

INSTRUCTION MANUAL

ELECTROPHORETIC BLOTTING SYSTEM

EBU-204

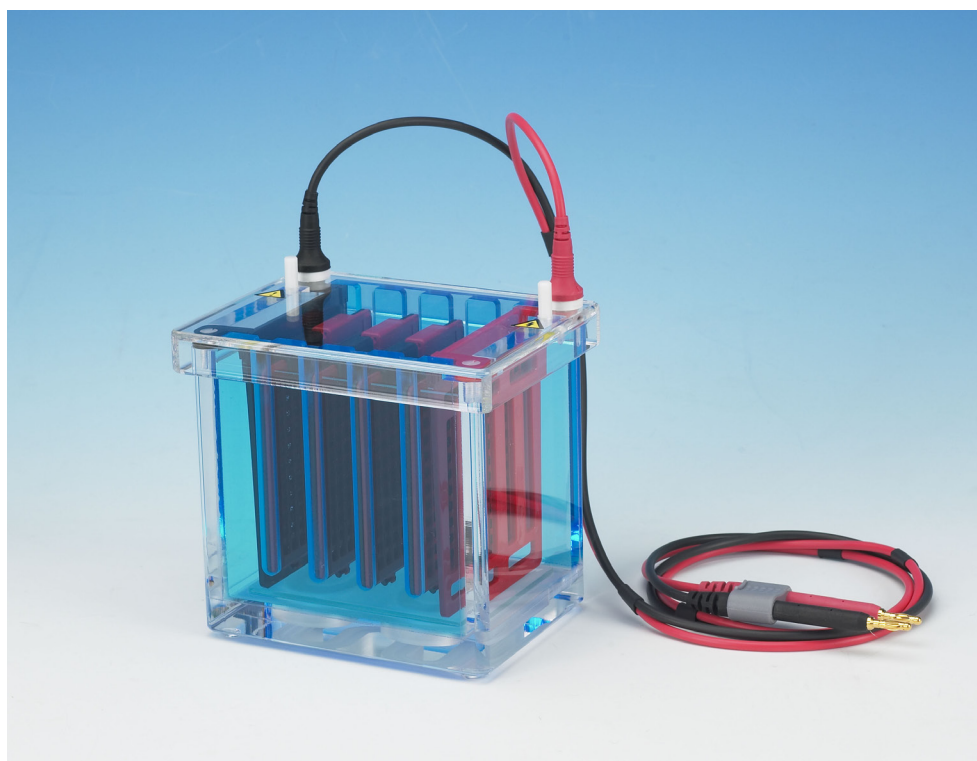


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IMPORTANT USER INFORMATION

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>FRANÇAIS INFORMATION IMPORTANTE A L'USAGE DES UTILISATEURS</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>GARANTIE ET RESPONSABILITÉ</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. Scientific garantit que le produit sera dépourvu de vices de matériaux et de main d'œuvre, en conditions de service normales, pendant un an à compter de la date d'expédition. Au cas où le produit s'avérerait défectueux pendant cette période de garantie, C.B.S. Scientific réparera ou remplacera le produit, à sa discrétion et gratuitement, si le produit lui est retourné port payé d'avance. La garantie ne couvre pas les dommages de transport; les dommages causés par l'imprudence, le manque de soins, l'abus ou la négligence; l'usure normale résultant d'une utilisation fréquente; les dommages causés par la corrosion des solvants; et les dommages causés par la manipulation inadéquate ou des changements apportés par l'utilisateur. La garantie ne couvre pas non plus les performances non satisfaisantes résultant de conditions hors du contrôle de C.B.S. Scientific. C.B.S. Scientific ne pourra en aucun cas être tenue responsable de dommages indirects, y compris, de manière non limitative, la perte de bénéfices, le manque à gagner, la perte d'occasions d'affaires, l'impossibilité d'usage ou tous autres dommages associés, quelle qu'en soit la cause, ni de dommages résultant de l'usage incorrect du produit.</p>	<p>ESPAÑOL INFORMACIÓN IMPORTANTE PARA EL USUARIO</p> <p>El presente instructivo explica la manera de usar este producto en forma segura y efectiva. 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>GARANTÍA Y RESPONSABILIDAD</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 período de servicio normal de un año a partir de la fecha de embarque. Si el producto resulta defectuoso durante este período, C.B.S. SCIENTIFIC lo reparará o lo repondrá, a criterio de C.B.S. SCIENTIFIC, libre de cargos, si se devuelve el producto a C.B.S. SCIENTIFIC porte pagado. 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Um höchste Sicherheit zu gewährleisten sollte dieses System in einem abgesonderten und besonders ruhigen Bereich eingesetzt werden und vor Unbefugten sicher sein.</p> <p>GARANTIE UND HAFTUNG</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>GARANZIA E RESPONSABILITÀ</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

Electroblotting is a faster, easier, and more efficient method to permanently transfer PAGE gels to nitrocellulose and nucleic acids to nylon, than capillary (passive diffusion) blotting. For a similar transfer, traditional “capillary blotting” can take 6-24 hours to complete compared to only 45 to 90 minutes or less using electroblotting. The C.B.S. Scientific Electrophoretic Blotting System comes equipped with; two electrode panels, four color-coded gel cassette assemblies with sponge pads, electrophoresis tank with cooling chamber and safety cover with attached power leads. The EBU-204 gel cassette will accept gel sizes up to 10 x 10cm and can transfer up to 4 gels at one time.

1.2 Specifications

Constructions:

Buffer chamber, safety cover	Acrylic
Electrode panels	Polycarbonate and platinum wire .012” diameter
Power cords	FR Urethane rated 7500VDC @ 200mA, 65°C
Gel cassette	Glass filled polycarbonate panels- red and black, and one sponge pad, polyether
Safety Certification	EN61010-1-1993 (IEC1010-1)

	EBU-204
Shipping Weight	5 lbs.
Overall Size (l)x(w)x(h) cm	14 x 13 x 16
Maximum Gel Size – cm	10 x 10
Distance between electrodes –cm	10
Recommended buffer volume	1.2 L

1.3 Safety



Power to the Electro-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

- 100 VDC
- 150 watts power
- 1500mA current
- 50°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

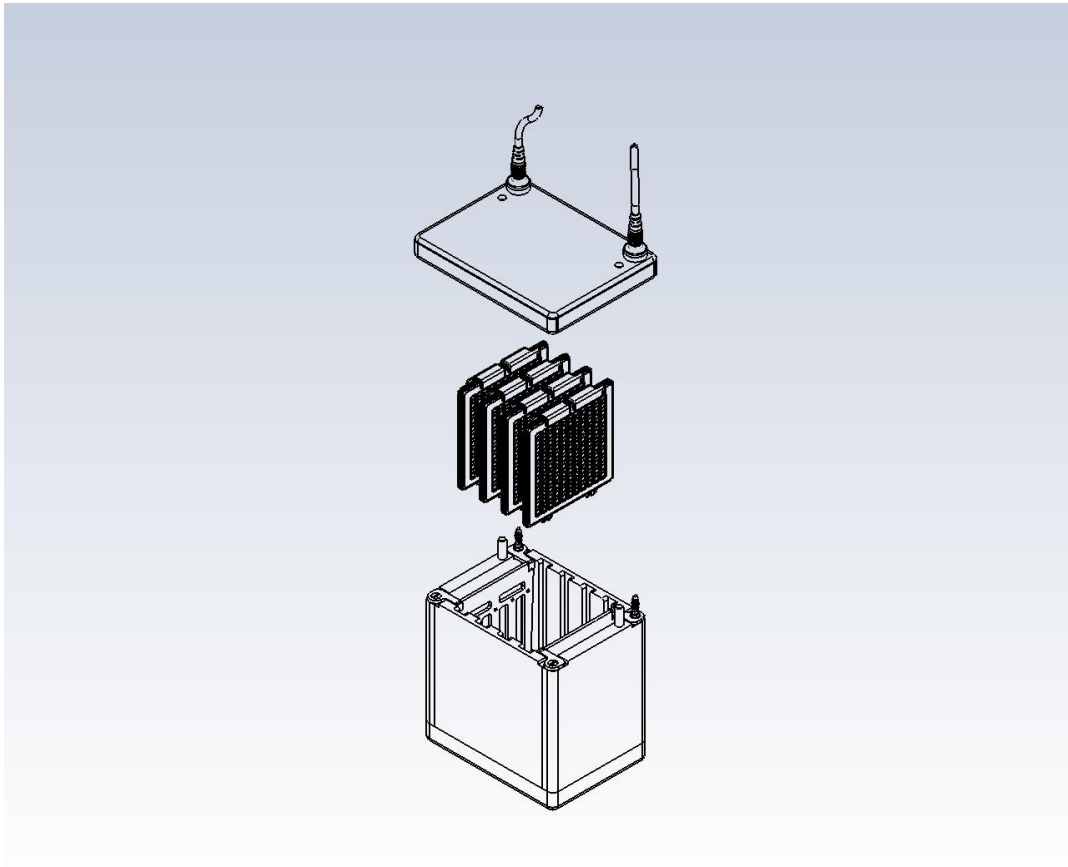
Description of Parts

1.1 Unpacking the EBU-204

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

- ◆ Blotting chamber
- ◆ Four Blotting cassettes with sponge pads
- ◆ Safety cover with attached power leads
- ◆ 2 electrode panels
- ◆ Tubing adapters for cooling base

2.2 Components/Assembly



SECTION 3

Instructions for Use

3.1 Blotting Unit Preparation

1. Place the blotting chamber on a level work surface in an authorized work area.
2. Consult applications table 4.1 for type of transfer, buffer system, membrane type and power settings.

Coolant Circulation:

For coolant circulation, connect a heavy wall tubing which will not kink (hose clamps are optional), to the tubing adapters supplied with the unit. Excessive pressure will damage the plexiglass in the base and create leaks. Attach a regulated pump or recirculating water bath (follow manufacturer's instructions), recommended flow is 500 ml per minute or 15psi. **Do NOT** use tap or house water as it normally delivers too much pressure and can be subject to large fluctuations in pressure.



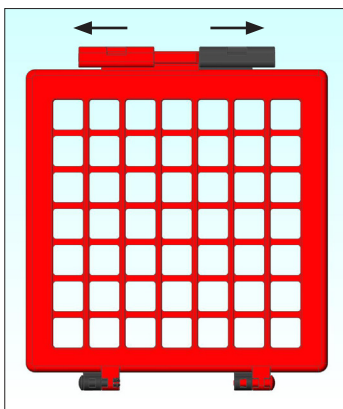
1. Place, do NOT drop, a magnetic spin bar (not supplied by C.B.S.) in the bottom of the tank. This maintains pH, buffer and heat circulation during the electroblot.
2. Place tank on top of the magnetic stirrer.
3. Connect to a cold water supply (see coolant circulation above).
4. Buffer should be pre-cooled to 10°C.

3.2 Preparing the Gel for Transfer

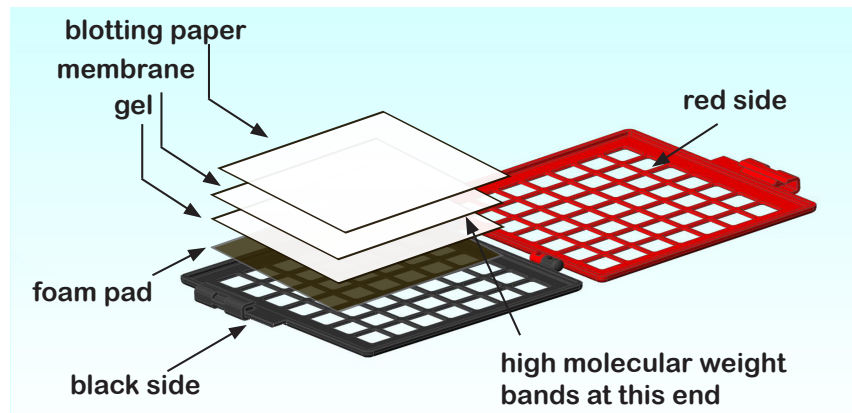
The EBU-204 can hold up to 4 gel cassettes simultaneously with space provided for a magnetic stir bar to allow buffer circulation and heat exchange for uniform transfers. When the resolving gel electrophoresis is complete, proceed with staining and photo-documentation if applicable. Cut one corner off the gel so that correct orientation is maintained throughout the procedure.

Preparation of Electroblotting Components

1. CBS membranes are supplied with the blotting paper cut to the correct size for CleaPAGE gel transfers. If not using CBS membranes, cut transfer membrane to size of gel or sufficient dimensions to cover the relevant bands. Also, pre-cut Whatman-type 3MM filter paper to same size and soak in transfer buffer until completely saturated (15 to 30 minutes).
2. Pre-wet membrane chosen according to manufacturer's instructions. Nylon or Nylon-supported nitrocellulose should be soaked in ddH₂O. PVDF in MeOH. Equilibrate all types in transfer buffer for 3 minutes. Submerge a single foam pad and gently tease to release trapped air bubbles until pad is completely saturated.
3. Pre-equilibrate gel in 1x transfer buffer to be used prior to electroblotting for 5 to 30 minutes depending on gel thickness.
4. Open the transfer cassette as shown below and lay it flat on the bench.



5. Assemble blotting stack as shown below. With cassette wide open assemble components on black side in the following order: buffer saturated sponge pad, gel equilibrated in transfer buffer, buffer saturated transfer membrane, then buffer saturated blotting paper. Smooth with gloved finger or roll with glass rod to be sure no bubbles exist between the gel and the transfer membrane.



6. Insert blotting cassettes into the lower reservoir. Red side of cassette should face toward red electrode panel.

3.3 Standard Electro-Blot Transfer

1. Fill the chamber with transfer buffer. (Buffers should be prepared fresh with reagent grade chemicals and pre-cooled). The transfer buffer should come up to the top of the platinum labyrinth. The buffer should not come in contact with the banana plugs when the gel cassette sandwich(es) are immersed in the unit.
2. Align safety cover over the unit and carefully attach. Begin cycling of coolant.
3. Connect the leads to the power supply, matching the color-coded (+) red to red and (-) black to black. **See Section 4.1 for recommended power conditions.**
4. Transfer times will vary according to several parameters. Optimization of electro-blotting transfers must be determined empirically. Keep in mind the following principles that govern the movement of molecules of gel electrophoresis:
 - ♦ Thicker or higher percentage gels will take longer to transfer than thinner or lower percentage gels.
 - ♦ Large molecules will need extended transfer times to completely transfer.
 - ♦ Actual transfer times for defined conditions can be approximated by running molecular weight standards.

3.4 Removing the Cassettes



1. Turn the power supply off and disconnect the leads from the power supply.
2. Remove the safety cover from the unit, by placing thumbs on white posts next to red & black connectors, then pushing down while pulling up with fingers under lid. **DO NOT pull on power cords.**



3. Gently lift the cassette from the unit. **Always wear gloves, eye protection and protective clothing** if buffer and/or gel contain Ethidium Bromide. Ethidium Bromide is a powerful mutagen; gloves, eye protection and protective clothing should always be worn when handling the gel or buffer solutions. See Material Data Safety Sheets.
4. Mark the orientation of the membrane with a pencil or by cutting off a corner and take apart the cassette carefully.
5. Process membrane according to type of transfer and manufacturer's recommendations.

SECTION 4

Applications & Running Conditions for Tank Type Electro-Blotting

4.1 Recommended Buffers, Membranes, Power Settings and Transfer Times

Gel Type	Blotting Buffer	Transfer Membrane	Power
denaturing: SDS-PAGE (Western)	ClearPAGE Transfer Buffer cat. # FB82500 as follows: for 1:20 dilution use 50ml ClearPAGE Transfer buffer, 200 ml Methanol and 770ml Ultrapure water ("Towbin": 25mM Tris/192mM Glycine/ pH 8.3, Methanol 20% (w/v)	nitrocellulose/nylon PVDF: ion-exchange	<ul style="list-style-type: none">◆ room temperature, 70V-100V, 200mA, 1.5- 4 hours◆ 4°C, 180V, @160mA, 1.5- 2 hours◆ Overnight, 30V, 75mA
non-denaturing: SDS-PAGE (acidic or neutral proteins)	25mMTris/192mM Glycine, pH 8.3 25mM sodium phosphate, pH6.5 15mM sodium borate, pH9.2	nitrocellulose; nylon PVDF; ion-exchange diazo-paper diazo-paper	<ul style="list-style-type: none">◆ room temperature, 60V, 200mA, 4 hours◆ 4°C, 125V, 400mA, 1.5 hours◆ Overnight, 30V, 100mA
IEF (native- basic proteins, acid urea gels)	0.7% acetic acid (w/v)*	nitrocellulose; nylon diazo-paper	<ul style="list-style-type: none">◆ room temperature, 70V, 400mA, 4 hours◆ 4°C, 100V, 600mA, 1.5 hours◆ Overnight, 30V, 200mA
DNA (Southern)	1XTAE or agarose 0.5X TBE, acrylamide	nylon; +charged nylon, neutral or +charged	<ul style="list-style-type: none">◆ room temperature, 30- 40V, 25-150mA, 2-4 hours◆ 4°C, 125V, 400mA, 1.5 hours
RNA (Northern)	19.6mM phosphate/5.4mM citrate pH3.0 or 25mM sodium phosphate pH 6.5	nylon nitrocellulose	<ul style="list-style-type: none">◆ room temperature, 60V, 200mA, 4 hours◆ 4°C, 125V, 400mA, 1.5 hours◆ Overnight, 30V, 200mA

4.2 References

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2. Burnette, W.N. (1981). Western blotting: Electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. *Anal. Biochem.* 112:195-203.
3. Peluso, R.W. and Rosenberg, G.H. (1987). Quantitative electrotransfer of proteins from sodium dodecyl sulfate polyacrylamide gels onto positively charged nylon membranes. *Anal. Biochem.* 162:389-398.
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6. Towbin, H., Staehelin, T., and Gordon, J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc. Natl. Acad. Sci. U.S.A.* 76: 4350-4354.
7. Sambrook, J., Fritsch, E.F., Maniatis, T. (1989). *Molecular Cloning. A Laboratory Manual*. 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. 18.47-18.61.

SECTION 5 Maintenance of Equipment

5.1 Care and Handling



The plastic components of the Electrophoretic Blotting units are fabricated from cell cast acrylic and polycarbonate. Electrodes and connectors are made from pure platinum, stainless steel, and chrome plated brass. As with any laboratory instrument, adequate care ensures consistent and reliable performance.

After each use, rinse buffer chamber, cassettes and sponge pads 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 undiluted 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. A brief rinse with DEPC-water is sufficient after a thorough wash, followed by a quick rinse in 70% ethanol.
- ◆ 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

<u>Cat. #</u>	<u>Item</u>
EBU-204	Mini-Electrophoretic Blotting system , 4 gel cassette capacity. Includes electrophoresis chamber with cooling base and four 10 x 10cm cassettes. Removable platinum electrode panels, safety cover and interlocking power leads.
EBX-BC-700	Accessory Blotting Cassette, includes foam pad
EBX-SP-700	Accessory foam pads, set of 4
EBX-EA-700	Replacement Anode
EBX-EC-700	Replacement Cathode

POWER SUPPLIES

<u>Cat. #</u>	<u>Item</u>
EPS-300-II	Mini Power Supply with timer, CE, CV or CC 10-300, 110V/50-60Hz. Current range: 4-500mA, 90 Watts
EPS-300-IIV	Mini Power Supply with timer, CE, CV or CC 10-300, 220V/50-60Hz. Current range: 4-500mA, 90 Watts
EPS-200-II	High Current Mini Power Supply with timer, CE, CV or CC 5-200 Volts, 110V/50-60Hz. Current range: 2000mA, 200 Watts
EPS-200-IIV	High Current Mini Power Supply with timer, CE, CV or CC 5-200 Volts, 220V/50-60Hz. Current range: 2000mA, 200 Watts

CONTACT INFORMATION



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