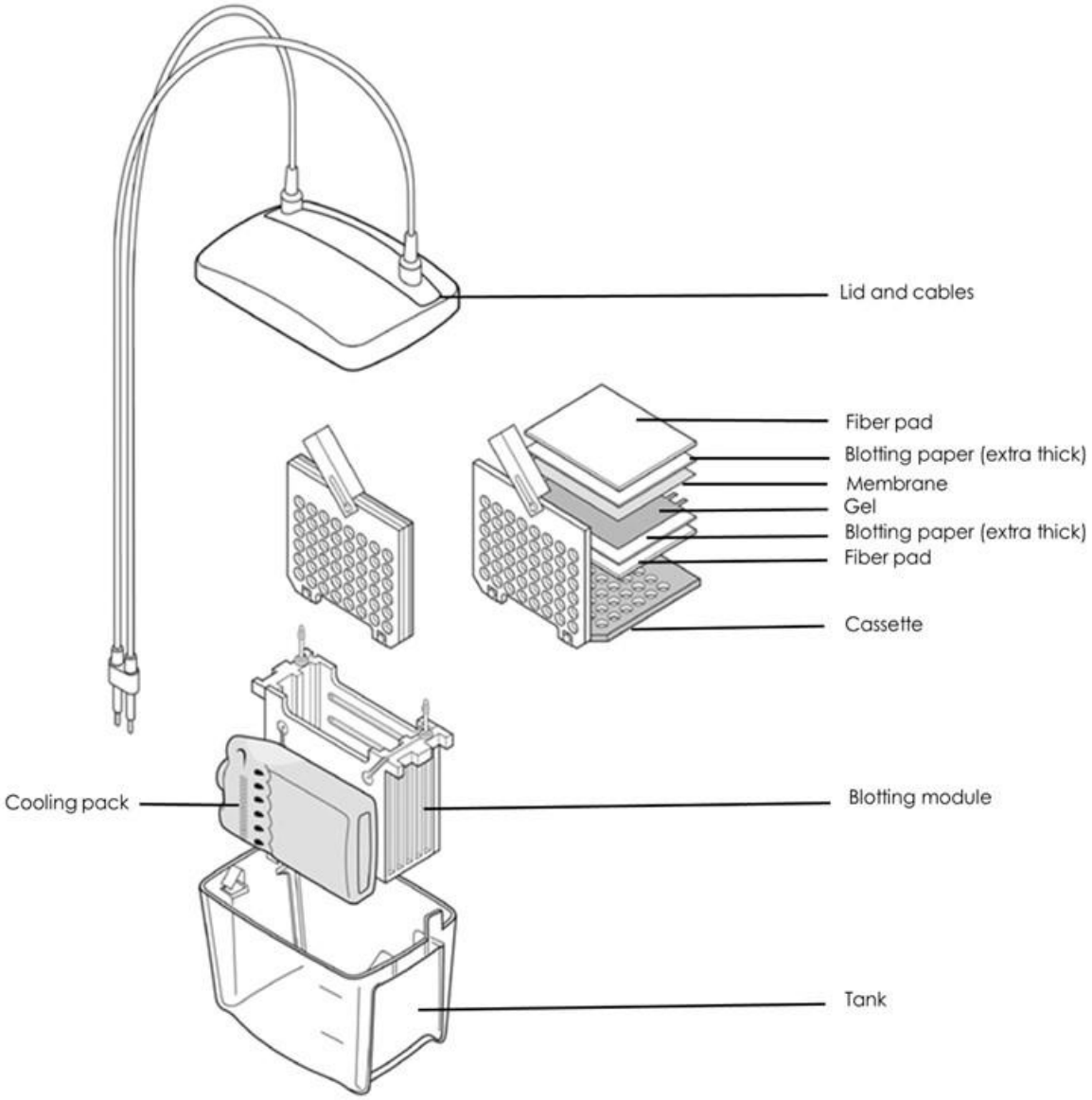
Date: _____

The packing lists should be referred to as soon as the units are received to ensure that all components have been included. The unit should be checked for damage when received.

Thistle Scientific is liable for all missing or damaged parts / accessories within 7 days after customers have received this instrument package. Please contact Thistle Scientific immediately regarding this issue. If no response within such period is received from the customer, Thistle Scientific will no longer be liable for replacement/damaged parts.

Please contact your supplier if there are any problems or missing items.

Specifications



Unit specifications	
Electrode module	Moulded polycarbonate
Gel holder cassettes	Moulded polycarbonate
Electrodes	Platinum wire (made from platinum ingot)
Tank and Lid	Moulded polycarbonate
Cooling unit	Polyethylene
Unit Dimensions	18 x 13 x 16 cm (L x W x H)
Gel clamp size	10 x 11 cm
Maximum gel size	7.5 x 10 cm
Buffer Volume	With cooling unit 950 ml Without cooling unit 1150 ml

Operating Instructions

Usage Guidance and restrictions

- Maximum altitude 2,000m.
- Temperature range between 4°C and 50°C.
- Maximum relative humidity 80% for temperatures up to 31°C decreasing linearly to 50% relative humidity at 40°C.
- Not for outdoor Use.
- Maximum Voltage Limit 150V DC
- Maximum power Limit 40W

This apparatus is rated POLLUTION DEGREE 2 in accordance with IEC 664.

POLLUTION DEGREE 2, states that: "Normally only non-conductive pollution occurs.

Occasionally, however, a temporary conductivity caused by condensation must be expected".

Casting Unit Preparation

Cleaning the Glass Plates

Clean a set of glass plates for each gel first with distilled water and then with 70 % ethanol. One set of glass plates constitutes one short glass plate and one plain glass plate with bonded spacers.

The plain glass plate is positioned outermost, then the short glass plate.

Note: All glass plates, gel casting modules, casting base and accessories must be completely dry before the set – up. Wet components are more likely to miss-align and cause leaks.

Gel Cassette and Casting Assembly

1. Place the casting frame upright with the pressure cams in the open position and facing forward on a flat surface.
2. Select a spacer plate of the desired gel thickness and place a short plate on top of it (see Figure 1a).

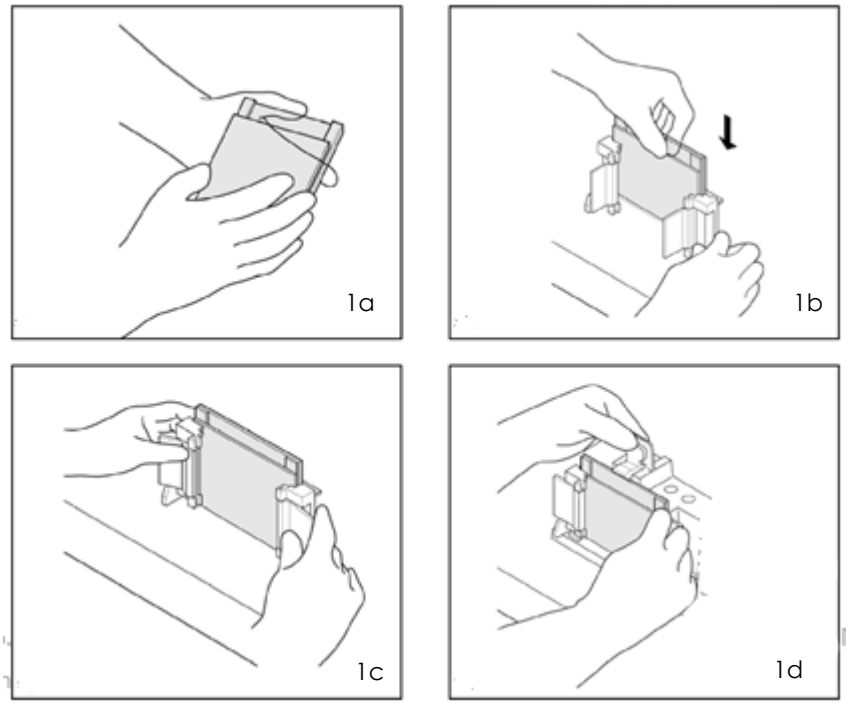
3. Orient the spacer plate so that the labelling is up. Slide the two glass plates into the casting frame, keeping the short plate facing the front of the frame (side with pressure cams) (see Figure 1b).

   Note: Ensure that both plates are flush on a level surface and that the labels on the spacer plate are oriented correctly. Leaking may occur if the plates are misaligned or oriented incorrectly.

4. When the glass plates are in place, close the pressure cams to secure the glass cassette sandwich in the casting frame (see Figure 1c). Check that both plates are flush at the bottom.

5. Place the casting frame into the casting stand by positioning the casting frame (with the locked pressure cams facing out) onto the casting gasket while engaging the spring-loaded lever of the casting stand onto the spacer plate (see Figure 1d).

6. Repeat steps 1 to 5 to cast another gel.



Gel Preparation

Care should be taken when selecting the pore size of the gel to be used. The pore size or % of gel determines the resolving ability given different sizes of protein.

Gel percentage for various protein sizes are shown below:

Acrylamide Percentage	Separating Resolution
5 %	60 - 220 KD
7.5 %	30 - 120 KD
10 %	20 - 75 KD
12%	17 – 65 KD
15 %	15 -45 KD
17.5%	12 – 30 KD

Gel Volumes for the proPAGE Mini Unit are shown below:

The volumes listed are required to completely fill a gel cassette. Amounts may be adjusted depending on the application (with or without comb, with or without stacking gel, etc.).

Number of gels	Gel Thickness (mm)	Volume (ml)
Single – one gel, one dummy plate	0.5 1.0 1.5 2.0	2.8 4.2 5.6 8.4

Prepare gel solutions as per tables below. These give the volumes of solutions from the standard stock solutions. These should be gently mixed avoiding generation of bubbles which will inhibit polymerization by removing free radicals.

10ml Resolving Gel:

Solution	5 %	7%	10 %	12%	15 %	17%
Distilled Water	5.7ml	5.1ml	4.1ml	3.4ml	2.4ml	1.7ml
30 % Stock Acrylamide Solution	1.7ml	2.3ml	3.3ml	4ml	5ml	5.7ml
4 X Resolving Tris Solution	2.5ml	2.5ml	2.5ml	2.5ml	2.5ml	2.5ml

Add 10 µl of TEMED and 100µl 10 % Ammonium Persulphate to the resolving gel solution **just before pouring** to initiate polymerisation

5ml of 5% Stacking Gel:

Solution	Volume
Distilled Water	2.87ml
30 % Stock Acrylamide Solution	0.83ml
4 X Stacking Gel Tris Solution	1.25ml

Add 50µl of 10 % Ammonium Persulphate and 5µl TEMED before pouring.

Preparation of denatured protein samples for loading

The instructions given below are for denatured samples. For Native samples, please consult a laboratory handbook.

1. Prepare the protein samples for loading. The volume of sample depends on the capacity of the wells.
2. Using a 0.5 ml micro-centrifuge tube or other convenient receptacle, combine the protein sample and 4 X sample buffer. It is always advisable to use protein markers in one of the end lanes to indicate sizes of bands. These should be prepared according to the manufacturer's instructions.
3. Heat the samples in a water bath or heating block for 2 minutes at 95^{o}C to denature the samples.
4. Centrifuge the samples in a micro-centrifuge for 20 seconds at 12,000 rpm. The protein samples are now ready to load.

Gel Pouring

Casting a gel with stacking layer

1. Place a comb into the gel cassette assembly with any gel and mark the glass plate below the comb teeth. This is the reference level to which the resolving gel should be poured.
2. Prepare the resolving gel solution. Mix well and avoid generating air bubbles.
3. Fill the glass plates smoothly up to the mark, avoiding generating any air bubbles. Filling must be performed quickly before the TEMED causes the gel to become too viscous.
4. Overlay the gel extremely carefully with 1 ml of Isobutanol, Isopropanol or distilled water. When using distilled water extra care must be taken to ensure there is no mixing with the gel solution.
5. Let the resolving gel polymerize. Usually this takes around 15 to 30 minutes, but this can vary due to the freshness of the reagents used. If polymerization is taking a lot longer than this, use fresher stock solutions or add more APS and TEMED.
6. Prepare the stacking gel solution.
7. Before casting the stacking gel, insert a piece of filter paper to dry the area in between the glass plates above the resolving gel. Take care not to touch the surface of the gel.
8. Carefully pour the stacking gel solution, avoiding generating air bubbles.
9. Carefully insert the comb making sure that no air bubbles get trapped under the ends of the comb teeth as these will inhibit sample progression.
10. Allow the stacking gel to polymerize.
11. Once the gel is polymerized it is ready for the electrophoresis run.

Casting a gel without stacking layer

- Prepare the resolving gel solution. Mix well and avoid generating air bubbles.
- Pour the solution smoothly into the glass plates avoiding any air bubbles until the top of the notched glass plate is reached.

- Carefully insert the comb making sure that no air bubbles get trapped under the ends of the comb teeth as these will inhibit sample progression.
- Let the gel polymerize. Usually this takes from 15 to 30 minutes, but this can vary due to the freshness of the reagents used. If polymerization is taking a lot longer than this, use fresher stock solutions or add more APS and TEMED.
- Once the gel is polymerized it is ready for the electrophoresis run.

Using Precast Gels

1. proPAGE mini is compatible with BioRAD mini precast gels.
2. Simply remove the precast gel from the storage pouch and remove the bottom tape to expose the bottom edge of the gel.
3. Gently remove the comb.
4. Insert the gel in the Running module

Note: If only one or three gels are required, use the dummy plate

Gel assembly and Sample loading

Note: when running 2 gels only, use the electrode assembly (the one with the banana plugs), not the companion running module (the one without the banana plugs). When running 4 gels, both the electrode assembly and the companion running module must be used, for a total of 4 gels (2 gels per assembly).

1. Set the clamping frame to the open position on a clean flat surface (see Figure 2a).
2. Place the first gel sandwich or gel cassette (with the short plate facing inward) onto the gel supports; gel supports are molded into the bottom of the clamping frame assembly; there are two supports in each side of the assembly. Note that the gel will now rest at a 30° angle, tilting away from the center of the clamping frame. Please use caution when placing the first gel, making sure that the clamping frame remains balanced and does not tip over. Now, place the second gel on the other side of the clamping frame, again by resting the gel onto the supports. At this point there will be two gels resting at an angle, one on either side of the clamping frame, tilting away from the center of the frame (see Figure 2b).

Note: It is critical that gel cassettes are placed into the clamping frame with the short plate facing inward. Also, the clamping frame requires 2 gels to create a functioning

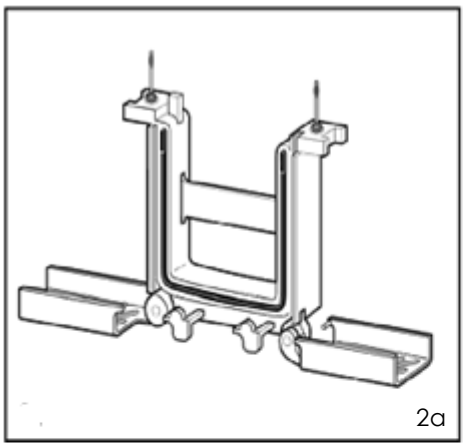
assembly. If an odd number of gels (1 or 3) is being run, you must use the buffer dam (see Figure 4b).

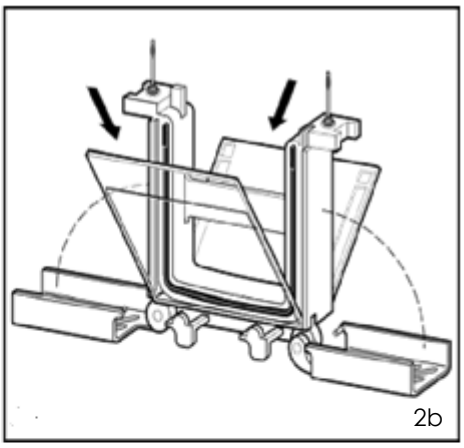
3. Using one hand, gently pull both gels towards each other, making sure that they rest firmly and squarely against the gaskets that are built into the clamping frame; make certain that the short plates sit just below the notch at the top of the gasket.
4. While gently squeezing the gel sandwiches or cassettes against the gaskets with one hand (keeping constant pressure and both gels firmly held in place), slide the purple arms of the clamping frame over the gels, locking them into place. Alternatively, you may choose to pick up the entire assembly with both hands, making sure that the gels do not shift, and simultaneously sliding both arms of the clamping frame into place (see Figure 2c). The arms of the clamping frame push the short plates of each gel cassette up against the notch in the gasket, creating a leak-proof seal.
5. At this point, the sample wells can be washed out with running buffer, and sample can be loaded (Figure 2d).

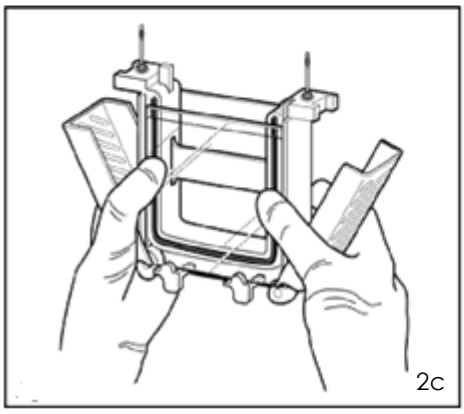
Note: If running more than 2 gels, repeat steps 1a–d with the companion running module.

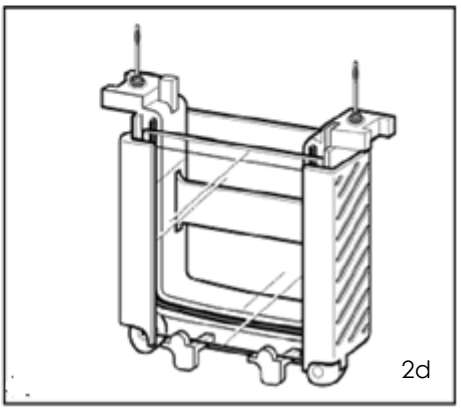
Important Note: Do not attempt to lock the purple arms of the clamping frame, without first ensuring that the gel cassettes are perfectly aligned and stabilized against the notches on the gaskets of the module. To prevent the gels from shifting during the locking step, firmly and evenly grip them in place against the core of the module with one hand.

Caution: When running 1 or 2 gels only, do not place the companion running Module in the tank. Doing so will cause excessive heat generation and prevent electrophoretic separation.









6. Transfer the Running module containing cast gels into the main tank in the correct orientation as indicated - +ve on the module aligned with +ve on the tank, -ve on the module aligned with –ve on the tank.
7. Fill the outer tank with 1X reservoir buffer. See "Solutions" for recommended running buffer solution.
8. Load the samples into the wells using a pipette tip taking care not to damage the wells or induce any air bubbles.
9. Fill any unused wells with 1 X sample buffer.

Note: It is a good idea to note the orientation and order the samples were loaded in. This can be done by noting which samples were loaded adjacent to each electrode.

Gel Running

1. Fit the lid and connect to a power supply.
2. Gels should be run at constant voltage, with voltage dependent on the size of the proteins undergoing separation. An initial setting of 100V with constant observation of migration and heat production is

recommended. An exploratory study may be required to determine the optimal settings for individual proteins.

Gel Removal

1. Turn the power supply off when the loading dye reaches the bottom of the gel, sooner if your proteins are below 4 kDa in size.
2. Remove the gel running module, first emptying the inner buffer into the main tank. Buffer can be re-used but this may affect run quality if continued.
3. Release the gels cassettes by opening the purple arms.
4. Remove the glass plates. Using gel releaser, separate the short and the plain glass plates. Place the wedged end of the releaser between the two plates and gently twist until the plates pull apart. The gel will usually stick to one of the plates and can be removed by first soaking in buffer and then gently lifting with a spatula.
5. The gel is now ready to be stained with Coomassie or silver stain or the proteins in the gel can be transferred to a membrane by electroblotting for specific band identification and further analysis.

Solutions (For SDS-PAGE)

Stock 30% Acrylamide Gel Solution:

30.0 g acrylamide

0.8 g methylene bisacrylamide

Distilled Water to 100ml

Stock 4 X Resolving Gel Tris (1.5 M Tris·HCl pH8.8, 0.4 % SDS)

To 110ml Distilled Water add 36.4 g of Tris base

Add 8ml of 10 % SDS

Adjust pH to 8.8 with 1N HCl

Adjust the final volume to 200ml with Distilled Water.

Stock 4 X Stacking Tris (0.5 M Tris·HCL pH6.8, 0.4 % SDS)

To 110ml Distilled Water add 12.12 g of Tris base

Add 8ml of 10 % SDS

Adjust pH to 6.8 with 1N HCl

Add Distilled Water to a final volume of 200ml

Stock 4 X Tris-glycine tank buffer - SDS

36 g Tris base

172.8 g glycine

Distilled Water to 3 L

1 x Tris-glycine tank buffer - SDS

750ml of 4 X Tris-glycine reservoir buffer - SDS

30ml of 10 % SDS

Distilled Water to 3L

10 % AP (ammonium persulphate solution)

0.1 g ammonium persulphate

1ml Distilled Water

TEMED

Stock 4 X Sample Buffer

4ml glycerol

2ml 2-mercaptoethanol

1.2 g SDS

5ml 4 X Stacking Tris

0.03 g Bromophenol blue

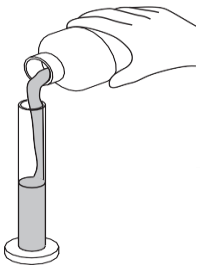
Aliquot into 1.5ml micro centrifuge tubes. Store at -20°C.

Blotting Procedure – miniBLOT Unit Setup

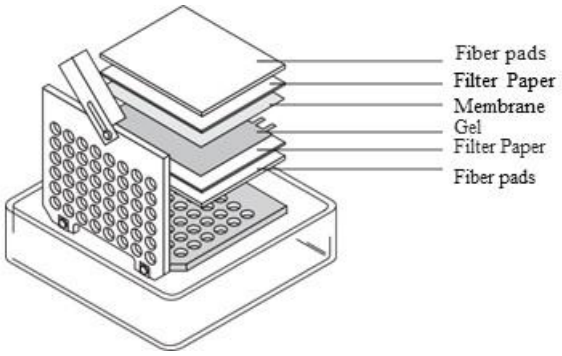
Setting up the blot sandwich

Note: Remember to always wear gloves when dealing with proteins to avoid contaminations!

1. Following electrophoresis, remove the glass plates and transfer the gel into Transfer buffer.
2. Prepare buffer solutions for blotting (See page 22 for recommended buffer solutions). Pre-chill the buffer to 4 °C to improve heat dissipation during electrophoresis.

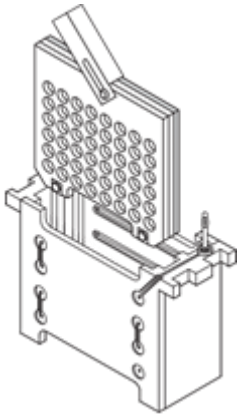


3. Cut the membrane of the same size of the gel and equilibrate in Transfer buffer as well, please wear the glove to avoid pollution. Equilibrate the gel and immerse the membrane and filter paper as well as the fiber pads (From 15 minutes to 1 hour, depending on the thickness of the gel).
4. Assemble the transfer sandwich by placing the sandwich clamp on a tray or clean surface with the black side facing downward (cathode side). Layer the components in the following order: first, place pre-wetted fiber pads on the black surface; then add a sheet of wet filter paper. Place the equilibrated gel on the filter paper, ensuring no air bubbles remain between the layers. Next, add the pre-soaked membrane directly on top of the gel and carefully remove any air bubbles. Cover the membrane with another sheet of wet filter paper, again removing air bubbles. Complete the assembly by placing the final fiber pad (if applicable) and securing the setup with the transfer lining.

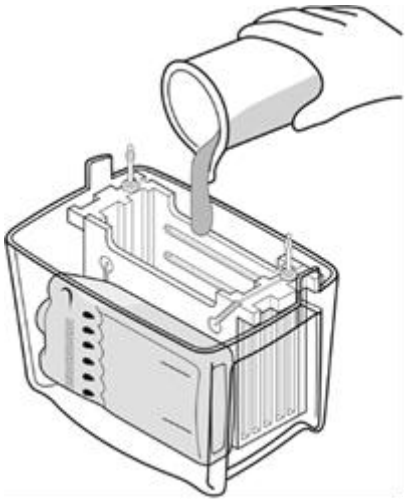


Note: To achieve optimal blotting results, it is essential to remove all air bubbles between layers during sandwich assembly. This can be effectively done by gently rolling a glass rod or stirring bar over the surface.

5. Secure the clamp by tightening it carefully, ensuring that the gel and filter paper sandwich remains properly aligned and undisturbed. Then, use the white sliding latch to lock the clamp in place.
6. Insert the assembled clamp into the blot electrophoresis core, ensuring proper orientation. If performing a double transfer, repeat the sandwich assembly process to prepare the second gel and insert it into the remaining slot in the core.



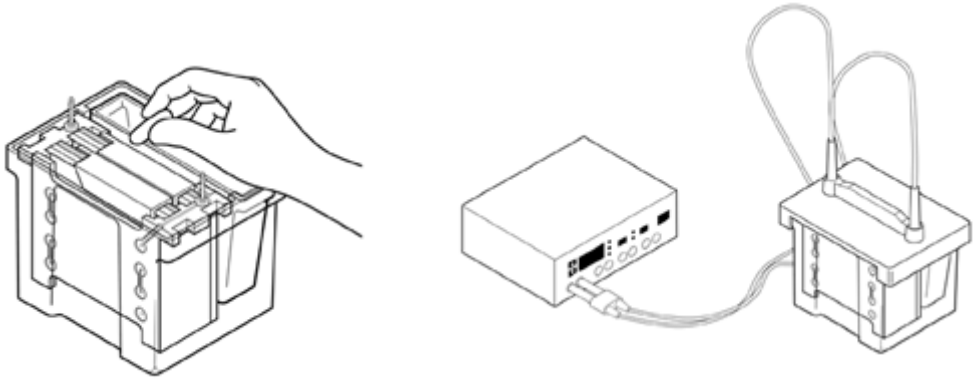
7. Place the pre-chilled cooling pack into the blotting tank, then fill the tank with transfer buffer until all components are fully submerged.



Note: Store the cooling pack in a -20 °C freezer before use. After completing the procedure, return the cooling pack to the freezer for proper storage.

8. Place a stirring bar into the transfer buffer to help maintain a stable temperature and ensure consistent ion strength. Set an appropriate stirring speed to promote even ion distribution without disturbing the transfer sandwich.

9. Close the safety lid, connect the power cables to the power supply, and begin the blotting process. For recommended voltage and transfer time settings based on different buffer systems, refer to **Condition for electrophoresis transfer** (see page xx).



10. After the electrophoresis is complete, carefully disassemble the sandwich and remove the membrane for the next step of the protocol. Clean all components - including the electrophoresis tank, blotting clamp, and fiber pads - first with a neutral detergent, then rinse thoroughly with deionized (ion-free) water to remove any residual detergent and buffer salts.

Note (for transfers under acidic conditions):

When performing protein transfer under acidic conditions, reposition the gel and membrane so that the membrane is placed on the cathode side (negative electrode).

This adjustment is necessary because, under acidic conditions, proteins migrate toward the negative electrode. do not reverse the electrode, as this may damage the equipment and compromise the transfer process.

Condition for Electrophoresis Transfer

Guideline for Buffer Transfer and Running Conditions

The table below provides the recommended voltage and transfer time settings for different buffer systems used in Western blotting.

Generally, higher voltage requires shorter transfer time, while lower voltage may require extended transfer durations to achieve efficient protein transfer.

Note: Always use the cooling pack during electrophoresis to prevent heat buildup, which can negatively affect transfer efficiency and membrane integrity.

Table: Guideline for buffer transfer and running condition

BUFFER	STANDARD FIELD STRENGTH TRANSFER OVERNIGHT	HIGH FIELD STRENGTH DISTANCE OF ELECTRODE IS 4CM TRANSFER TIME 1 HOURE
SDS-PAGE Gels	**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% MeOH and 0.25%–0.1% SDS.	30 V 90 mA	100 V 350 mA

B: 48 mM Tris, pH 9.2, 39 mM glycine, with or without 20% MeOH and 0.25%–0.1% SDS. C: 10 mM NaHCO3, 3 mM NaCO3, pH 9.9, with or without 20% MeOH and 0.25%–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	30 V 100 mA	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 V

The cautions for electrophoresis transfer

The following changes will change resistance and current.

Changes in the preparation of buffer: such as, the SDS ratio is increased, ratio of the acid or the alkaline is increased when adjusting the buffer, in this case the strength of ion is changed accordingly.

The pH value, the strength of ion and percentage of acrylamide of gel, especially in the case of gel, has not been properly equilibrated.

Quantity of gel: the increased quantity leads to the slightly augmented current.

Buffer volume: The increased volume leads to augmented current accordingly.

Quality of platinum electrode: The improved quality of platinum electrode leads to augmented current.

Temperature of transfer: The increased temperature leads to the augmented current.

Time of transfer: The capacity of buffer weakens and the current augments with the running of electrophoresis.

The pre-equilibrium of gel

All the gels should be pre-equilibrium by the transfer buffer before conducting the electrophoresis transfer, which can remove the salt and neutralize the salt which denatures the nucleic acid, otherwise a lot of heat is generated, and the conductivity of buffer is increased. Meanwhile, the low concentration gel (<12%) shrinks in the methyl alcohol solution, so the pre-equilibrium activity adjusts the gel to the best size eventually before the electrophoresis transfer.

The usage of stirring bar during transfer

All the stirring bars should be put inside miniBLOT Units during the application of blot in order to stir the buffer in the course of the experiment, which is useful to maintain uniform conductivity of buffer and temperature. It leads to poor macromolecular transfer and fosters the potential safety hazard if the temperature is not adjusted efficiently.

pH value of transfer buffer

Do not adjust the pH value of transfer buffer unless it is necessary. The adjustment of transfer buffer will result in an increase of conductivity, which is proved by the output current higher than the expected and decrease of resistance. It is recommended to use electrophoresis power supply and check the initial current value before any electrophoresis.

Recommendation of transfer buffer

Please use high-quality methyl alcohol. The polluted methyl alcohol will lead to the increase conductivity of transfer buffer and the failure of transfer of macromolecular. Do not reuse or dilute the transfer buffer below the recommended concentration. It is not recommended to reuse the transfer buffer because the buffer cannot maintain a stable pH value during electrophoresis. Diluting the buffer below the recommended concentration will reduce its buffer capacity.

The maximum voltage

The voltage should not exceed the set value mentioned above, **the Guideline for buffer transfer and running condition**, during the overnight electrophoresis. The conductivity of the buffer should be close to the current mentioned in the table. The upper limit of current should be set in electrophoresis power supply.

Electrophoresis should be done by the high voltage once the low voltage is not available, however, the time must be shortened otherwise it leads to potential safety hazard.

Buffer Preparation Guidelines

The miniBLOT Unit requires approximately 650 mL of buffer when using a cooling unit, and 850 mL without it. Do not adjust the pH by adding excess acid or base, as this may alter the buffer's ionic strength and effectiveness. Use high-purity methanol when preparing buffers. Low-grade methanol may contain metal contaminants, which can damage the electrodes over time.

Note: Some pH electrodes are not sensitive to Tris-based buffers. If the measured pH appears inaccurate, verify that the electrode is compatible with Tris. If the electrode is functioning correctly but the buffer pH is still below 8, the buffer should be reformulated.

25mM Tris, 192 mM glycine, 20% v/v methyl alcohol, pH 8.3

Mix 3.03g Tris, 14.4 glycine and 200ml methyl alcohol, add 1 liter of ion-removing distilled water (dd H2O)

25mM Tris, 192 mM glycine, pH 8.3

Mix 3.03g Tris, 14.4 glycine, add 1 liter of ion-removing distilled water (dd H2O)

48mM Tris, 39 mM glycine, 20% v/v methyl alcohol, pH 9.2

Mix 5.82g Tris, 2.93g glycine and 200ml methyl alcohol, add 1 liter of ion-removing distilled water (dd H2O)

48mM Tris, 39 mM glycine, pH 9.2

Mix 5.82g Tris, 2.93g glycine and add 1 liter of dd H2O

10Mm NaHCO3, 3 mM NaCO3,20% methyl alcohol pH 9.9

Mix 0.84g NaHCO3 and 0.318g NaCO3 in the ddH2O, add 200ml methyl alcohol, and set the volume of ddH2O to 1 liter.

1.0x TBE (Tris-boric acid EDTA), pH 8.3

90mM Tris-boric acid 1 mM EDTA

5x storage solution

54 g Tris base

27.5 boric acid

20 ml 0.5 M EDTA (pH 8.0)

Add 200ml 5x storage solution into 800ml ddH2O 1.0x buffer solution

1x TAE (Tris-acetic acid EDTA)

40mMTris-acetic acid 1 mM EDTA

50x storage solution

242 g Tris base

57.1 ml glacial acetic acid

100 ml 0.5 M EDTA (pH 8.0)

1x buffer: add 20 ml 50x storage solution until reaching 980ml ddH2O.

Procedure for Optimizing Electrophoresis Conditions

Optimizing protein transfer

Generally, the quantitative transfer of high molecular weight proteins can be challenging.The following methods - used individually or in combination - can help improve transfer efficiency for large proteins:

Compositions of different gel

The graded gel makes it easier to elute the protein with wide range of molecular weight

than that of the single-concentration gel.

The gel with more pores can be made by decreasing the total concentration of polymer monomer.

The diameter of pore of the gel with 5.26% cross-linking agent methene-bisacrylamide (%C) is shortest regardless of what the concentration of acrylamide is. The resolution is decreased in case of increase of pore diameter caused by the increase or decrease of %C accordingly.

$$\%C = \frac{\text{Methyl-bisacrylamide (g)}}{\text{Methyl-bisacrylamide(g)+ acrylamide (g)}} \times 100\%$$

Increase the transfer time

The initial controlling condition determines the total time of electrophoresis transfer. The time ranges from 30 minutes to overnight based on different controlling conditions. The voltage of overnight electrophoresis transfer should be set 30V with a view to reducing the heat.

Strengthen the electric field intensity

The setting of the initial condition should guarantee the required transfer rate (V/cm) as well as the temperature of transfer. The increased temperature changes the nature of protein and resistance of buffer as well as the field stress. The transfer rate is affected eventually.

Lowering the buffer strength

The diluted buffer lowers the current under the fixed voltage and does not cause much more heat in case of higher voltage.

Different buffer and pH

Changing the type of buffer and pH maximizes the ratio of charge to weight. The SDS seems to be separated from protein via ethyl alcohol in the SDS buffer. Assume that the basic protein in the buffer such as Tris, glycine, methyl alcohol, pH 8.3 remains in the condition of equal-neutral-electricity, which makes that

protein cannot transferred. Such property applies to the lysozyme. The buffer with pH 9.5 to 10 makes the basic protein, such as lysozyme and histone, transfer better.

Various buffer features different transfer efficiency even in case of similar electric field intensity (V/cm). General speaking, the transfer efficiency of Tris buffer is better than that of acetic acid and phosphate buffer.

Increasing the volume of detergent

It is reported by literature that the transfer efficiency is improved by adding 0.1% SDS in the buffer of Tris/ glycine/ methyl alcohol. However, the initial temperature is slightly higher because of the sediment of SDS in case of being under 10℃ with the increased current and electric field intensity as well as the heat. The SDS also affects the antigenicity of some protein. To elute protein, the SDS is added into the buffer, meanwhile the binding efficiency of protein and nitrocellulose membrane is lowered.

Removing alcohol from buffer

The alcohol transferring buffer is to improve bending of SDS protein and nitrocellulose membrane. Elimination of alcohol results in transfer efficiency improved but the binding efficiency is lowered. The transfer efficiency is improved because the alcohol shrinking the pore of gel, and the big-sized protein remains inside. The PVDF membrane is applied to eliminate demand of alcohol and create a reasonable strategy for the big-sized protein and hard-transferable protein. The PVDF membrane must be wet by 100% methyl alcohol and then put into the buffer without methyl alcohol.

Dealing with limited protease

It is reported in the literature that limited digestion of proteins during the transfer process to enhance transfer efficiency without decreasing the immunological activity of proteins.

Type of membrane

As said above, the PVDF membrane makes the transfer electrophoresis go under the condition of no methyl alcohol.

Type of gel

If possible, use non-denaturing gradient gel for separation of the proteins with different molecular weight. The isoelectric focusing gel or native gel are recommended unless the protein sample must be separated according to the molecular weight.

Enhance contact of gel and membrane

The phenomenon that the effective combination of protein molecule and membrane fails because of poor contact of gel and membrane is always treated mistakenly as the futile elution. The poor contact is caused by the surplus liquid between the gel and membrane. Complete contact is done by the technique of employing the test tube and glass bar as the best tool. Good compression can be done by suitable filter paper. The gel and membrane should be equilibrated from 30 minutes to 1 hour in the buffer before the electrophoresis is conducted, which prevents the shrinkage and remove the urea and SDS and other reactant from gel during the electrophoresis.

Optimizing DNA and RNA Electrophoresis

Nucleic Acid Elution Challenges

Elution efficiency can be improved by adjusting gel concentration. However, quantitative transfer of genomic DNA remains difficult due to its size and abundance.
The following methods may enhance transfer efficiency:

Change of gel composition

It is useful for transferring of high molecular weight DNA by lowering the percentage of monomer and cross-linking agent in polyacrylamide and lowering the percentage of agarose gel.

Change of DNA denaturant

The denature of oxalaldehyde allows the elution of DNA more efficient than that of sodium hydroxide. The excellent result can also be achieved by DNA denatured by boiled acrylamide. The polyacrylamide gel would be softened and stuck to membrane by alkaline denaturation.

Option of Transfer Membrane

Protein blot membrane

Nitrocellulose membrane

Nitrocellulose membrane is widely used in protein binding and detection, total protein detection can be done by protein gel dye such as Amido black, Coomassie blue, Ponceau S, Fast green FCF etc.) or the more sensitive colloidal gold dye. The nitrocellulose membrane is also used to analyze the radioimmunoassay, fluoroimmunoassay and enzyme-linked immunoassay. The nitrocellulose membrane does not need to be pretreated because of the binding volume of 80-100μg/cm2. The Connected point of specific proteins is easily closed to avoid the problem of background. The protein with low molecular weight (less than 20 KD) is easily lost during the washing after electrophoresis transfer, thus, leads to the decreased detection sensitivity. However, the nitrocellulose membrane with a smaller diameter (0.2μm) of pore can eliminate such phenomenon. The big-molecular (more than100KD) protein denatured by SDS is hard to be transferred because of the existence of methyl alcohol in the transfer buffer. Methyl alcohol improves the binding of SDS protein and nitrocellulose, meanwhile, it shrinks the pore of gel. The combination is obviously decreased if the methyl alcohol is removed from SDS protein. The transfer rate is increased by adding SDS (until reaching 0.1%) into the buffer, but the binding is decreased. The SDS also enhances the conductivity of current and generates much more heat during the transfer.

PVDF membrane

PVDF (polyvinylidene fluoride) is the ideal support for amino-terminal sequencing, amino acid analysis, immunoassay of blotted proteins and can reserve the protein in extreme conditions, such as immersed in acid and alkali environment, emerging in organic solvent. The feature of strength is helpful to improve repeatability and increase the possibility of obtaining the protein with low abundance during the process of sequencing. Besides, the PVDF membrane could improve the binding efficiency in buffer contained SDS. The PVDF membrane must be soaked with 100% methanol before use and then used in a methanol free buffer solution.

DNA and RNA blot membrane

Zeta-Probe® nylon membrane

Nitrocellulose membranes are not suitable for nucleic acid transfer following electrophoresis due to their requirement for high-salt binding conditions (e.g., >10× SSC). Nucleic acids with molecular weights below 500 base pairs cannot effectively bind even under these high-salt conditions. Additionally, the high ionic strength of the buffer lowers resistance, allowing excessive current to flow. This can lead to increased power dissipation, potentially damaging the nitrocellulose membrane — even at low voltages. Inefficient transfer can also occur due to insufficient electric field strength (V/cm), which prevents proper migration and binding of nucleic acids to the membrane.

In contrast, Zeta-Probe nylon membranes enable efficient binding of DNA and RNA of various sizes in low-ionic-strength buffers. Compared to nitrocellulose, Zeta-Probe membranes offer greater stability during post-transfer washing and approximately tenfold higher signal detection efficiency.

There are several types of blotting membranes used for immunoblotting, each offering unique advantages depending on the specific requirements of the experiment. When selecting the most suitable transfer conditions, both the physical properties and performance of the membrane should be carefully evaluated.

Table: Guide to Protein Blotting Membranes

Membrane	**Pore Size**	**Binding Capacity (µg/cm2)**	**Remarks**
Nitrocellulose	0.45 µm 0.2 µm	80–100	Universal protein blotting membrane
Supported Nitrocellulose	0.45 µm 0.2 µm	80–100	Pure nitrocellulose cast on the inert synthetic support to improve strength and make the operation convenient, and the color appears easily.
PVDF	0.2 µm	170–200	Improve the strength and stability for the

			protein sequencing and blotting. Enhance binding in SDS Immersed by methyl alcohol in advance.
Nylon	0.2 µm	170	Nucleic acid is recommended.

Note: RNA should not be transferred to nitrocellulose membranes using electrophoretic transfer. Instead, Zeta-Probe® membranes must be used for optimal RNA binding and retention.

Troubleshooting

Electrophoresis Transfer Troubleshooting

Problem: Sample Preparation	**Cause**	**Solution**
Laemmli sample buffer turns yellow	Sample buffer too acidic	Add Tris base until buffer turns blue again.
Sample very viscous	High DNA or carbohydrate content	Fragment DNA with ultrasonic waves during cell lysis and protein solubilization. Add endonucleases Precipitate protein with TCA/acetone to diminish carbohydrate content.
Problem: Gel casting and sample loading	**Cause**	**Solution**
Poor well formation	Incorrect catalyst used Monomer solution not degassed (oxygen inhibits polymerization)	Prepare Fresh catalyst solution. Increase catalyst concentration of stacking gel to 0.06% APS and 0.12% TEMED. Degas monomer solution immediately prior to casting stacking gel.
Webbing; excess acrylamide behind the comb	Incorrect catalyst concentration	Prepare a fresh catalyst solution. Increase catalyst concentration of stacking gel to 0.06% APS and 0.12% TEMED.
Gel does not polymerize	Too little or too much APS or TEMED Failure to degas Temperature too low	Use 0.05% APS and 0.05% TEMED. Degas monomer solutions 10-15min. Cast at room temperature, warming glass plates if necessary. Use electrophoreses-grade reagents

	Poor quality acrylamide or bis Old APS	Prepare fresh APS.
Swirls in the gel	Excess catalysts; polymerization time < 10min Gel inhibition; polymerization time >2hr	Reduce APS and TEMED by 25% each. Increase APS and TEMED by 50%; degas.
Gel feels soft	Low %T Poor quality acrylamide or bis Too little cross-linker	Use different %T. Use electrophoresis- grade reagents. Use correct %C.
Gel turns white	Bis concentration too high	Check solutions or weights.
Gel brittle	Cross-linker too high	Use correct % cross-linker
Sample floats out of the well	Sample is not dense enough Pipetting, loading error	Include 10% glycerol in sample to make it denser than surrounding buffer. Slowly pipette sample into well. Do not squirt sample quickly into well as it may bounce off bottom or sides and flow into next well. Do not remove pipette tip from well before last of sample has left the tip.
Problem: Electrophoresis	**Cause**	**Solution**
Current zero or less than expected and samples do not migrate into gel	Tape at the bottom of precast gel cassette not removed Insufficient buffer in inner buffer chamber Insufficient buffer in outer buffer chamber Electrical disconnection	Remove tape. Fill buffer chamber with running buffer. Fill inner and outer buffer chambers to ensure wells are completely covered. Check electrodes and connections.
Gels run faster than expected	Running buffer too concentrated and gel temperature too high; incorrect running buffer concentration or type used Running or reservoir buffer too dilute Voltage too high	Check buffer composition and type. Check buffer protocol and concentrate if necessary. Decrease voltage by 25-50%.
Gels run slower than expected	Incorrect running buffer composition or type Excessive salt in sample	Check buffer composition and type. Desalt sample.
Buffer leaking from inner chamber	Incomplete gasket seal	Set up again with sliding clamps tighter.
Problem: Total Protein Staining	Cause	Solution

Bands not visible	No protein in gel Imaging system malfunctioning Incorrect imaging parameters were used	Stain with another method to confirm there is protein. Check instrument manual for troubleshooting or contact imaging instrument manufacturer. Check Instrument manual.
Poor staining sensitivity	Dirty staining trays Insufficient stain volume Insufficient staining time Overuse of staining solution	Clean staining trays and other equipment with laboratory glassware cleaner. Follow recommendations for stain volume (appropriate to gel size). Increase staining time. Repeat staining protocol with fresh staining solution.
High or uneven background staining	Staining trays or equipment dirty Too much time in staining solution Reagent impurities	Clean staining trays and other equipment with laboratory glassware cleaner. Restrict duration of incubation in staining solutions as recommended in protocol. Wash gel in water or retrospective destaining solution for >30min. Use high-purity water and reagents for staining.
Speckles or blotches in gel image	Particulate material from reagents, staining tray, dust or gloves	Clean staining trays thoroughly. Decrease time that gels and staining solution are exposed to open air. Use dust-free gloves and handle gels only by edges.
Uneven staining	Insufficient shaking during staining	Agitate gel during staining.
Gel shrinkage	Gel dehyrated	Transfer gel to water for rehydration.
Problem: Evaluation of Separation	**Cause**	**Solution**
Diffuse or broad bands	Poor quality acrylamide or bis-acrylamide incomplete polymerization Old SDS or sample buffer Gel temperature too high	Use electrophoresis-grade reagents. Check polymerization conditions. Prepare fresh solutions. Use external cooling during run or run at a lower voltage.
Bands 'smile' across gel, band pattern curves upward at both sides of gel	Excess heating of gel; centre of gel runs hotter than either end Power conditions excessive	Check buffer composition; buffer not mixed well, or buffer in upper chamber too concentrated. Prepare new buffer, ensuring thorough mixing, especially when diluting 5x or 10x stock. Do not exceed recommended running conditions. Decrease power setting from 200V to 150V or fill lower

	Insufficient buffer	chamber to within 1cm of top of short plate. Fill inner and outer buffer chambers to ensure that wells are completely covered.
Smiling or frowning bands with gel lane	Overloaded proteins Sample preparation/ buffer issues Incorrect running conditions	Load less protein. Minimize salts, detergents and solvents in sample preparation and sample buffers. Use correct voltage.
Skewed or distorted bands, lateral band spreading	Excess salt in samples Ionic strength of sample lower than that of gel Insufficient sample buffer or wrong formulation Diffusion prior to turning on current Diffusion during migration through stacking gel Uneven gel interface	Remove salts, from sample by dialysis or desalting column prior to sample preparation. Use same buffer in samples as in gel. Check buffer composition and dilution instructions. Minimize time between sample application and power start-up. Increase %T of stacking gel to 4.5% or 5%T. Increase current by 25% during stacking. Decrease polymerization rate. Overlay gels carefully. Rinse wells after removing comb to remove residual acrylamide.
Vertical streaking	Overloaded samples Sample precipitation	Dilute sample. Selectively remove predominant protein in sample (fractionate). Reduce voltage by 25% to minimize streaking. Centrifuge samples to remove particulates prior to sample loading. Dilute sample in sample buffer.
Fuzzy or spurious artefactual bands	Concentration of reducing agent too low	Use 5% BME or 1% DTT in sample buffer.
Protein bands do not migrate down as expected	Bands of interest may be neutral or positively charged in buffer used; pH of bands must be -2pH units more negative than pH of running buffer	Use SDS-PAGE or a different buffer system in native PAGE or IEF.

Blotting Transfer Troubleshooting

Poor electrophoresis transfer (detected by staining gel) -- Protein

1. Transfer time is too short.

Increase the time of transfer

2. Power is too low.

Always check the current at the beginning of transfer. The specific setting voltage makes the current too low. If the buffer is not formulated properly, the conductivity is too low to lead to a shortage of force.

Re-formulate the buffer or increase the voltage.

Try high-strength transfer.

3. The Blotting unit is assembled incorrectly, causing proteins to migrate in the wrong direction.

The wrong order of gel and sandwich membrane, or the reversed inserting direction of sandwich clamp in the buffer tank.

4. The charge-to-mass ratio is incorrect

Try the transfer buffer with more acid and alkaline to increase the transfer rate of protein, The buffer pH that is close to iso-electric point of protein makes the transfer fail. It is generally recommended that the buffer pH should be less or more than that of protein by two values of pH to increase the transfer efficiency.

5. Protein is precipitating in gel

Have a try to add SDS to the buffer. The SDS could improve transfer efficiency, meanwhile decrease the binding rate and affect the reaction of certain protein and antibody.

6. The power supply does not work, or the improper one was used.

Check the fuse and ensure the output of current and voltage of power supply matches the electrophoresis equipment.

7. Methanol in the transfer buffer can reduce protein elution from the gel, potentially limiting transfer efficiency.

Reducing the methanol concentration in the transfer buffer can improve the transfer efficiency of proteins from the gel, especially high molecular weight proteins. However, it may also decrease protein binding to nitrocellulose and PVDF membranes.

8. Gel percentage too high.

Reduce %T (total monomer) or %C (Bis cross-linking agent). 5%C (Bis cross-linking agent)

could make the smallest sized pore, reduce the concentration and enlarge the size of pore to improve the transfer rate.

Poor transfer -- nucleic acid

1. Gel percentage is too high.

Reduce the percentage of total monomer or cross-linking agent in polyacrylamide gel as well as the percentage of agarose in Sepharose gel.

Before transfer, 0.25M diluted hydrochloric acid was used for cleaving DNA or dilution NaOH for RNA.

2. Transfer time is too short, or power conditions are too low.

Increase the transfer time or try high intensity transfer.

3. DNA and RNA cannot be transferred to nitrocellulose membrane via electrophoresis because high salting concentrations are required for efficient binding to the membrane.

Use the Zeta-Probe membrane to replace the nitrocellulose membrane.

The warp or lost band: diffusion transfer

1. Air bubbles or excess buffers trapped between the membrane and gel can prevent proper contact, resulting in incomplete or uneven transfer.

Use a test tube or pipette to roll over the surface of membrane in different directions until the air bubble and surplus buffer are removed between gel and membrane to ensure complete contact.

Use thicker filter paper in the gel-membrane sandwich.

Replace the sponges. The sponges would be thin after the lone-term squeezing; thus, it will not hold the membrane and gel.

2. Power conditions are too high.

Always check the current at the beginning of transfer. The specific voltage leads to high current. The surplus force would be supplied to electrophoresis cells in case of high current conductivity caused by improper formulation of buffers.

3. The membrane is not immersed completely or has dried out.

White spots on the nitrocellulose membrane indicate the dry areas where protein will not bind. The membrane should be immersed completely and equilibrated by buffer for the usage by heating the distilled water just under the boiling point if the immersed membrane doesn't get wet immediately in the buffer.

The PVDF membrane should be wet completely by methyl alcohol before equilibrated into a watery buffer because of its hydrophobic property. Please follow the guide of the instruction manual.

4. The possible errors in gel electrophoresis

The abnormal electrophoresis may be caused by poor gel binding, improper electrophoresis condition, the polluted buffer, overload of sample etc.

The pattern of gel clamp is transferred on the blotting membrane

1. The polluted or too-thin transfer fiber cushion is used.

Replace the sponge or complete rinse the polluted sponge.

2. There is an excess of protein or SDS in buffer. Protein may penetrate the blotting membrane without binding to it and dissociate in the electrophoresis cell.

Reduce the amount of protein in the gel and SDS in the buffer. Add a second membrane to binding excessive protein.

3. The transfer buffer is polluted.

Re-formulate the buffer.

Poor binding to the membrane -- nitrocellulose membrane

1. The nitrocellulose membrane requires 20% methyl alcohol to optimize protein binding in buffer.

Ensure that there is proper methyl alcohol in buffer.

2. The protein may be passing through the nitrocellulose membrane without binding.

The PVDF or nylon membrane (high-combined volume) is recommended or decreases the diameter of pore of nitrocellulose membrane (0.2μm).

Lower the voltage or change it into the standard transfer (if the high-strength transfer is required.)

3. Poor protein binding occurs when using mixed cellulose acetate membranes, as they are not optimized for protein retention

The pure nitrocellulose membrane is recommended.

4. Low molecular weight proteins (<15 kDa) may not bind effectively to 0.45 μm nitrocellulose membranes and are at risk of being washed away during subsequent assay steps.

To enhance binding stability, the protein can be crosslinked to nitrocellulose membrane with glutaraldehyde.

The PVDF or nylon membrane with high-binding capacity is recommended.

Use the Tween-20 as the detergent during the process of cleansing and antibody incubation to reduce or remove the condition of strong cleaning.

5. The SDS in the buffer would reduce the binding efficiency of protein.

Reduce or remove the SDS from the buffer.

6. Blotting membrane is incompletely wet.

White spots on the nitrocellulose membrane indicate the dry areas where protein will not bind. The membrane should be immersed completely and equilibrated by buffer for the usage by heating the distilled water just under the boiling point if the immersed membrane doesn't get wet immediately in the buffer.

Poor binding to the membrane -- PVDF membrane

1. Incomplete wetting of the membrane can result in poor or uneven protein binding.

The PVDF membrane should be wet completely by methyl alcohol before equilibrated into a watery buffer because of its hydrophobic property. Please follow the guide of the instruction manual.

2. The membrane dried completely during the operation.

The appearance of a complete immersed membrane is gray or semi-transparent.

White spots formed on the membrane indicate that it will be dry. Please re-wet the membrane by methyl alcohol and re-equilibrate membrane by transfer buffer because the protein doesn't bind to the dry points.

Detection of immunological features

The high general background

1. Blocking conditions are not suitable.

The block material must match the membrane. For example, the degrease dry milk is usually adopted to block the PVDF membrane and nylon membrane fully.

Increase block concentration and the length of time according to the specific requirement.

The block material should be pure protein. The block material may be polluted by the material that can be combined with nonspecific probe.

2. The rinse project with poor performance is adopted.

Increase the time of washing, length of wash period, or the strength of washing. Use the stronger detergent gradually. The strength of SDS is stronger than that of NP 40 and Tween-20.

3. The blotting membrane is soaked in blotting substrate for a long period.

Remove the blotting membrane from substrate solution when the ratio of info-to-noise can be accepted. The image should not be excessive, put the blotting membrane in the double-distilled water to stop the reaction.

4. Contamination from the transfer or electrophoresis steps can cause background noise or poor results

Discard the gel or buffer.

Replace or thoroughly rinse the sponge because excess protein or SDS in the buffer can cause proteins to pass through the membrane without binding; reduce protein and SDS concentrations and consider using a second membrane to capture surplus protein.

5. High concentration of primary antibodies or second antibodies

Increase the dilution of antibody and optimize the working concentration of experiment of mark-blotting.

6. The incubation tray is contaminated, which can affect the experiment's results. Rinse thoroughly or use a disposable tray.

There is no specific reaction between binding protein and probe.

1. Primary antibodies or second antibodies are polluted by IgG in non-specific reaction and cross reaction.

Use the purified IgG as primary antibody to adjust the purified blotting-level second antibody.

2. Monoclonal antibody may react nonspecifically to SDS denatured proteins Comparing other monoclonal antibodies or polyclonal antibodies Blotting with non-denatured protein

3. The meaningless mutual action caused by more acidic protein binding to the membrane. Such as antibiotic protein, glycoprotein binding to more acidic protein on the membrane.

Improve the strength of ion in incubation buffer. Increase the time of washing, length of wash period, or the strength of washing. Use the stronger detergent gradually. The strength of SDS is stronger than that of NP 40 and Tween-20. Put Tween-20 in antibody diluent to reduce the non-specific binding.

No reaction or weak signal

1. Insufficient sample

The sample may be required to be concentrated before its loading. Or use more sensitive detection methods.

2. The antigen binding to the membrane is not enough.

Dye the gel after transfer or assess the transfer efficiency by pre-dye. Please refer to the previous chapters to improve.

3. Either primary antibody or second antibody is inactivated or unsaturated.

The storage conditions of the reagent must meet the requirement. Avoid multigelation, bacterial pollution and heat inactivation.

The detergent affects the antibody activity. Remove the detergent from the system except block washing.

Optimize the concentration by spot-blotting experiment if the efficiency of antibody is too low.

Increase the period of antibody incubation.

4. Enzyme compounds are inactivated or unsaturated.

Detect the activity of reagent (refer to the table below).

Ensure reagent storage conditions meet requirements to avoid gelation, bacterial contamination, and heat inactivation.

Sodium azide is an effective inhibitor of horseradish peroxidase. Use Thimerosal sodium thiosalicylate (Thimerosal) as antibacterial agent.

The impure water will also cause enzyme deactivation, use distillation deionized water completely. Optimize the concentration by spot-blotting experiment if the efficiency of enzyme compound is too low.

5. Chromogenic reagent inactivation.

Detect the activity of reagent (refer the following info), re-formulate if it is required.

Detect the activity of detection reagent

1. Activity detects of colored solution

The chromogenic reaction is triggered once mixing 1.0ml chromogenic liquid with second antibody compound. The chromogenic reagent is inactivated if the color is not changed after several minutes, thus re-formulated.

2. Activity detection of coenzyme solution

Mix 1.0ml of color solution with 1.0ml 1"3000 diluted coenzyme solution. The light blue light appears within 15 minutes. There are some problems with the coenzyme solution if the light does not appear. Re-experiment with fresh coenzyme solution.

3. Activity detects of primary antibody

Test the reaction of antibody and antigen by ELISA, radioimmunoassay, double immunodiffusion, method of precipitation. If possible, try to use the primary antibody with different dilution rates to repeat the experiment.

Detection of protein

Colloidal gold TP dye -- high background

1. Insufficient or missing block steps

Use 0.3% Tween-20 TBS to block 3 times each 20 minutes.

2. The membrane is not suitable for this dye

The nylon membrane with positive charge cannot be used for colloidal gold dye, replaced by the biotin-blotting TP detection.

3. It is polluted in the previous step. Such as the step of transfer or electrophoresis.

Discard the gel and buffer.

Replace or completely wash the sponge.

4. There is excessive protein on the gel or too much SDS is mixed in buffer.

The protein may penetrate the blotting membrane without binding to it, and dissociate in the electrophoresis cell.

Reduce the protein in gel and SDS in buffer. Increase the binding of surplus protein with second membrane.

5. The colloidal gold dye solution was contaminated.
The dye reagent can be recycled. Store the used reagent in independent, clean and plastic container and put the container in the refrigerator. Discard the reagent that sticks to the bottom of the bottle. If the solution is light blue but not the deep red wine color, the solution is contaminated by the salt in buffer. The salt in the buffer reacts to the gel in the golden solution and causes the non-specific sediment of reagent on the membrane. Just discard them.

Colloid gold TP dye -- low low sensitivity

1. Increase the incubation period of low-testing signal.

Try overnight incubation, the background signal is strengthened accordingly.

2. The transfer is not done.

Refer to the relative chapter about the content of failure to improve the efficiency of transfer.

3. The dye period is too long, and the deep red wine color is lost to prove that the dye is invalid.

Discard reagent.

4. The salt contaminated in the buffer, the deep red wine color is replaced by light blue.

Discard the reagent.

5. The sample volume is too small (compared with the detection reagent)

Test the 10pg protein on each band by gold enhancement kit

Biotin-Blotting TP detection -- high background

1. Insufficient block condition

Match the block material with a membrane. The nylon requires to be added into several solutions like MPO. Refer to the special detailed information in the biotin-blotting manual.

2. The membrane is left in chromogenic reagent for a long time.

Remove the membrane from chromogenic reagent and transfer it to the distilled water to stop the reaction when the signal appears while the background does not appear.

3. There is excessive protein on the gel or too much SDS is mixed in buffer. The protein may penetrate the blotting membrane without binding to it, and dissociate in the electrophoresis cell.

Reduce the protein in gel and SDS in buffer. Increase the bonding of surplus protein with second membrane.

Biotin-Blotting TP detection -- no reaction or weak color

1. Incomplete transfer

Refer to the chapter relates to the content of failure to improve the efficiency of transfer.

2. The sample volume is too small (compared with the detection reagent)

Increase the volume of sample of protein in gel.

3. NHS- inactivation of biotin solution

NHS Biotin is hydrolyzed in an aqueous solution. The temperature of reagent bottle must be adjusted to room temperature before opening it in order to avoid the condensation of water vapor. Use sterile syringes to prevent contamination.

Add boric acid-Tween in NHS-biotin reagent before usage.

4. The competition of biotin reagent and amine in buffer salt.

Washing the membrane completely in boric acid-Tween to remove the residual buffer salt during the transfer and electrophoresis.

5. Inactivation of Avidin-HRP compound

Detect whether the reagent is inactivated or not according to the procedures of activity detection.

6. Inactivation of color solution

Detect whether the reagent is inactivated or not according to the procedures of activity detection.

Anion dye -- high background

1. Insufficient decoloration

Increase the time of washing and period of washing in decoloration solution.

2. Concentration of dye solutions is too high.

Re- formulate the buffer.

3. The nylon membrane is not compatible with dye.

Use the box of biotin-blotting protein detection kit.

Anion dye -- low sensitivity

The anion dye cannot detect the protein with less than 100ng band.

Use the more sensitive dye. Such as Colloid gold total protein detection or biotin-imprint testing box.

Increase the volume of sample to reach the level of anion dye.

Care and Maintenance

Cleaning Vertical Units

Units are best cleaned using warm water and a mild detergent. **Water at temperatures above 60°C can cause damage to the unit and components.**

The tank should be thoroughly rinsed with warm water or distilled water to prevent build-up of salts, but care should be taken not to damage the enclosed electrode, and vigorous cleaning is not necessary or advised.

Air drying is preferable before use.

The units should only be cleaned with the following:

Warm water with a mild concentration of soap or other mild detergent.

Compatible detergents include dishwashing liquid, Hexane and Aliphatic hydrocarbons

The units should not be left in detergents for more than 30 minutes.

The units should never come into contact with the following cleaning agents, these will cause irreversible and accumulative damage:

Acetone, Phenol, Chloroform, Carbon tetrachloride, Methanol, Ethanol, Isopropyl alcohol, Alkalis.

RNase Decontamination

This can be performed using the following protocol:

Clean the units with a mild detergent as described above.

Wash with 3% hydrogen peroxide (H2O2) for 10 minutes.

Rinse with 0.1% DEPC- (diethyl pyro carbonate) treated distilled water,

Caution: DEPC is a suspected carcinogen. Always take the necessary precautions when using.

RNaseZAP™ (Ambion) can also be used. Please consult the instructions for use with acrylic gel tanks.

Ordering information

Catalogue No.	Product description	Standard Quantity
618202	ProPAGE2 2 gel mini vertical System, with 0.75mm bonded space	1
618201	ProPAGE2 2 gel mini vertical System, with 1.0mm bonded space	1
618203	ProPAGE2 2 gel mini vertical System, with 1.5mm bonded space	1
618205	ProPAGE4 4 gel mini vertical System, with 0.75mm bonded space	1
618204	roPAGE4 4 gel mini vertical System, with 1.0mm bonded space	1
618206	roPAGE4 4 gel mini vertical System, with 1.5mm bonded space	1
618229	Comb 10 sample, 1.0mm thick	5
618232	Comb 15 sample, 1.0mm thick	5
618228	Comb 10 sample, 0.75mm thick	5
618231	Comb 15 sample, 0.75mm thick	5
618230	Comb 10 sample, 1.5mm thick	5
618233	Comb 15 sample, 1.5mm thick	5
618222	10 x 8cm Plain Glass Plates with 0.75mm Bonded Spacers	5
618223	10 x 8cm Plain Glass Plates with 1mm Bonded Spacers	5
618224	10 x 8cm Plain Glass Plates with 1.5mm Bonded Spacers	5
618226	10X 7cm Short Plates	5
618211	casting stand	1
618212	casting frames with clamps	1
618210	Silicone mat	5
618221	Dummy Plate	1
618227	Gel Releasers	5
618216	U-sealing Gasket Strip	4

618214	proPAGE - Inner Running Module with Electrode Banana plugs	1
618215	proPAGE - Inner Running Module without Electrode without Banana plugs	1
618219	CVS8 lid (Black & Red cables included)	1
618220	CVS8Tank	1

Catalogue No.	**Product description**
618250	miniBlot Mini 10 x 8cm Blotting System
618251	High Intensity miniBOLT Blot Mini insert
618252	miniBLOT Blot Clip
618554	miniBLOT Macroporous Sponge
618253	miniBLOT Cooling Block

Warranty

The Thistle Scientific Ltd. (TSL) proPAGE Vertical Electrophoresis units have a warranty against manufacturing and material faults of twelve months from date of customer receipt.

If any defects occur during this warranty period, TSL will repair or replace the defective parts free of charge.

This warranty does not cover defects occurring by accident or misuse or defects caused by improper operation.

Units where repair or modification has been performed by anyone other than TSL or an appointed distributor or representative are no longer under warranty from the time the unit was modified.

Units which have accessories or repaired parts not supplied by TSL or its associated distributors have invalidated warranty.

TSL cannot repair or replace free of charge units where improper solutions or chemicals have been used. For a list of these please see the Care and Maintenance subsection.

This warranty does not apply to parts listed below:

1. Platinum Electrode Wires.

If a problem does occur, then please contact your supplier or Thistle Scientific Ltd:

Thistle Scientific Ltd.

Unit 41, Somers Road Industrial Estate

Rugby, Warwickshire, CV22 7DH

Tel: +44 (0)1788 565300

Email: enquiries@thistlescientific.co.uk

Or support@thistlescientific.co.uk