IBI SCIENTIFIC

OPERATION MANUAL

Semi-Dry Blotters

16cm x 16cm | 24cm x 30cm

IB44000 | IB45000

Important Safety Information!

- Please read this manual carefully before operating your new IBI Semi-Dry Blotter.
- This manual contains important operating and safety information.
- To best use this product, please read the entire manual carefully prior to use.
- To avoid possible injury, this product should only be used for its intended purpose.

Package Contents | Unpacking

Upon receiving this product, please verify all of the noted parts and accessories are contained in this package. Awareness of the stated cautions and warnings contained within this manual, compliance with recommended operating parameters, and maintenance requirements are important for safe and satisfactory operation.

- Semi-Dry Blotter; based on size IB44000 or IB45000
- Top cathode (black) and bottom anode (Gray) assemblies.
- Power Cord Set - Black & Red (To connect Semi-Dry Blotter to Power Supply)
- Operation Manual

Save all packing material, and these instructions, if the Semi-Dry Blotter is received damaged. This shaker was carefully packaged and thoroughly inspected before leaving the factory. Responsibility for its safe delivery was assumed by the carrier upon acceptance of the shipment; therefore, claims for loss or damage sustained in transit must be made with the carrier.

Note: Carefully inspect all items in the package to insure no items are broken or missing. If there are items broken, please inspect the package carefully for signs of shipping damage. If there is ANY sign of shipping damage, please contact the carrier and file a claim with them immediately. Contact the distributor from which you purchased the item or IBI Scientific directly for assistance at (800) 253-4942 or (563) 690-0484.

Product Specifications

IB44000

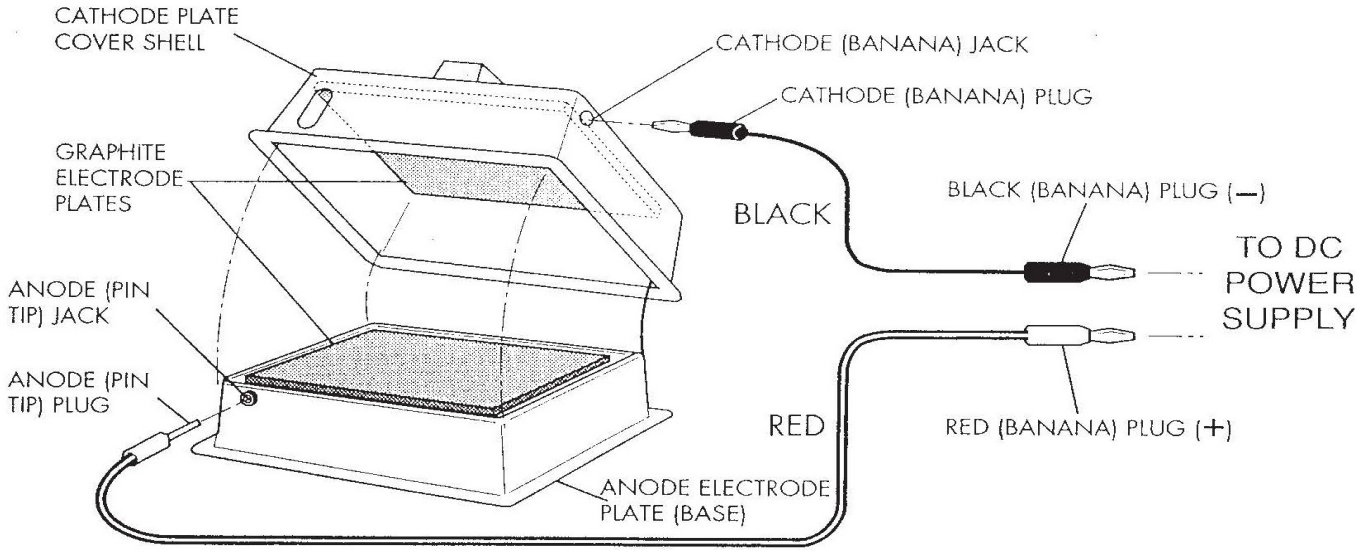
Unit Dimensions:	9.25”(l) x 9.25”(w) x 5.25”(h)
Working Surface Dimensions:	7” [16cm] x 7” [16cm]
Weight:	3.75lbs [1.7Kg]
Power Cords (Red & Black)	36” & 36”
Recommended Trans Units:	1 - 6

IB45000

Unit Dimensions:	14.25”(l) x 12”(w) x 3”(h)
Working Surface Dimensions:	9.5” [24cm] x 11.8” [30cm]
Weight:	9.5lbs [4.3Kg]
Power Cords (Red & Black)	36” & 36”
Recommended Trans Units:	1 - 3

Product Set-up::

The diagram shown below shows how to set up your new Semi-Dry Blotter. Make certain you do NOT have the power cords connected to the DC power supply until you are ready to proceed with an actual transfer.



Principles of Operation

Mixtures of biomedical products can be separated on the basis of their molecular sizes and ionic characteristics by polyacrylamide or agarose gel electrophoresis. Different proteins will appear as bands on the gels after staining. In order to further identify the characteristics of these proteins, components can be transferred and immobilized on a solid support in order that further detection can be achieved using radioisotopic or immunological methods. The transfer of these components from gels to a solid support requires either an electrical current, vacuum suction or diffusion to move the components from the gel to the solid support. The IBI Semi-Dry Blotting apparatus is a device that can migrate these components from the gel to a solid support by way of electrical current.

The fastest method for the transfer of proteins, RNA, and DNA from a gel to a solid support is electrophoretic transfer. To do this, the gel is layered in contact with the membrane sandwiched between several layers of filter paper soaked with buffer solutions forming a stack or single trans unit. A constant current is applied perpendicular to the gel and filter papers causing the sample to migrate from the gel on to the membrane. The membrane is then ready for staining or other developmental methods utilizing a PTS instrument, autoradiography, or enzymatic methods.

The IBI Semi-Dry Blotting apparatus utilizes a semi-dry electrophoretic method for transferring molecules of interest from gels to solid platforms/membranes. This method is safer and considerably more efficient and economical than wet tank methods. It can also be used for transfer of proteins from isoelectrofocused gels or slab gels.

The IBI Semi-Dry Apparatus

The IBI Semi-Dry apparatus consists of a specially designed, graphite covered, cathode plate and anode plate. The cathode plate is part of the lid assembly (black) and the anode plate is part of the base assembly (gray). A Trans-Unit stack is formed by the gel and the membrane being in contact and sandwiched between filter papers soaked in buffer solution.

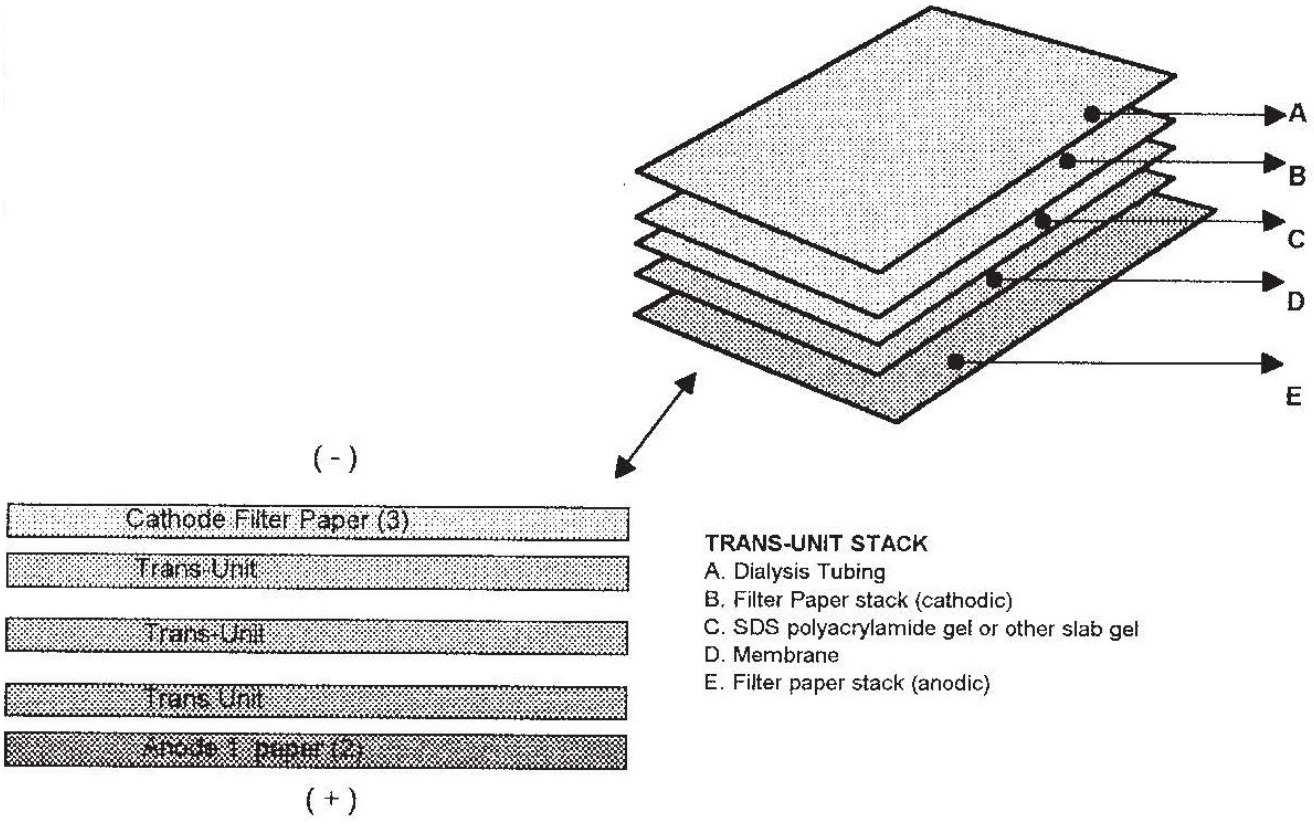
An electric current is applied perpendicular to the gel and filter papers. The cathode paper is saturated with the cathode buffer and the anode paper is soaked with anode buffer. In electrotransblotting, a pH gradient between the cathode and anode side of the trans unit is established so that negatively charged molecules can migrate toward the positively charged side of the trans unit. The bands from the gels migrate out of the gels and bind to the nitrocellulose membrane (or other membrane supports depending on which type of blotting is being accomplished). Once the bands reach the membrane they are immobilized until the portion of the membrane is saturated with the migrated molecules. Excess unbound molecules will migrate into the anode filter paper until it reaches the dialysis membrane which will block further migration of the molecules into the next trans unit. Several trans units can be stacked on top of each other in order to increase the throughput of the apparatus. The IB44000 Semi-Dry unit is designed to accommodate as many as 6 trans units stacked on top of each other and run simultaneously. The IB45000 Semi-Dry unit is designed to run 2 – 3 trans units simultaneously.

The graphite covered cathode and anode serve as the electrodes for the electrophoretic transfer and are designed to dissipate heat during the process. The time needed to transfer bands from various types of gels to membranes using various current densities are listed in Table 1.

Table 1. Recommended current settings for electro-transblotting proteins

Current Densit	Trans Units	Time Limit
0.8mA/cm2	1 – 6	1 – 2 hours
2.5mA/cm2	1 – 6	30 – 45 minutes
4.0mA/cm2	1 – 6	10 – 30 minutes

Building a Trans Unit Stack



Items not included but required to construct a Trans Unit:

- Polyacrylamide or Agarose gel that has been through electrophoresis.
- **IB95011** - Thick Blot paper for absorbing cathode or anode buffer – cut 10cm x 10cm – (pack of 10)
- **IB95020** - 0.2μm Nitrocellulose Membrane cut 10cm x 10cm. (pack of 10 membranes)
- **IB95031** - 0.45μm PVDF membrane cut 10cm x 10cm. (pack of 10)
- **IB95050** - Filter pads for inserting between trans units.

If you are running larger gels you will need to purchase the above items from another supplier as we work to provide trans unit materials for larger gels.

IBI Power supply:

- SH-500XL - 500v / 500mA power supply with 4 outputs
- SH-300XL - 300v / 400mA power supply with 4 outputs

Buffer Solutions for SDS PAGE Proteins:

- Anode Buffer Solution #1: 0.3M TRIS, 20% methanol, pH 10.4
- Anode Buffer Solution #2: 0.025M TRIS, 20% methanol, pH 10.4
- Cathode Buffer Solution: 0.025M TRIS/0.04M amino caproic acid, 20% methanol, pH 9.4
- IBI TRIS Powder: **IB70142**(500gm), **IB70144**(1Kg), **IB70145**(5Kg)

Operational Procedures and Techniques for Western Blotting

Warning: Be sure to wear gloves, safety glasses and lab coat/apparel when working with gels, buffers and semi-dry apparatus.

Trans Unit Assembly:

- Pre-soak the gel in anode buffer for 2 to 10 minutes at room temperature.
- Pre-cut blotting/filter paper and the solid support membrane to the same size as the gel.
- Pre-soak the membrane in distilled water at room temperature. If membrane is PVDF, soak in methanol.
- Place 2 sheets of thick blot paper/filter paper, saturated with anode buffer #1 on to the anode plate (base plate) of the semi-dry device. Now place an additional blot paper soaked with anode buffer #2 on top of the other two blot papers. (see recipes for anode buffer #1 and #2 above)
- Lay the wet membrane from step 3 on top of the blot papers.
- Carefully lay the pre-soaked gel on top of the membrane making certain not to have any air bubbles between the gel and the membrane.
- Place a thick blot paper soaked in cathode buffer on top of the gel.
- If stacking another trans unit on top, lay a dialysis membrane or **IB95050** fine filter pad, pre-soaked in distilled water and cut to the same size as the blot paper, on top of the cathode blot paper and proceed to build another stack of trans unit.
- When you have reached the top of the final trans unit stack, place two blot papers on top soaked in cathode buffer.
- Place the cathode plate cover (Black) on the assembled trans unit stack. Be sure to align the anode relief slot.
- Connect the red anode lead wire to the red jack on the semi-dry apparatus. Connect the black cathode lead wire to the black jack on the base of the semi-dry apparatus.
- Connect the anode and cathode lead wires to the corresponding jacks on the DC power supply.
- Turn on the power supply and set the unit for constant current. Adjust the current setting on the power supply to match the amperage requirements as determined by Table 1.
- When transfer is completed: **TURN OFF** power supply and unplug power cords.
- Remove the cathode cover plate
- Carefully peel off the blot/filter paper and discard
- Carefully peel off the gel which can now be stained or discarded.
- Peel off the membrane with forceps and ready for fixation and post transblotted development process.

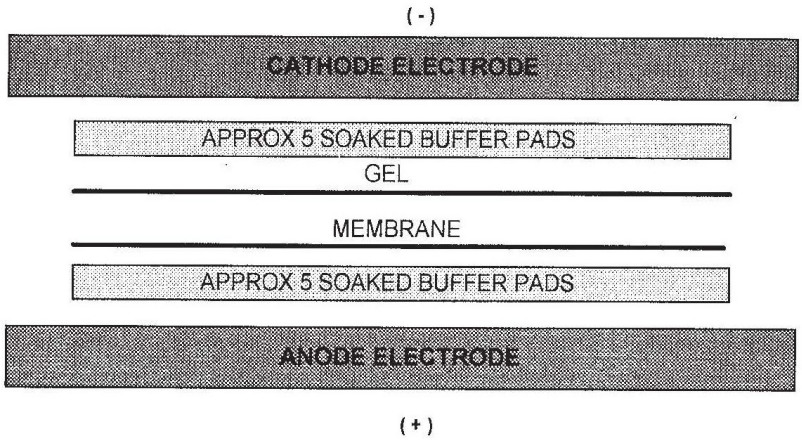
Operational Procedures and Techniques for Semi-Dry DNA Blotting

If the user is not using an automated developing device and is developing blots and gels by a manual method, the following is an overview of suggested solutions and methods. **NOTE:** The filter pads and blotting paper noted below are 10cm x 10cm. If you need larger you will need to source elsewhere.

- DNA is separated on an IBI Molecular Biology agarose gel (0.7 – 1%) poured with 1X TBE buffer or IBI Electrophast buffer. For genomic DNA use 0.7% and for plasmid digest use 0.8% - 1%
- After electrophoresis trim away non-essential portions of the gel. Prepare 2 buffer pads (**IB95050** Fine Filter pads – 10cm x 10cm)) and 2 thick blot papers (**IB95011** – 10cm x 10cm) and one charged modified nylon membrane cut to the exact size of the trimmed gel.
- Soak gel twice in 2 gel volumes of DI water (**IB42110** – 500ml Molecular Biology Grade Water) for 10 minutes.
- Soak gel twice in two gel volumes of denaturing solution #1 for 30 minutes at room temp with constant shaking.
- Soak gel twice in two gel volumes of neutralizing solution #2 for 30 minutes at room temp with constant shaking.
- Soak gel twice in two gel volumes of solution #3 – 1X TBE for 30 minutes. (**IB70150** – 10X TBE buffer in 1L)
- Wet one blotter filter pad and one thick blot paper in solution #3 (1X TBE). Place the filter pad on the anode plate and press down to minimize bubbles. Place the thick blot paper, soaked in 1X TBE on top of the filter pad. Roll out to eliminate bubbles
- Equilibrate the charge modified nylon transfer membrane in solution #3. 1X TBE, for at least 10 minutes and place it on top of the thick blot paper and eliminate air bubbles. Do not move the gel once it is in place.
- Carefully place the equilibrated gel on top of the membrane making certain to eliminate air bubbles.
- Wet a second blotter filter pad and thick blot paper in solution #3, 1X TBE. Place the thick blot paper on top of the gel then place the filter pad on top of the blot paper, eliminate air bubbles.
- Carefully lower the cathode / top of the semi-dry apparatus on to the gel sandwich.
- Connect the semi-dry blotter to the DC power supply and set transfer conditions between 1.5 – 3.5 mA/cm2 of gel area. Example: A gel measuring 7cm x 7cm has an area of 49cm2 and will require between 17 and 73mA constant current.

Power conditions and transfer times will vary with DNA/RNA type and size, thickness of the gel and size of the electroblot sandwich. Typically 20 – 60 minutes will be required at current densities of 1.5 – 3.5mA/cm^2

During the electrotransfer the voltage should be monitored for large fluctuations. During the transfer the voltage will slowly increase to maintain a constant current. If the voltage is lower than 15V the transfer time will lengthen. If the voltage increases significantly, such as greater than 25V, the buffer capacity has probably been depleted and the run should be terminated. If the run is not stopped, the gel will over heat and eventually melt. If the voltage indicated is significantly lower than normal, for instance less than 15V, the buffer solution may be more concentrated than 1X and therefore less volume may be required to maintain the specific current setting. If this is the case, the run can be completed as long as the current is adjusted to the specific setting. CARE MUST BE TAKEN NOT TO OVER HEAT THE GEL OR THE BLOTTING DEVICE. Monitor the procedure closely.



Operational Procedures and Techniques for Semi-Dry RNA Blotting

Denaturing Formaldehyde Agarose gels

- RNA is separated on a 1.2% Agarose gel with 1X MOPS and 1.8% formaldehyde. (**IB70042** – Molecular Biology Grade Agarose & **IB70175** – 10X MOPS w/DEPC water.)
- The Total RNA sample is heated to 65C for 10 – 15 minutes and added to RNA loading buffer. Loading buffer consists of 50% formamide, 6.5% formaldehyde and 1X MOPS. Typically 5 – 10μg of RNA are loaded per/lane. The samples are then heated to 55C for 10 – 15 minutes and mixed with 1/10 bromophenol blue.
- The gels are electrophoresed in a submarine apparatus in 1X MOPS buffer for 3 – 4 hours at 4 – 5v/cm^2
- Following electrophoresis, the gel is soaked in 5 gel volumes of 1X TBE buffer containing 0.1ug/ml EtBr for 30 minutes. Then soak the gel in 1X TBE buffer without EtBr for another 30 minutes.
- The gel can remain in the 1X TBE until just prior to semi-dry blotting.
- Trim away non-essential portions of the gel. Prepare 2 buffer pads, 2 spacer pads and one charge modified nylon membrane cut to the exact size of the trimmed gel.
- Saturate the trimmed buffer pads, two spacer pads and nylon membrane in 1X TBE buffer.
- Place one blotter pad on the semi dry blotter anode (base) plate. Roll out any air bubbles that may be trapped beneath the pad. Place a spacer/thick blot paper on top of the pad. Make certain there are no air bubbles beneath the blotting paper.
- Be certain the membrane has been in TBE buffer for 10 minutes to insure equilibration. Place the membrane on top of the blotting paper making certain there are no air bubbles beneath the membrane.
- Carefully place the equilibrated gel on top of the membrane making certain there are no air bubbles beneath the gel.
- Place a second blot paper/spacer on top of the gel. Place the second blotter pad on top of the blotting paper. Make certain there are no air bubbles.
- Carefully lower the semi-dry blotter cathode (black top assembly) on to the gel stack.
- Connect the semi-dry apparatus to the power supply and set transfer conditions to 2 – 3.5mA/cm^2 typical transfer time should 20 – 35 minutes.

Non-denaturing Formaldehyde Agarose gels

- Prior to electrophoresis, heat samples in RNA sample/denaturing buffer (4.5μl RNA in ultrapure water (**IB42200** – DEPC water – 125ml), 2μl 5X TBE buffer 3.5μl formaldehyde and 10μl formamide).
- RNA is separated on a 1 – 1.5% agarose gel, omitting formaldehyde in gel.
- The gel is electrophoresed in a submarine apparatus in 1X TBE buffer.
- Following electrophoresis the gel is soaked in 5 gel volumes of 1X TBE buffer containing 0.1μg Ethidium Bromide for 30 minutes and then soaked in 1X TBE buffer without Etidium Bromide for 30 minutes.
- The gel should remain in the 1X TBE buffer prior to semi-dry blotting.
- Follow the remaining steps above for RNA blotting.
- 1X MOPS buffer can be substituted for 1X TBE buffer.

Recommended Solutions for DNA / RNA Semi-Dry Transfers

DNA Solutions:

• Solution 1	Denaturing solution	0.5M NaOF, 1.5M NaCl
• Solution 2	Neutralizing solution	1.0M TRIS-Hcl/pH 8.0 1.5M NaCl
• Solution 3	1X TBE	0.089M TRIS 0.089M Boric Acid 0.002M EDTA, pH 8.3
	10X TBE	108gm TRIS base 55gm Boric acid 40ml 0.5 EDTA, pH 8.0
	1X MOPS	4.186gm MOPS (20mM MOPS) 41gm sodium acetate (5mM Sodium acetate) 2μl of 0.5M EDTA pH 8.0 (1mM EDTA) Dissolve the MOPS, sodium acetate and EDTA into 880ml of DI water.

- 37% formaldehyde stock solution
- 1.8% formaldehyde stock solution – 5ml of 37% stock into 100ml of DI water
- 6.5% formaldehyde stock solution - 1.75μl of 37% stock into 10μl of DI water.

Requirements for Electro-Transblotting DNA and RNA

Method	Recommended Current	Gel Size	Recommended Time
DNA/RNA from Acrylamide or agarose gels	1.5 – 3.5mA/cm^2	9 x 9cm 16 x 16cm	30 – 60 minutes 1 hour
RNA from Agarose	2.0 – 3.5mA/cm^2	9 x 9cm 16 x 16cm	30 – 40 minutes 30 – 40 minutes

Gel thickness and buffer solutions will affect run time.

Troubleshooting

Problem	Cause	Solution
Poor electrophoretic Transfer of DNA or RNA	Buffer may be too concentrated causing gel to carry too much much current.	Reduce buffer concentration to 0.5X to reduce current
	Power conditions during transfer may have changed If the voltage is no set high enough the current draw will drop below optimal range for good electrophoretic transfer	Set Voltage higher Optimum transfer of plasmid, vector and PCR DNA appear to occur when amps are set between 3 – 3.55mA/cm^2 for 10 to 15 minutes. Optimum Transfer of RNA is 2.5 to 3mA/cm^2 for 30 - 40 minutes.

Maintenance and Care of Apparatus

- After each use the device should be cleaned by thoroughly rinsing the plates with DI water, then dry.
- Similarly, salt deposits or crystal should be rinsed off of the surfaces of the electrodes before the next use.
- DO NOT USE organic solvents such as acetone, toluene, or petroleum to clean the device
- DO NOT IMMERSE the device in any solution
- DO NOT place strong acids or strong bases in contact with the electrode plates. Rinse the device immediately with DI water if exposed to corrosive soluitions
- BE CERTAIN to place the top cover (cathode) on the base (anode) prio to connecting to the power supply.
- TURN OFF power source prior to disconnecting electrode wires from the device or taking the device apart.

Limited Warranty

Our limited warranty for all life science equipment is one (1) year to the original buyer only (nontransferable) from the original date of purchase.

Our limited warranty as noted above extends to the direct end user of IBI Scientific products only. This warranty is in lieu of all other warranties whether expressed or implied, including warranties of merchantability or fitness for a particular purpose. In no situation shall IBI Scientific be liable for any incidental or consequential damages of any kind, even though IBI Scientific has been advised of the possibility of such damages arising out of, or resulting from, the products or the use or modification thereof or due to the breach of this warranty or any other obligation of IBI Scientific to the customer, whether based on contract, tort, or any other legal theory. In no such event shall IBI Scientific be liable for damages which exceed the purchase price of any products.

For further assistance contact IBI Scientific Technical Service at **(800) 253-4942**, **(563) 690-0484** or visit us on the web at **ibisci.com**

Accessories | Replacement Parts

Accessories

Catalog #	Description
IB95011	Thick Blot Paper (10cm x 10cm) - 10/Pk
IB95020	0.2mm Nitrocellulose Membrane (10cm x 10cm) - 10/Pk
IB95031	0.45mm PVDF Membrane (10cm x 10cm) - 10/Pk
IB95050	Filter pads (Fine) - 4/Pk

Replacement Parts

Catalog #	Description
IB45500	Replacement Power Cord Set (Red and Black)

IB44000

IB45000

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