韦克斯科技（北京）有限公司

WIX TECHNOLOGY BEIJING CO., LTD

WIX-miniBLOT Mini Vertical Blot

Instruction Manual

WIX TECHNOLOGY BEIJING CO.,LTD

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Unpacking remarks

1. WIX-miniBLOT is packaged by the imported corrugated paper with clean appearance outside and quality environmental friendly lining inside. The product label is labeled on the outer package. Please check the outer package carefully to make sure it is in the best condition, please contact us in case of any damage.

2. Packing list

1) One piece of buffer tank and one piece of upper lid with special cable.
2) One piece of blot core.
3) Two pieces of gel sandwich clamps.
4) Four pieces of blot cushions.
5) Two piece of freezing module.
6) One piece of instruction manual.

3. To insure the best performance and optimize the condition of experiment, please read this instruction manual carefully to get acquainted with every component and function of electrophoresis system. The correct operation is required to avoid a damage and prolong the usage of the electrophoresis.

4. It is recommend that the customer use the neutral detergent to cleanse all the components and then to cleanse them again by distilled water before using the WIX-miniBLOT.

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Content

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Chapter 1 General Instructions

1.1 Introduction

WIX-miniBLOT is part of WIX-mini electrophoresis system, which contains the SDS-PAGE and Native-PAGE gel WIX-miniPRO vertical electrophoresis cell.

WIX-miniBLOT accommodates 2 blot clamps and can be used for blotting protein and RNA sample in the polyacrylamide and sepharose gel.

Cooling module belongs to the standard accessories of WIN-miniBLOT, which after being freezed to absorb the heat generated during the operation of blotting. The built-in cooling module avoids the trouble of connection and the cost of installing the external cooling-recycling system. Other features include easy-to-use lock mechanism installed in gel clamp, gel clamp with color marker, direction positioning, special-designed plug-in-out mechanism. All these features make the WIX-miniBLOT a perfect electrophoresis system that is easy to use.

1.2 Technical specification

Electrophoresis core	Polycarbonate
Gel sandwich clamp	Polycarbonate
Electrophoresis electrode	Platinum wire (made from platinum ingot)
Buffer tank and upper lid	Polycarbonate
Cooling module	Polyethylene and cold storage agent
Cell size	16 cm (L) x 12 cm (W) x 18 cm (H)
Gel clamp size	10 cm x 11 cm
Maximum gel area	7.5 cm x 10 cm
Buffer volume (with cooling module)	850 ml
Buffer volume (without cooling module)	650 ml

Cleanse:

Use the neutral detergent and warm water to cleanse the electrode, gel clamp and buffer tank. Be careful to cleanse the electrode and avoid extending or breaking off the platinum wire. Do not use the abradant and strong detergent to cleanse the instrument. Warm water is to rinse the fiber lining cushion and then make it clean by distilled water and ion-removing water.

Chemical reagent compatibility:

All the components of WIX-miniBLOT electrophoresis cell should be kept clear of hydrochloric ether (such as chloroform), arene (such as methylbenzene, benzene) and acetone. The damage caused by organic reagent is not covered by guarantee.

1.3 Safety Instructions

WIX-miniBLOT is powered by the external-connected direct current that must be isolated from the external-ground wire with the view to assure that the current is conducted through electrophoresis cell. All the WIX branded instruments meet such safety standard. The following are the maximum value regardless of what power supply are applied,

150V(direct current)	Maximum input voltage
40W	Maximum input power
50℃	Maximum temperature

The current is inputted via the upper lid, which supplies the customer with the safe-mutual lock. The current is disconnected when the upper lid is opened. Be sure to power off before removing or opening the upper lid. Do not try to operate the instrument without upper lid.

Note: The safety standard is met from designing stage to manufacturing stage. It is safe to operate the instrument according strictly to the instruction manual. Do not mend or improve the instrument by any means. Otherwise, it leads the following result,

The quality guarantee is valid;

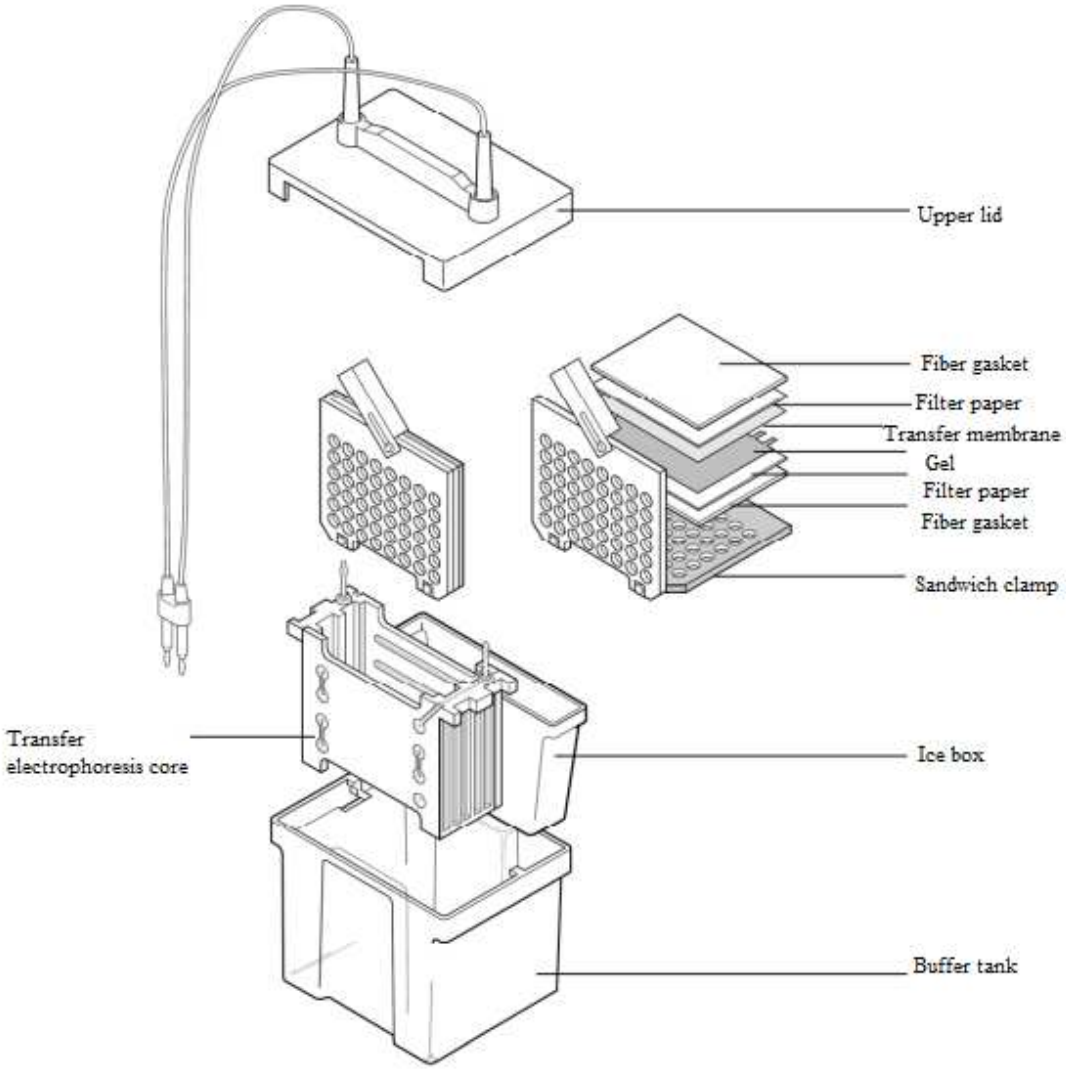
Damage the safety standard;

Cause the potential safety hazard.

WIX company is irresponsible for and damage and loss caused by any improper usage intentionally and unauthorized improvement.

Chapter 2 Installation and preparation for electrophoresis cell

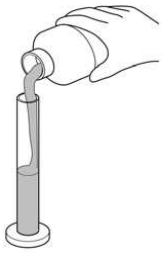
2.1 The component of WIX-miniBLOT



2.2 Preparation for blot

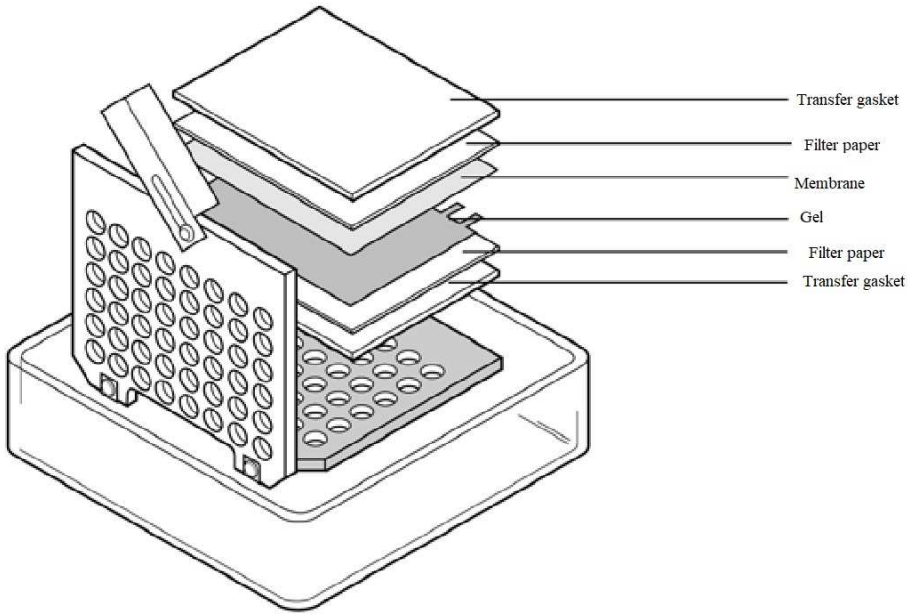
Put ice box into the refrigerator with temperature -20°C, and put back after usage.

1. Prepare the buffer. (Please refer to formula mentioned in section 3.3, it is easy to diffuse the heat when the buffer is freeze to 4°C)



2. Cut the filter paper and film according to the size of gel, please wear the glove to avoid pollution. Balance the gel and immerse the film and filter paper as well as the lining (From 15 minutes to 1 hour, depended on the thickness of the gel).

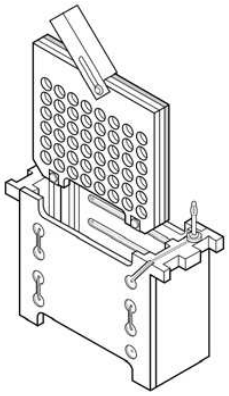
3. Prepare the sandwich clamp and put on the clean desk with the black side downward. Put a ready-wet transfer lining on the black part of clamp. Put the wet filter paper on the transfer lining. Put the balanced gel on the filter pater (Remove the bubble between the gel and filter paper). Put the immersed film on the gel (Remove the bubble between the film and gel). Put the filter paper on the film and remove the bubble, add the transfer lining.



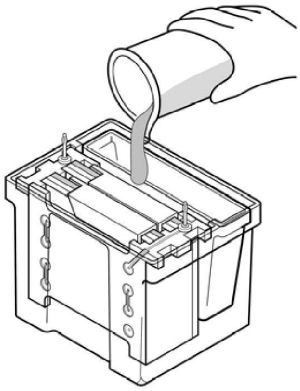
Note: In order to achieve the best blot result, it is key to remove the bubble by glass-stick stirring.

4. Tighten the clamp and do not move the gel and filter paper sandwich, then use the white sliding block to lock the clamp.

5. Insert the clamp into the blot electrophoresis , and repeat the process to make another sandwich gel.

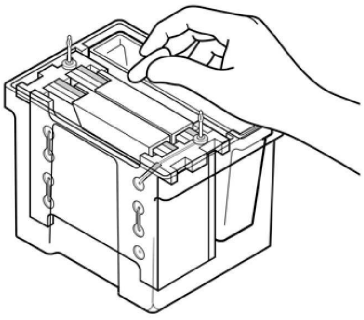


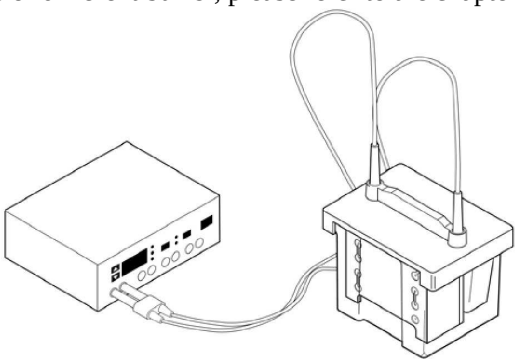
6. Put the cold ice box in the tank and fill up the tank by buffer.



7. Put the stirring material into the buffer to keep the temperature and balance the ion strength, set the most suitable speed to distribute the ion evenly.

8. Close the safety lid and insert the power supply wire into the socket and start the electrophoresis cell. The set of voltage and time of different buffer, please refer to the chapter 3.





9. Disassemble the sandwich when the electrophoresis ends, take out the film and continue the

next step. Use the neutral detergent to rinse the electrophoresis cell, clamp, lining, and then again use the ion-removing water to rinse.

2.3 Acid transfer

If the transfer is being done under acidic conditions, switch the position of the gel to the membrane and place the membrane on the cathode of the gel.

Under acidic conditions, the protein migrates to the direction of cathode in the acid condition, do not reverse the electrode otherwise it may be damaged.

Chapter 3 Condition for electrophoresis transfer

3.1 Guideline for buffer transfer and running condition

The table 3.1 indicates the different power supply and time needed according to different buffer. The general rule is that shorter time needed, the higher voltage is. The ice box should be used during the process of electrophoresis.

Table 3.1 Guideline for buffer transfer and running condition

		High field strength
	Standard field strength	Distance of electrode is 4cm
Buffer	Transfer overnight	Transfer time 1 hour
SDS-PAGE Gels	Buffer A or B or C	Buffer A or B or C
A: 25 mM Tris, pH 8.3, 192 mM	30 V	100 V
glycine, with or without	90 mA	350 mA
20% MEOH and .025%–0.1% SDS.		
B: 48 mM Tris, pH 9.2, 39 mM glycine,		
with or without 20% MEOH and		
.025%–0.1% SDS.		
C: 10 mM NaHCO3, 3 mM NaCO3,		
pH 9.9, with or without 20% MEOH		
and .025%–0.1% SDS.		

DNA and RNA		
TAE: 20 mM Tris, pH 7.8, 10 mM	30 V	80 V
sodium acetate, 0.5 mM EDTA	100 mA	500 mA
TBE: 50 mM Tris, pH 8.3,		
50 mM sodium borate, 1.0 mM EDTA		
Native Gels		
25 mM Tris, pH 8.3,	30 V	100 V
192 mM glycine. No methanol.	90 mA	350 mA
Isoelectric Focusing, Native Gels,		
Basic Proteins, Acid Urea Gels		
0.7% acetic acid	30 V	100 V
	100 mA	350 mA

3.2 The cautions for electrophoresis transfer

The following change changes the resistance, and the current is changed accordingly.

Changes in the preparation of buffer: such as, the SDS is increased, 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 unbalance of gel.

Quantity of gel: the increased quantity leads to the slight 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-balance of gel

All the gels should be pre-balanced by the transfer buffer before conducting the electrophoresis, 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-balance activity adjust the gel to the best size eventually before the electrophoresis.

The usage of stirring material during transfer

All the stirring material should be put into WIX-miniBLOT during the application of blot in order to stir the buffer in the experiment, which is useful to keep the conductivity of buffer and temperature in the best harmony. It leads to the poor performance of macromolecular transfer and foster 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 leads to the increase of conductivity, which is proved by the output current higher than the expected and decrease of resistance. It is advised to use the Power B elctrophoresis power supply and check the initial current value before any electrophoresis.

Recommendation of transfer buffer

Please use the high-quality methyl alcohol. The polluted methyl alcohol leads 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 can not 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 in table 3.1 during the overnight electrophoresis. The conductivity of buffer should be close to the current mentioned in the table. The upper limit should be set in electrophoresis power supply. The electrophoresis should be done by the high voltage once the low voltage is not available, however, the time must be shortened otherwise it leads potential safety hazard.

3.3 Formula of buffer

WIX-miniBLOT electrophoresis cell requires around 500ml buffer.

Do not adjust the pH value by increasing the acid or alkali. The methyl alcohol should be pure otherwise the metal pollution in the low-purity methyl alcohol will damage the electrode.

Note: Some pH electrodes are not sensitive to Tris, please check whether the pH electrode is suitable for Tris buffer if the pH of the buffer is off. The buffer needs to be formulated again if the pH electrode is right but the pH of buffer is less than 8.

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 (Tri-boric acid EDTA), pH 8.3

90mM Tris- boric acid 1 mM EDTA

5x storage solution

54 g Tris alkali

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 (Tri- acetic acid EDTA)

40mMTri- acetic acid 1 mM EDTA

50x storage solution

242 g Tris alkali

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.

Chapter 4 Procedure for optimizing condition of electrophoresis

4.1 Optimizing protein transfer

Generally speaking, it is difficult to wash high molecular weight protein. The following methods used individually or by combination are useful to improve the transfer rate.

Compositions of different gel

The graded gel makes it easier to wash 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 monadelphous polymer.

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.

	Methyl-bisacrylamide (g)	
%C=		X 100%
	Methyl-bisacrylamide(g)+ acrylamide (g)	

Prolonging the transfer time

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

Strengthen the electric field intensity

The setting of 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 efficiency

The diluted buffer lowers the current under the fix 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 the protein can not migrate. Such property applies to the lysozyme. The buffer with pH 9.5 to 10 makes the basic protein, such as lysozyme and histone, migrate 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 that of acetic acid and phosphate buffer.

Increase 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. In order to realize the elution of protein, the SDS is added into the buffer, meanwhile the combination efficiency of protein and nitrocellulose membrane is lowered.

Removing alcohols from buffer

The purpose of transferring the alcohols is to promote the combination between SDS protein and nitrocellulose membrane, the transfer efficiency is accordingly improved but the combination efficiency is lowered. The transfer efficiency is improved on the basic of fact that the alcohols shrinking the pore of gel and the big-sized protein remains inside. The PVDF film is applied to eliminate the need of alcohols and create the reasonable strategy for the big-sized protein and hard-transferable protein. The PVDF film has to be wetted 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 the transfer efficiency without decreasing the immunological activity of proteins.

Type of film

As said above, the PVDF film makes the transfer electrophoresis go under the condition of no

methyl alcohol.

Type of gel

If possible, use the non-degeneration gradient gel to separate the protein 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 the contact between the gel and the film

The phenomenon that the effective combination of protein molecule and film fails because of poor contact of gel and film is always treated mistakenly as the futile elution. The poor contact is caused by the surplus liquid between the gel and film. The complete contact is done by the technique of employing the test tube and glass stick as the best tool. The good compression can be done by the suitable filter paper. The gel and film should be balanced 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.

4.2 Optimize DNA and RNA electrophoresis

The problem of elution of nucleic acid can be solved by the adjusting the percentage of gel. The difficulty remained there is the big volume of DNA in the fixed transfer genome. The following methods can be adopted.

Change gel composition

It is useful to promote the electrophoresis of high molecular weight DNA by lowering the percentage of single monomer and cross-linking agent in polyacrylamide and lowering the percentage of agarose gel.

Change the DNA denaturant

The denature of oxalaldehyde is more useful for the elution of DNA than that of sodium hydroxide. The perfect result can also be achieved by DNA denatured by boiled acrylamide. The polyacrylamide gel would be softened and stuck to film by alkaline denaturation.

Chapter 5 Option of transfer membrane

5.1 Protein blot membrane

Nitrocellulose membrane

Nitrocellulose membrane is widely used in combination of protein and testing, whose total protein testing can be done by protein gel dye such as amino black, coomassie blue, ponceau S, fast green FCF etc.) or the more -sensitive collaurum gel dye. The nitrocellulose membrane is also used to analyze the radioactivity immunoassay, fluoroimmunoassay and enzyme-linked immunoassay. The nitrocellulose membrane needs not to be pretreatment because of the volume of 80-100μg/cm^2. The Connected point of specific proteins is easily closed so as to avoid the problem of

background. The protein with low molecular weight (less than20 KD) is easily lost during the washing after electrophoresis transfer, thus, leads to the decreased detection sensitivity. However, the nitrocellulose membrane with the 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. The methyl alcohol improves the combination 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 combination is decreased. The SDS also enhances the conductivity of current and generates much more heat during the electrophoresis.

PVDF film

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

5.2 DNA and RNA blot membrane

Zeta-Probe® nylon film

The nitrocellulose membrane is not suitable for medium of nucleic acid electrophoresis transfer because of combination requirement of high concentration salt (>10 x SSC). The nucleic acid with molecular weight ≤500bp is impossible to be combined even in the condition of high concentration salt. The lower resistance caused by high current going through the high salt solution leads to the high potential current and electrical power that would damage the nitrocellulose membrane under the condition of low voltage. The invalid transfer would exist in the required combination because of elution of lowering voltage (V/cm) per centimeter. Zeta-Probe film allows all the AND and RNA with different size of chain to combine efficiently in the buffer with low strength ion. As for the nitrocellulose membrane. Zeta-Probe film is the ideal option to analyze the nucleic acid because of the stability during the washing after transfer and about ten-fold reappear rate.

Table 5.1 Guide for protein imprinting membrane

There are many kinds of imprinting membrane for immunoblotting, each of which features the unique advantage according to the specific requirement of different experiments. The physical property and feature should be assessed when choosing the most suitable transfer conditions.

		Volume	
Membrane	Pore Size	(μg/cm2)	Remark
Nitrocellulose	0.45 μm	80–100	Universal protein imprinting membrane
	0.2 μm		
Supported	0.45 μm	80–100	Inject the pure nitrocellulose into the inertia supporter to improve the strength and make the operation convenient and the color appears easily.
Nitrocellulose	0.2 μm		
PVDF	0.2 μm	170–200	Improve the strength and stability for the protein sequencing and imprinting. Increase bonding in SDS
			Immersed by methyl alcohol in advance.
Nylon	0.2 μm	170	The nucleic acid is recommended.

Note: The RNA should not be transferred to nitrocellulose membrane via electrophoresis, instead, the Zeta-Probe film must be used.

Chapter 6 Trouble shooting

6.1 Electrophoresis transfer

The poor performance of electrophoresis transfer (detected by gel dye)--Protein

1. Too short time

Increase the time of transfer

2. Lower power

Detect 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 shortage of force.

Re-formulate the buffer or increase the voltage.

Have a try of high-strength transfer.

3. Incorrect installation of transfer equipment, the wrong direction of protein.

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

4. The wrong ratio of charge to weight

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

5. Protein sediment in gel

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

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

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

7. The methyl alcohol in the buffer restricts the elution.

The decreased methyl alcohol improves the transfer rate of protein in gel, meanwhile , decreases the combination rate of protein and nitrocellulose membrane as well as PVDF film.

8. Too high percentage of gel

Decrease %T (total monomer) or %C (Bis cross-linking agent). 5%C (Bis cross-linking agent) could make the smallest sized pore, decrease the concentration and enlarge the size of pore so as to improve the transfer rate.

Poor performance of transfer-nucleic acid

1. Too high percentage of gel

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

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

2. Too short transfer time and too low power

Increase the transfer time or try to use a high intensity transfer.

3. DNA and RNA can not be transferred to nitrocellulose membrane via electrophoresis because of high salting strength which combines the film.

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

The warp or lost band: diffusion transfer

1. Poor contact between film and gel, the bubble or surplus buffer exists between imprinting and gel.

Make the test tube or pipette to roll the surface of film in different directions until the bubble and surplus buffer is expelled out completely so as to ensure the complete contact.

Use the thicker filter pater in gel-film sandwich.

Replace the fiber cushion. The cushion would be thin after the lone-term squeezing, thus it can not compress the film and gel.

2. High power requirement

Check the current at the beginning of transfer. The specific voltage leads to the high current. The surplus force would be supplied to electrophoresis cell in case of high current conductivity caused by improper formulation of buffer.

3. The film is not immersed completely or dry

The white spot on the nitrocellulose membrane indicates the dry place where protein can not be combined. The film should be immersed completely and balanced by buffer for the usage by heating the distilled water to the point below the boiling point if the immersed film can not be wetted immediately in the buffer.

The PVDF film should be wetted completely by methyl alcohol before being balanced in watery buffer because of its hydrophobic property. Please follow the guide of instruction manual.

4. The possible error in gel electrophoresis

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

The pattern of gel clamp is transferred on the imprint film

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

Replace the cushion or complete rinse the polluted cushion.

2. There are an excess of protein or SDS in buffer. The protein can penetrate the imprint film without bonding 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 bonding excessive protein.

3. The transfer buffer is polluted.

Re-formulate the buffer.

Failure to attach to the film- nitrocellulose membrane

1. The nitrocellulose membrane needs 20% methyl alcohol to optimize the absorption of protein in buffer.

Ensure that there is proper methyl alcohol in buffer.

2. The protein penetrates the nitrocellulose membrane possibly

The PVDF or nylon film (high-combined volume) is recommended, or decrease 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 needed)

3. Poor combination of mixed acetate cellulose and protein.

The pure nitrocellulose membrane is recommended.

4. The less-than-15000 daltons protein's absorption by 0.45μm nitrocellulose membrane is decreased or is washed away during the analyzed process.

In order to enhance the stability of combination, the protein can be absorbed to nitrocellulose membrane by glutaraldehyde .

The PVDF or nylon film with high-combined volume is recommended.

Use the Tween-20 as the detergent during the process of cleanse and anti-body incubation so as to reduce or remove the condition of strong cleaning,.

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

Reduce or remove the SDS in the buffer.

6. Incompletely wetted imprint film

The white spot on the nitrocellulose membrane indicates the dry place where protein can not be combined. The film should be immersed completely and balanced by buffer for the usage by heating the distilled water to the point below the boiling point if the immersed film cannot be wetted immediately in the buffer.

Failure to attach to the film-PVDF film

1. Film is not wetted completely

The PVDF film should be wetted completely by methyl alcohol before being balanced in watery buffer because of its hydrophobic property. Please follow the guide of instruction manual.

2. The film is dry during the operation

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

The white spot formed on the surface of film indicates that it will be dry. Please re-wet the film by methyl alcohol and re-balance film by transfer buffer because the protein doesn't attach to the dry points.

6.2 Detection of immunological feature

The high general background

1. Improper lock condition

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

Increase the concentration and the length of time according to the specific need.

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

2. Adopt the project of washing with poor performance.

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

3. The imprint film is suspended in substrate for a long time.

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

4. There is pollution in the previous step, such as the step of transfer or the step of electrophoresis. Discard the gel or buffer.

Replace or rinse the fiber cushion completely. There are an excess of protein or too much SDS in buffer. The protein can penetrate the imprint film and stay in the electrophoresis cell without bonding to the film. Reduce the volume of SDS in the gel protein or buffer. Increase the bonding of the second film and 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-imprinting

6. The incubation tray is polluted.

Rinse the tray or use the single-usage tray

There is no specific reaction between bonding protein and probe.

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

reaction.

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

2. Monoclonal antibody may react non specifically to SDS denatured proteins

Comparing other monoclonal antibodies or polyclonal antibodies

Imprinting with non-denatured protein

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

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

No reaction or weak signal

1. Insufficient sample

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

2. The antigen bonding to the film is not enough

Dye the gel after transfer or assess the transfer efficiency by pre-dye or kaleidoscope standard.

Please refer to the previous chapters to improve.

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

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

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

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

Increase the period of antibody incubator.

4. Enzyme compound is inactivated or unsaturated.

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

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

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

The impure water can also cause enzyme deactivation, use distillation deionized water completely.

Optimize the concentration by spot-imprint experiment if the efficiency of enzyme compound is too low.

5. Chromogenic reagent inactivation

Test the activity of reagent (refer the following info), re-formulate if needed.

Test the activity of detection reagent

1. Activity test of colored solution

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

2. Activity test 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 is something wrong with the coenzyme solution if the light does not appear. Re-experiment with fresh coenzyme solution.

3. Activity test 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 rate to repeat the experiment.

6.3 Detection of protein

Colloid total protein dye- high background

1. Insufficient or missing locking steps

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

2. The film is not suitable for this dye

The nylon membrane with positive charge can not be used for collaurum dye, use the biotin-imprinted total protein for 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 fiber cushion.

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

The protein can penetrate the imprint film without bonding to it, and dissociate in the electrophoresis cell.

Decrease the protein in gel and SDS in buffer. Increase the bonding of surplus protein with second film.

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 cause the non-specific sediment of reagent on the film. Just discard them.

Colloid total protein dye-low low sensitivity

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

Try the 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 period of dye 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 the light blue. Discard the reagent.

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

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

Biotin- Imprinting total protein detection-high background

1. Insufficient locking condition

Match the blocked material with film. The nylon needs to be added into several solutions like MPO. Refer to the special detailed information in the biotin-imprinting manual.

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

Remove the film 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 can penetrate the imprint film without bonding to it, and dissociate in the electrophoresis cell.

Decrease the protein in gel and SDS in buffer. Increase the bonding of surplus protein with second film.

Biotin- Imprinting total protein detection-no reaction or weak color

1. Incomplete transfer

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

2. The sample volume is too small (compare 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 level before opening it in order to avoid the condensation of water vapor. Use sterile syringes to prevent contamination

Put the boric acid-Tween in NHS-biotin reagent before usage.

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

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

5. Inactivation of Avidin-HRP compound

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

6. Inactivation of color solution

Test 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.

1. Solution concentration of dye solution is too high

Re- formulate the buffer.

2. The nylon film is not compatible with anion dye.

Use the box of biotin-imprint protein detection kit.

Anion dye- low sensitivity

The anion dye can not 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.

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