

# INSTRUCTION MANUAL

## Semi-Dry Blotting Systems

**EBU-4000**  
**EBU-6000**



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## IMPORTANT USER INFORMATION

This Instruction Manual will explain how to use this product safely and effectively. Please read and carefully follow the instruction manual in its entirety.



The triangle/exclamation mark symbol alerts the user of the product to important operational, maintenance, and/or warranty requirements.



The triangle/lightning bolt symbol alerts the user of the product to potentially hazardous electrical exposure.



Failure to adhere to the instructions could result in personal and/or laboratory hazards, as well as invalidate any warranty. Always turn off the DC power source prior to disconnecting power cords from the product. Disconnect power cords from the power source first and then from the product. For maximum safety, always operate this system in an isolated, low traffic area, not accessible to unauthorized personnel. Never operate damaged or leaking equipment.

## WARRANTY AND LIABILITY

This product was produced utilizing the highest practical standards of materials, workmanship, and design. C.B.S. Scientific warrants that the product has been tested and will meet or exceed published specifications. This warranty is valid only if the product has been operated and maintained according to the instructions provided.

C.B.S. Scientific warrants this product to be free from defects in materials and workmanship under normal service for one year from date of shipment. If the product proves defective during this period, C.B.S. Scientific will repair or replace it at our option, free of charge, if returned to us postage prepaid. This warranty does not cover: damage in transit, damage caused by carelessness, misuse or neglect, normal wear through frequent use, damage caused by solvent corrosion, damage caused by improper handling or user alteration, nor unsatisfactory performance as a result of conditions beyond our control. C.B.S. Scientific shall in no event be liable for incidental nor consequential damages, including without limitation, lost profits, loss of income, loss of business opportunities, loss of use and other related damages, however caused, nor any damage arising from the incorrect use of the product.

<p><b>FRANÇAIS INFORMATION IMPORTANTE À L'USAGE DES UTILISATEURS</b></p> <p>Le présent manuel d'utilisation explique la manière de se servir efficacement du produit en conditions de sécurité. Il est recommandé de soigneusement lire la totalité du manuel, avec ses consignes et ses instructions.</p> <p> Le triangle avec point d'exclamation est un symbole destiné à avertir l'utilisateur du produit de l'importance de certaines exigences relatives au fonctionnement, à l'entretien et/ou à la garantie.</p> <p> Le triangle avec flèche en zigzag est un symbole destiné à avertir l'utilisateur du produit de la possibilité d'exposition à des décharges avec danger de secousses électriques.</p> <p> Tout manquement à l'observation des consignes et des instructions peut exposer les personnes et les biens à des dommages corporels et/ou matériels et peut annuler toute garantie. Il faut toujours interrompre l'alimentation de courant continu avant de déconnecter les cordons d'alimentation du produit. Déconnecter d'abord les cordons d'alimentation branchés sur la source de tension (alimentation de secteur) puis ceux branchés sur le produit. Pour une sécurité maximum, il faut toujours faire fonctionner ce système dans un lieu isolé, peu fréquenté, où le personnel non autorisé n'a pas accès. Ne jamais faire fonctionner un matériel endommagé ou affecté par des fuites.</p> <p><b>GARANTIE ET RESPONSABILITÉ</b></p> <p>Le produit a été fabriqué conformément aux normes applicables les plus exigeantes en matière de matériaux, de main d'œuvre, de conception et d'ingénierie. C.B.S. Scientific garantit que le produit a subi des essais et que ses performances rempliront les conditions des spécifications publiées ou leur seront même supérieures. La présente garantie n'est valide que si le produit a fonctionné et a été entretenu conformément aux consignes et instructions fournies.</p> <p>C.B.S. 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Sírvase leerlo en su totalidad y seguir detenidamente las indicaciones que contiene.</p> <p> El símbolo del triángulo con exclamación llama la atención del usuario a requisitos importantes para el uso y mantenimiento del producto, así como para la validez de la garantía.</p> <p> El símbolo del triángulo con rayo llama la atención del usuario a la posibilidad de riesgos eléctricos.</p> <p> El incumplimiento de las instrucciones aquí señaladas podría dar lugar a riesgos a la persona, al laboratorio o a ambos y podría anular toda garantía. Siempre apague la fuente de corriente continua antes de desenchufar los cables eléctricos del producto. Primero desconecte los cables de la fuente de energía y después del producto. Para mayor seguridad, siempre use este sistema en un área aislada, de poco movimiento de personas e inaccesible a personal no autorizado. Jamás use equipo que presenta algún daño o fuga.</p> <p><b>GARANTÍA Y RESPONSABILIDAD</b></p> <p>Este producto fue fabricado de acuerdo con las normas más estrictas que sean factibles en cuanto a materiales, mano de obra y diseño. C.B.S. Scientific garantiza que se sometió el producto a pruebas y que cumplirá o excederá las especificaciones publicadas. Esta garantía será válida únicamente si se usa y se da servicio de mantenimiento al producto de acuerdo con las instrucciones señaladas.</p> <p>C.B.S. Scientific garantiza que este producto se encontrará libre de defectos de materiales y mano de obra por un periodo de servicio normal de un año a partir de la fecha de embarque. Si el producto resulta defectuoso durante este periodo, C.B.S. Scientific lo reparará o lo repondrá, a criterio de C.B.S., libre de cargos, si se devuelve el producto a C.B.S. porte pagado. 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Um höchste Sicherheit zu gewährleisten sollte dieses System in einem absonderten und besonders ruhigen Bereich eingesetzt werden und vor Unbefugten sicher sein.</p> <p><b>GARANTIE UND HAFTUNG</b></p> <p>Dieses Produkt wurde unter Anwendung von Produkten mit höchster Qualität und aus Materialien mit bester Verarbeitung und modernstem Design hergestellt. C.B.S. Scientific garantiert, daß das Produkt getestet wurde und alle publizierten Spezifikationen übertrifft. Diese Garantie ist jedoch nur gültig, wenn das Produkt nach der beigefügten Bedienungsanleitung bedient und gewartet wurde.</p> <p>C.B.S. Scientific garantiert, daß dieses Produkt bei normaler Bedienung aus fehlerfreiem Material besteht und fehlerfrei in der Ausführung ist. Diese Garantie gilt für ein Jahr ab Lieferdatum. Sollte das Produkt in diesem Zeitraum fehlerhaft werden, bietet C.B.S. Scientific eine kostenlose Reparatur bzw. kostenlosen Ersatz, einschließlich freiem Rückporto. 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Si preghi di leggere e seguire con cautela le istruzioni di ogni parte di questo manuale.</p> <p> Il triangolo contenete il simbolo di un punto esclamativo avverte l'utente di importanti requisiti relativi al funzionamento, manutenzione e/o garanzia del prodotto.</p> <p> Il triangolo contenete il simbolo di un lampo avverte l'utente del prodotto della possibilità di pericoli dovuti a corrente elettrica.</p> <p> La mancata osservanza delle istruzioni può essere causa di pericolo alla propria persona ed al laboratorio, oltre a poter annullare la garanzia. Prima di distaccare il cordone d'alimentazione dal prodotto, spegnere sempre la sorgente di corrente continua. Distaccare i cordoni d'alimentazione prima dal lato della sorgente di tensione e poi dal lato del prodotto. Per maggior sicurezza, mettere sempre in funzione il prodotto in un'area isolata con poco traffico che non sia accessibile al personale non autorizzato. Non mettere mai in funzione un'apparecchiatura che sia danneggiata o abbia perdite.</p> <p><b>GARANZIA E RESPONSABILITÀ</b></p> <p>Questo prodotto è stato fabbricato seguendo gli standard più elevati per i materiali, la manodopera e la progettazione. La C.B.S. Scientific garantisce il prodotto è stato sottoposto a prova e raggiunge o supera i valori pubblicati per i dati tecnici. Questa garanzia è valida solo se il prodotto è messo in esercizio e soggetto a manutenzione secondo le istruzioni fornite.</p> <p>La C.B.S. Scientific garantisce che questo prodotto è libero di difetti di materiali e manodopera, in normali condizioni d'esercizio, per la durata di un anno dalla data di spedizione. Se, in questo periodo, il prodotto si dimostrerà difettoso, la C.B.S. Scientific, a suo giudizio, lo riparerà o sostituirà. 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# SECTION 1

## General Information

### 1.1 Introduction

The C.B.S. Scientific Semi-Dry Blotting Systems will transfer Western, Northern, or Southern blots reliably and quickly. These blotting systems have transfer capabilities for gel dimensions up to 20cm x 20cm (Cat. # EBU-4000) or 35cm x 45cm (Cat. # EBU-6000). The stainless steel cathode and platinum-coated titanium anode are durable and corrosion-resistant. These electrodes create a uniform electric field producing excellent transfers onto nitrocellulose, supported nitrocellulose, nylon, charged nylon or activated paper substrates. The upper electrode housing is separate from the base, allowing it to be secured at the desired height with side hand screws. This unique design permits several gels to be transferred simultaneously.

In the Semi-Dry system, blotting is accomplished by layering transfer membrane and gel between buffer saturated blotting paper, minimizing the amount of buffer required. To prevent drawing current away from the gel during a transfer, the uncovered portion of the cathode is shielded with a Mylar® mask. This shield insures that all applied current passes directly through the gel. Use of the mask will increase transfer efficiency and reduce transfer times up to 50%.

### 1.2 Specifications

#### Constructions:

Buffer chamber, safety cover	Acrylic
Electrode panels	Stainless steel, platinum-coated titanium
Electrode support panel	Styrene
Power cords	FR Urethane rated 7500VDC @ 200mA, 65°C
Mylar masks	Mylar

	EBU-4000	EBU-6000
Shipping weight	7lbs	15lbs
Overall Size (l)x(w)x(h) cm	9 ¾ x 9 ¾ x 2 ¾	19 ¾ x 17 x 2 ½
Maximum Gel Size - cm	20cm x 20cm	35cm x 45cm
Distance between electrodes -cm	1cm	1cm

### 1.3 Safety



Power to the Semi-Dry Blotter is to be supplied by an external DC voltage power supply that must be ground isolated so that the DC voltage output floats with respect to ground. For any power supply used, the maximum specified operating parameters for the units are:

#### Maximum Limits

EBU-4000  
50 VDC  
500mA current  
55°C ambient temperature

EBU-6000  
50 VDC  
1200mA current  
55°C ambient temperature



Current to the unit, provided from the external power supply, must enter the unit through the lid assembly, providing a safety interlock to the user. Current to the unit is broken when the lid is removed. **Do not attempt to use the unit without the safety lid, and always turn the power supply off before removing the lid, or when working with the unit in any way. Follow safety precautions specified by the power supply manufacturer.**

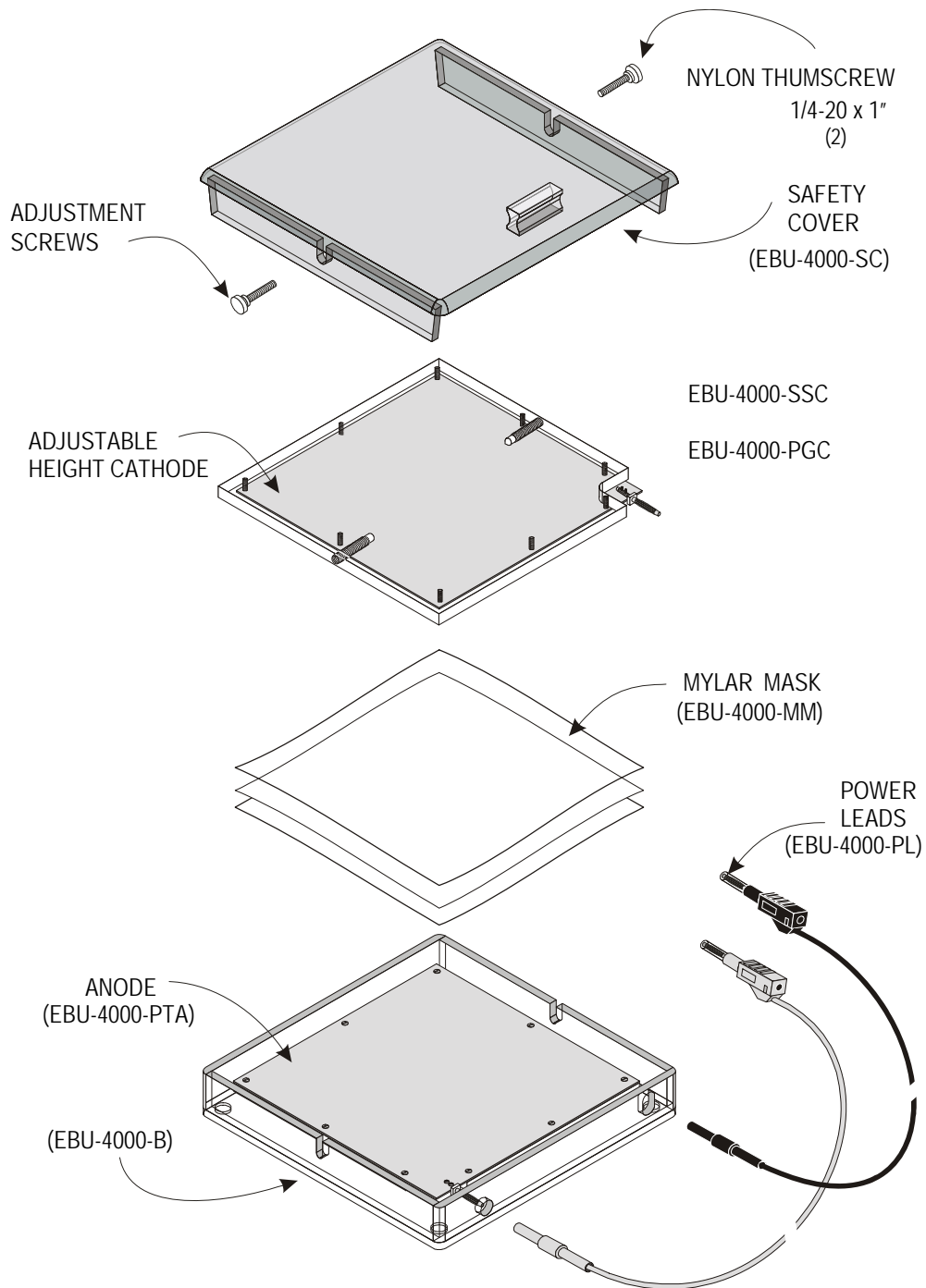
## Section 2 Description of Parts

### 2.1 Unpacking the EBU-4000 or EBU-6000

Please verify that your unit comes complete with the following components:

- Blotting chamber with stainless steel cathode, and platinum-coated anode
- Safety cover with power leads
- Mylar sheets (for shielding uncovered portion of anode)

### 2.2 Components/Assembly



## SECTION 3

**Instructions for Use** --- The instructions are divided into the following three categories:

- A. General Considerations
- B. Semi-Dry Electro-Blotting of DS DNA from Agarose Gels (Southern)
- C. Semi-Dry Blotting of Proteins (Western)
- D. Semi-Dry Blotting of RNA (Northern)

### 3.1 Blotting Unit Preparation

Place the blotting chamber on a level work surface in an authorized work area.

#### A. General Considerations

- Because transfer efficiency depends on many factors (e.g. gel concentration and thickness, size, shape and net charge of molecule) results may vary. For resolving gels, the % acrylamide range from 8 to 10% will separate with close to 100% efficiency the transfer of 14 – 66 kD.
- Polyacrylamide gels have pore sizes that are too small for effective capillary diffusion of DNA. These types of gels must be blotted electrophoretically requiring a transfer buffer of low ionic strength (0.5 to 1X TBE). This is why nylon membranes are generally used (uncharged nylon being preferable to positively charged nylon). Nylon is the membrane of choice because it will bind DNA fragments as small as 50bp and can be irradiated to covalently linking the DNA to the membrane.
- The general “rule of thumb” for electrophoretic transfer in semi-dry blotting units is application of a maximum current of  $0.8\text{mA}/\text{cm}^2$  of gel area. For gels used in the EBU-4000 (15x15cm up to a max. of 20x20cm) this translates to upwards of 300mA. In the larger EBU-6000, this can increase to 1200mA. Please ensure that an adequate power supply is being used to power the transfer.
- In general, most runs should take between 15 to 60 minutes depending on the size and type of the gel and they type of transfer, i.e., Southern, northern or western.
- After setting the current at mA range, one can usually switch to the voltage panel to obtain the voltage being applied. Most power supplies allow you to switch back and forth between the amperage and voltage.

<u>Percent of acrylamide (resolving gel)</u>	<u>Size range transferred (approx. 100% efficiency)</u>
5-7	29-150 kD
8-10	14-66 kD
13-15	<36 kD
18-20	<20 kD



## B. Semi-Dry Electro-Blotting of DS DNA from Agarose Gels (Southern)

### 1. Preparation for Southern Blotting

- a. Agarose gels 20cm x 20cm and up to 6mm thick may be run in this system. Thicker gels require longer transfer times. Prior to transfer, gels should be stained with Ethidium Bromide. Ethidium bromide is a Mutagen. **ALWAYS** wear gloves. Dispose of solutions in accordance with the safety regulations of your institution.
- b. Optionally, depurinate the gel by soaking in 0.25M HCl for 20 minutes. Depurination can increase transfer efficiency. To denature the DNA in the gel before transfer, soak in 0.5M NaOH, 1.0M NaCl for 20 minutes. Neutralize 2X for 15 minutes in 0.5M Tris-HCl, pH 7.4, 1.5M NaCl.
- c. Membrane selection: Nylon or nylon supported nitrocellulose. **ALWAYS** use forceps to handle membranes. If nylon is not base resistant, denature DNA prior to transfer. If it is, you may base wash the DNA after transfer instead of denaturing before. Base wash for 30 min. in 0.1N NaOH followed by suspension in 0.2M Tris-base, pH 8.0, 0.5% SDS for 30 minutes to neutralize the membrane.



### 2. Preparation of the Transfer Materials

- a. Required Materials: Membrane, cut to exact size of gel; saturate in ddH<sub>2</sub>O for ten minutes, then in 0.1X TAE or 0.3X TBE for 20 minutes.
- b. Gels should be soaked in running buffer, 0.1XTAE or 0.3XTBE for 20-60 minutes depending on thickness (3-6mm).
- c. Cut eight sheets of 3mm blotter paper (Whatman™) the exact size of the gel. Saturate all eight sheets in running buffer.
- d. Mylar Mask: Prepare a mask by cutting a rectangular hole that is slightly smaller (1-2mm) than the gel dimensions.
- e. For transfer of ssDNA (acrylamide): use 0.5 –1X TBE buffer. Equilibrate in transfer buffer for 15 minutes.

### 3. Stacking of components into Electro-Blotter Apparatus:

- a. Insert the mylar mask over the bottom platinum coated titanium electrode (anode +). **Note:** The bottom electrode is the anode and is fixed in place, while the cathode is adjustable and within the lid of the apparatus. Place 3-4 pieces of saturated filter paper carefully centered over the cut-out. Flatten the filter paper by rolling a glass rod over the surface.
- b. Apply a saturated membrane on the top of the paper.
- c. Carefully place the gel on the membrane, making sure no bubbles remain between the gel/membrane interface.
- d. Stack the remaining 3-4 sheets of blotting paper on the top of the gel. Add one sheet at a time and be sure to 'smooth' with glass rod to remove air bubbles.
- e. Close the lid which contains the cathode (-). Holding the two white nylon thumbscrews, place the lid over the stack and secure while applying some downward pressure (gently!).

### 4. Power Setting

Connect the unit to the power supply using the power cords supplied. **Warning:** C.B.S. has designed this device such that it cannot be opened unless the power cords are disconnected. DO NOT attempt to open the transfer apparatus without disconnecting the power cords and shutting off the power supply.

- a. Transfer DNA of agarose gels (Southern) requires some special precautions. Excessive heat build-up can melt agarose if too much power is applied. To avoid this, try to use higher percentage gels made from high melt agarose.
  - b. With this type of agarose, run at low current (100-200mA for 15-30 minutes, depending on the gel thickness).
  - c. If you are using low melt agarose or low percentage agarose, use 50mA for 45-60 minutes.
5. Single stranded DNA from 6% Acrylamide
    - a. 100-125mA @ constant current for 45-60 minutes (0.5-1XTBE)

## B. Semi-Dry Blotting of Proteins (Western)

1. Preparation for Western Blotting
  - a. Acrylamide gels 20cm x 20cm and up to 3mm thick may be transferred in these systems.
  - b. Buffer System
 

192mM Glycine	Towbin reference
25mM Tris, pH 8.3	
0.0013M SDS	
10-20% MeOH	
39mM Glycine	Maniatis reference
48mM Tris, pH 8.3	
0.0375% (w/v) SDS	
20% (v/v) Methanol	
  - c. If the gel is to be stained with Coomassie Blue prior to blotting, refer to Perides, et. al. (1986) for an alternate protocol before blotting.
2. Prior to completion of SDS-PAA electrophoresis:
  - a. Prepare a Mylar™ mask by cutting a rectangular hole that is slightly smaller (1-2mm) than the gel dimensions.
  - b. Prepare 8 pieces of Whatman 3mm chromatography paper the exact size of the gel. Larger pieces of filter paper may promote a 'short circuit' by allowing current to bypass the gel and therefore reduce the efficiency of the transfer. Soak the filter paper in transfer buffer until saturated. The 'ready for use' thickness should be around 1mm (dry paper is approximately .35mm).
  - c. Hydrate the Nitrocellulose by first floating in a tray of de-ionized water (capillary absorption) followed by 5 minutes of total immersion.
3. Stacking of components into Electro-Blotter Apparatus
  - a. After SDS-PAA electrophoresis of proteins, separate the gel plate sandwich and briefly soak the gel in a tray of de-ionized water. Remove the stacking gel with a spatula or razor blade and discard.
  - b. Wet the anode and cathode plates by wiping with a lint free paper towel. No puddles should be left in the unit.
  - c. Insert the Mylar mask over the bottom platinum coated titanium electrode (anode +). **Note:** The bottom electrode is the anode and is fixed in place, while the cathode is adjustable and within the lid of the apparatus. Place 3-4 pieces of saturated filter paper carefully centered over the cutout. Flatten the filter paper by rolling a glass rod over the surface.

- d. Apply a saturated membrane on the top of the paper.
  - e. Carefully place the gel on the membrane, making sure no bubbles remain between the gel/membrane interface.
  - f. Stack the remaining 3-4 sheets of blotting paper on the top of the gel. Add one sheet at a time and be sure to 'smooth' with glass rod to remove air bubbles.
  - g. Close the lid which contains the cathode (-). Holding the two white nylon thumbscrews, place the lid over the stack and secure while applying some downward pressure (gently!).
4. Power Settings
- a. Calculate power input by using 0.8mA/sq. cm of gel for larger gel transfers. Mini-gels (10 x 11cm) may be transferred at up to 100mA (5-25V) total current. Remember, smaller proteins (<20kd) require less time than medium size (<80kd) or larger proteins.
  - b. At the conclusion of the run, turn power supply off and disconnect leads from blotter. Cover will not release unless power cord is removed. Remove the cover slowly to catch any part of the gel sandwich sticking to the cathode. After each use, clean the electrodes of buffer salts by rinsing in distilled water.

### C. Semi-Dry Blotting of RNA (Northern)

1. Electro-Blotting RNA
  - a. Buffer System:
    - 1X TAE (40mM Tris-acetate, 1mM EDTA, pH8.0)
    - 4.84g Tris-base
    - 1.14ml glacial acetic acid
    - 0.37g Na<sub>2</sub>EDTA-2H<sub>2</sub>O
    - pH to 8.0 with HOAC

or

    - 1XTBE: (89mM Boric Acid, 2.5mM EDTA, pH 8.4)
    - 10.8g Tris-base
    - 5.5g Boric Acid
    - 0.93g Na<sub>2</sub>EDTA-H<sub>2</sub>O
    - H<sub>2</sub>O to 1 liter
2. Preparation of the Gel
  - a. Formaldehyde must be removed from the gel by soaking in 0.1XTAE for at least an hour at room temperature.
  - b. Standard electrophoresis buffers for RNA are usually 1XTAE or 1XTBE. Electro-blotting transfer occurs in low ionic strength buffer. Exchange the 1X electrophoresis buffer by soaking in 0.1X buffer for 20-30 minutes.



3. Preparation of the Apparatus

- a. Prepare 10 sheets of blotting paper cut to the same size of the gel and soak in 0.1XTAE. Cut a nylon membrane to the same size and wet in ddH<sub>2</sub>O for 5 minutes followed by equilibrate in 0.1XTAE for 20-30 minutes.
  - b. Prepare one of the clear plastic Mylar sheets (provided in the kit) by cutting a hole in the sheet which is 1-2mm smaller than the gel dimension.
  - c. Insert the Mylar mask over the bottom electrode anode. Place 5 pieces of saturated filter paper carefully centered over the cut-out in the Mylar mask. If prepared correctly, the filter paper will overlap the mask cut-out slightly. Flatten the filter paper by rolling a glass rod over the surface. Apply the equilibrated Nylon membrane to the stack of filter paper followed by the gel and 5 more pieces of saturated filter paper. Again roll a glass rod over the filter paper stack to compress and remove trapped air bubbles. The lid of the apparatus containing the cathode should be placed directly over the stack. While holding the two white nylon thumb screws, place the lid over the stack and secure while applying some downward pressure.
4. Electro-blotting transfer
- a. Once the lid is in place, insert the power cords into the apparatus. Plug the ends of the power cords into the Power Supply.
  - b. Set the Power Supply at 250mA and transfer for 30 minutes at constant current.
  - c. After the run, turn off the power supply and disconnect the power cords from the apparatus. The lid cannot be lifted unless the power cord is unplugged from the anode. Carefully lift the lid and watch for filter paper sticking to the top electrode. Remove the Nylon membrane and process according to protocol. Rinse the inside of the apparatus and the mask with ddH<sub>2</sub>O.

## SECTION 4

### References

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## SECTION 5 Maintenance of Equipment

### 5.1 Care and Handling



The plastic components of the Semi-Dry Blotting units are fabricated from acrylic and polycarbonate. Electrodes and connectors are made from pure platinized titanium, and stainless steel. As with any laboratory instrument, adequate care ensures consistent and reliable performance.

After each use, rinse with de-ionized water. Wipe dry with a soft cloth or paper towel, or allow to air dry. Whenever necessary, all components may be washed gently with water and a non-abrasive detergent, and rinsed and dried as above. *Never* use abrasive cleaners, glass cleaning sprays or scouring pads to clean the components, as these will damage the unit and components.

Additional precautions:

- Do not autoclave or dry-heat sterilize the apparatus or components.
- Do not expose the apparatus or components to phenol, acetone, benzene, halogenated hydrocarbon solvents or alcohols.
- Avoid prolonged exposure of the apparatus or components to UV light.
- Do NOT treat with diethylpyrocarbonate (DEPC)-treated water for extended periods at 37°C.
- Electrostatic charge and heat affix the conductive platinum surface covering the titanium. It can be scratched off or damaged by using sharp objects or scouring pads. This will disturb the continuity of the electric field and have a negative effect on transfers.
- The stainless electrode can be permanently damaged by corrosion if the polarity between anode and cathode is reversed. CAUTION: Be certain of power connections before initiating transfer.

### 5.2 Maintenance

The following inspection and maintenance procedures will help maintain the safety and reliable performance of the Blotting systems. Replacement parts can be ordered by calling 1-858-755-4959 or by contacting your local distributor.



- Banana plugs and power cords should be inspected regularly. If the banana plugs become loose or do not feel friction tight replace the plugs or power cords.
- Should power cord assemblies (connectors, wire or shrouds) show any signs of wear or damage (e.g. cracks, nicks, abrasions, or melted insulation), replace them immediately.
- The platinum wire is secured to the banana jack by compression between a stainless washer and the jack nut. The nut/washer interface should be tight and free of corrosion.

**SECTION 6**  
**Equipment and Accessories**

<b>Cat. #</b>	<b>Item</b>
<b>EBU-4000</b>	<b>Semi-Dry Blotting System.</b> Includes power leads and safety interlock, 20cm x 20cm.
<b>EBU-6000</b>	<b>Semi-Dry Blotting System.</b> Includes power leads and safety interlock, 35cm x 45cm.
MM-4000	Mylar® Mask for EBU-4000, package of 4 each
MM-6000	Mylar® Mask for EBU-6000, package of 6 each

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## NOTES

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# CONTACT US



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