

Warranty Information

THE OWL SEPARATION SYSTEMS WARRANTY

A three-year quality and material warranty covers all products manufactured by Owl Separation Systems. Owl will repair or replace any equipment found to be defective at no cost. This warranty does not cover equipment damage due to misuse or abuse. After the warranty expires, Owl will repair products at a reasonable cost. All shipping claims must be made within 48 hours from date received.

To activate your warranty, complete and return the enclosed postage paid warranty card. Please note that the card must be completely filled out in order to process your warranty.

RETURNING EQUIPMENT

Be environmentally friendly – and speed up your return – by saving all packing materials cartons and documents until you have thoroughly inspected your shipment. Should you find that your order is incorrect or damaged, verify the problem with the shipper, save all packing material, and call Owl for return instructions within 48 hours. All returns, exchanges, and credits must be pre-approved by Owl.

IMPORTANT DOCUMENTS ENCLOSED

Model #: _____
Serial #: _____
C.T.: _____



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(800) 242-5560
F. (603) 559-9258

Website: www.owlsci.com
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Thank You!

We at Owl Separation Systems thank you for your order and appreciate your business. Please contact us regarding our complete line of electrophoresis equipment and reagents for DNA, RNA and protein separations. While innovation and quality are our foremost objectives, we pride ourselves on exceptional customer response and service.

Owner's Manual



The Panther™ Semidry Electroblotter

Models HEP-1 and HEP-3

Apogent.

Rev. Date: 3/2003

owl 
Separation Systems

Safety Information

Important Safety Information! Please read carefully before operating!



• *This manual contains important operating and safety information. In order to benefit from the use of this apparatus, you must carefully read and understand the contents of this manual prior to use of this apparatus.*

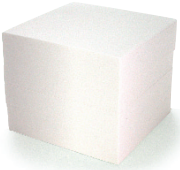


• *To avoid the risk of personal shock, always disconnect the gel box from the power supply. Further, the power supply must be equipped with a shut-down-on-disconnect circuit.*

- *Statement of Proper Use: Use this product only for its intended purpose as described in this manual. Do not use this product if the power leads are damaged or if any of its surfaces are cracked.*
- *Running conditions for this unit should not exceed the name plate readings found on the lower buffer chamber.*
- *Do not move the unit unless the power source to the unit has been disconnected.*
- *This Owl System is designed to meet IEC 1010-1 safety standards (IEC 1010-1 is an internationally accepted electrical safety standard for laboratory instruments).*

Optional Equipment

Contact the customer service department at Owl to order replacement parts 800-242-5560.



Accessories	Catalog No.
Blotting Filter Paper, 20cm x 20cm (pkg of 100)	FP-1
Blotting Filter Paper, 35cm x 45 cm (pkg of 100)	FP-2
Blotting Filter Paper, 46cm x 57 cm (pkg of 100)	FP-3
Blotting Filter Paper, 9cm x 9cm (pkg of 100)	FP-4
Blotting Filter Paper, 10cm x 10cm (pkg of 100)	FP-6
Blotting Filter Paper, 12cm x 16cm (pkg of 100)	FP-7
Power Supply Leads	PSL-5
Buffer Kit (recommended for Western Blotting)	ER-35



ER-35 Electroblot Buffer Kit

For Optimal Performance on Western blotting applications we recommend ER-35 electroblot buffer kit. These three different buffers (anode 1, anode 2 and cathode 1) that vary in buffering capacity and increase the transfer efficiency.

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A Few Tips About Caring for Your System

WARNING!

Organic solvents cause acrylic to “craze” or crack. Clean all Owl acrylic systems with warm water and a mild detergent. Do not use ethanol or other organic solvents to clean Owl products. Do not autoclave, bake, or microwave your unit. Temperatures over 50°C can do damage to the acrylic.

NOTE:

If an RNase free electrophoresis system is desired, there are various methods to rid the system of RNA contamination. For fast and easy decontamination, use RNase Away®*. Spray, wipe or soak labware with RNase Away® then wipe or rinse the surface clean; it instantly eliminates RNase. RNase Away® eliminates the old methods that include treatment with 0.1% Diethyl Pyrocarbonate (DEPC) treated water and soaking in dilute bleach. DEPC is suspected to be a carcinogen and should be handled with care. This electrophoresis system should never be autoclaved, baked, or placed in a microwave.

To order RNase Away® (not available through Owl), contact Molecular BioProducts 800-995-2787 (U.S. and Canada) or 858-453-7551:

Part Number	
7000	250ml bottle
7002	475ml spray bottle
7003	1 liter bottle
7005	4 liter bottle

**Rnase AWAY® is a registered trademark of Molecular BioProducts*

Care of Acrylic

The following chemical compatibility chart is supplied for the convenience of our customers. Although acrylic is compatible with most solvents and solutions found in the biochemical laboratory, some solvents can cause substantial damage. Keep this chart handy to avoid harm to your apparatus by the use of an inappropriate solvent.

Codes:

S—Safe (No effect, except possibly some staining)

A—Attacked (Slight attack by, or absorption of, the liquid)

(Slight crazing or swelling, but acrylic has retained most of its strength)

U—Unsatisfactory (Softened, swollen, slowly dissolved)

D—Dissolved (In seven days, or less)

Table 7-1 Chemical Compatibility for Acrylic-Based Products

Chemical	Code	Chemical	Code	Chemical	Code
Acetic acid (5%)	S	Ethyl alcohol (50%)	A	Naptha	S
Acetic acid (Glacial)	D	Ethyl alcohol (95%)	U	Nitric acid (10%)	S
Acetic Anhydride	A	Ethylene dichloride	D	Nitric acid (40%)	A
Acetone	D	Ethylene glycol	S	Nitric acid concentrate	U
Ammonia	S	2-Ethylhexyl Sebacate	S	Oleic acid	S
Ammonium Chloride (saturated)	S	Formaldehyde (40%)	S	Olive oil	S
Ammonium Hydroxide (10%)	S	Gasoline, regular, leaded	S	Phenol 5% solution	U
Hydroxide (10%)	S	Glycerine Heptane (commercial grade)	S	Soap solution (Ivory)	S
Ammonium Hydroxide concentrate	S	Hexane	S	Sodium carbonate (2%)	S
Aniline	D	Hydrochloric acid (10%)	S	Sodium carbonate (20%)	S
Benzene	D	Hydrochloric acid concentrate	S	Sodium chloride (10%)	S
Butyl Acetate	D	Hydrofluoric acid (40%)	U	Sodium hydroxide (1%)	S
Calcium chloride (saturated)	S	Hydrogen peroxide (3% solution)	S	Sodium hydroxide (10%)	S
Carbon tetrachloride	U	Hydrogen peroxide (28% solution)	U	Sodium hydroxide (60%)	S
Chloroform	D	Isooctane	S	Sodium hydrochlorite (5%)	S
Chromic acid (40%)	U	Isopropyl alcohol (100%)	A	Sulfuric acid (3%)	S
Citric acid (10%)	S	Kerosene (no. 2 fuel oil)	S	Sulfuric acid (30%)	S
Cottonseed oil (edible)	S	Laquer thinner	D	Sulfuric acid concentrate	U
Detergent Solution (Heavy Duty)	S	Methyl alcohol (50%)	A	Toluene	D
Diesel oil	S	Methyl alcohol (100%)	U	Trichloroethylene	D
Diethyl ether	U	Methyl Ethyl Ketone	U	Turpentine	S
Dimethyl formamide	U	Methylene chloride	D	Water (distilled)	S
Diocyl phthalate	A	Mineral oil (white)	S	Xylene	D
Ethyl acetate	D				

This list does not include all possible chemical incompatibilities and safe compounds. Owl's acrylic products should be cleaned with warm water, a mild detergent such as Alconox™, and can also be exposed to a mild bleach solution (10:1). In addition, RNAse removal products are also safe for acrylic. Please contact Owl's Technical Service at 1-800-242-5560 with any questions.

General Information

The Panther semi-dry electroblotter offers rapid transfer of proteins or nucleic acid molecules from polyacrylamide or agarose gels to membranes. Solid plate platinum/titanium and stainless steel electrodes are highly conductive and allow transfer at low voltages without external cooling systems. Plate electrodes also provide a uniform electric field for efficient, even transfers.

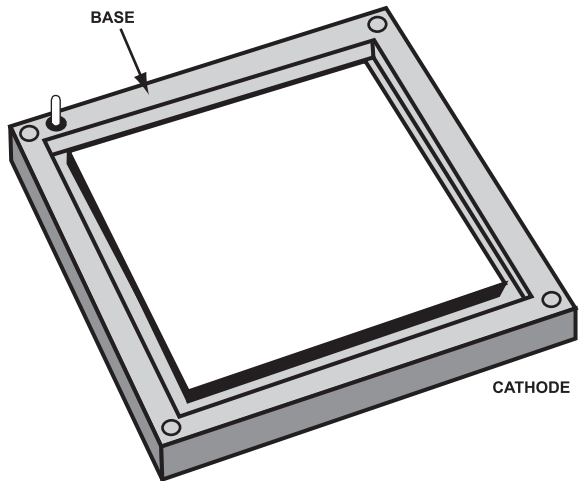
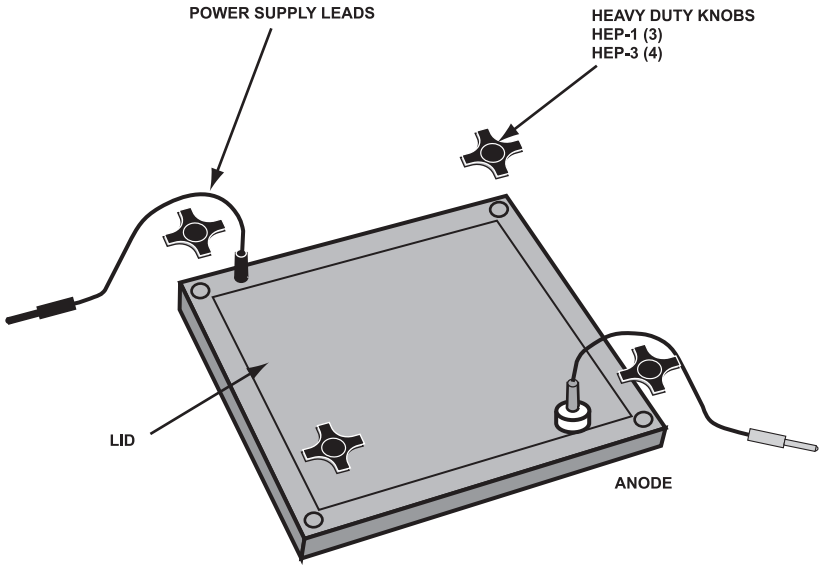
Before starting, unpack the unit and inventory your order. If any parts are missing, refer to the warranty section of this manual and contact Owl immediately. **You have 30 days from date of shipment to make any shipment claims!**

If you are missing any parts, call Owl at 800-242-5560 or 603-559-9297.

Item Description	Catalog No.
Complete System Transfer Area: 20cmW x 20cmL Footprint: 25cmW x 25cmD x 5cmH	HEP-1

Item Description	Catalog No.
Complete System Transfer Area: 35cmW x 45cmL Footprint: 40cmW x 51cmD x 5cmH	HEP-3

General Information



- 1 base with stainless steel cathode
- 1 lid with platinum/titanium anode
- 1 attached pair of power supply leads
- 3 knobs for the HEP-1, 4 knobs for the HEP-3

1X Three Buffer System for Semidry Electroblotting

This buffer is used with the HEP-1 Semidry Electroblotter

Final 1X composition:

Anode 1 Buffer: 0.3M Tris Base, 20% MeOH, pH 10.4

Anode 2 Buffer: 0.025M Tris Base, 20% MeOH pH 10.4

Cathode Buffer: 0.025M Tris Base, 0.04M Caproic Acid,
20% MeOH pH 9.4

NAQ Northern Transfer Buffer (8)

For transfer of RNA from agarose gels. With its high buffering capacity and low ionic strength, this buffer is more efficient than TAE, TBE or MOPS from agarose gels.

50X:

0.2M morpholinopropanesulfonic acid (MOPS)

50mM sodium acetate

5mM EDTA

pH 7.0

NAQ Southern Transfer

Transfer of DNA from agarose gels

50X

1M ethanolamine-glycine buffer, pH 11

NAQ Transfer Buffer

10X

0.8M Tris

1.18M borate

24mM EDTA pH 8.3

CAPS Buffer

PH11

This buffer can be used to improve transfer of some proteins. 10mm

CAPS (3-[cyclohexylamino]-1-10% methanol propanesulfuric acid, adjust to pH 11 with sodium hydroxide.

Recipes for buffers

1X Tris-Borate EDTA Buffer (TBE)

1X or 0.5X TBE is used for agarose gel electrophoresis and semidry electroblotting of nucleic acids

Final 1X composition:

89mM Tris Base
89mM Boric Acid
2mM disodium EDTA
pH 8.3

1X Towbin Buffer

1X Tris-glycine buffer (Towbin buffer minus the methanol) is used for agarose and polyacrylamide gel electrophoresis of nucleic acids. Towbin buffer (containing 20% methanol) is a commonly used buffer for semidry transfers.

0.025M Tris Base
0.192M Glycine
20% MeOH
pH 8.3

1X Tris-Borate EDTA Buffer (TAE)

1X TBE is used for agarose and polyacrylamide gel electrophoresis and semidry electroblotting of nucleic acids:

Final 1X composition:
0.04M Tris Acetate
0.001M disodium EDTA
pH 8.0

1X Tris-Glycine-SDS Buffer (TGS)

1X TGS buffer is used for denaturing polyacrylamide gel electrophoresis of proteins.

Final 1X composition:
0.025M Tris Base
0.192M Glycine
0.1% SDS
pH 8.3

Introduction

Once the proteins (or nucleic acids) in a sample aliquot have been separated on a slab gel, the resulting bands may be transferred to a solid support membrane. The primary reason for this type of blot is one of localization and secondarily, concentration of discrete bands. Although many have used alternative cross linking agents such as DATD (N,N'-dihydroxyethylene-bis Acrylamide) to allow for the accessibility of gel bound proteins, this still represents an impediment to radio enumeration due to the quenching by the gel matrix itself.

The most common solid support membrane is nitrocellulose. A second type of membrane is PVDF (Polyvinylidene difluoride) which is generally used when a transferred protein is to be sequenced, additionally, it has a 2x binding capacity. Also used for nucleic acid capture are Nylon membranes. In either case, the proteins are transferred from the gel to the matrix in an electric field perpendicular to the gel (initial running direction).

Tris based buffers are employed in the transfer. Methanol and SDS are modifiers often use in protein transfer buffer. These components however are antithetical in their effects both in terms of movement and adsorption. Methanol restricts protein movement from the gel but is often required to support the ionic nature of protein to nitrocellulose binding. SDS aids in protein elution, but can also inhibit binding of small molecular weight proteins (Mozdzanowski, J., High yield electrophoresis, Electrophoresis, 1992, Vol 13.,p.59-64).

Materials Needed

In order to use this blotting device you will need:

Power supply - Blotting requires a power supply that can operate at a fairly high current setting and low voltage. If an inappropriate power supply is used, the power supply may blow a fuse, shut itself off, display a no load or short load message or even have a short circuit. It is very important to be sure that the power supply you will be using will work with this device. Some power supplies that will work with this device are Owl's OSP-135, Owl's OSP-300, EC Apparatus EC135 and EC570, and Bio-

Rad's PowerPac 200. If your power supply is not among those listed, please contact the manufacturer of your power supply to determine if it will work for tank blotting applications. Contact Owl for power supply recommendations if you do not have an appropriate power supply.

Blotting buffer - See page --- of this manual for the appropriate buffer. The most commonly used buffer for protein blotting from polyacrylamide gels is Towbin buffer. Small amounts of buffer may be needed for equilibrating the gel and membrane prior to blotting, in addition to the buffer in the transfer tank. Should be cooled to 4°C.

Filter paper - Sometimes called blotting paper, it used in the blotting sandwich. It can come as pre-cut, thicker blotting sheets such as Owl Separation Systems part number FP-4 or large sheets or rolls of Whatman 3MM paper can be cut and used.

Blotting membrane - Nitrocellulose and PVDF (Polyvinylidene difluoride) can be used for Proteins, while charged Nylon membranes can be used for nucleic acids. The choice depends upon the user's preference and sometimes the detection method to be used. Owl offers Immobilon-P PVDF transfer membrane in a mini gel size (10 x 10cm), catalog #TM151-5.

Setting up the Blot

There are three types of gels, each requiring a different handling procedure before they can be added to the blotting sandwich. **Protein** and **agarose** gels set up are arranged together, with **sequencing** gel transfer protocol following separately

DNA/RNA: If these gels were not run in 1XTBE, they should be equilibrated for 10 minutes in this buffer.

Protein Gels.

After electrophoresis, remove the gel assembly from the apparatus and remove the spacers.

Open the gel cassette by gently rocking a spatula between the plate, forcing separation of the plate from the gel. The gel will normally remain affixed to the bottom plate. Remove the top (notched) plate by slowly lifting it from the

Semidry Blotting-General References

1. Bjerrum, O.J. and Schafer-Nielsen, C. in: Dunn, J.J. (ed.) *Electrophoresis '86* VCH Weinheim 1986, pp. 315-327. These authors compare results using different transfer buffers (Towbin buffer vs. the three buffer system).
2. Khyse-Anderson, J. 1984. Electroblothing of multiple gels. A simple apparatus without buffer tank for rapid transfer of proteins from polyacrylamide gels to nitrocellulose. *J. Biochem. Biophys. Methods* 10: 203-209. This paper describes a the semidry blotter with a 3 buffer system (Owl ER35) which is effective for transfer of proteins.

Protein Blotting

3. Castora, Frank J., "Western Blotting of Proteins", *Clinical Biotechnology* 1: 43-49 (1989). This review article on Western Blotting gives a good overview of factors such as transfer buffers, types of membranes, and post-membrane stains. Although written for standard tank blotting, much of this is applicable also to semidry blotting.
4. Eckerskorn, Christoph and Lottspeich, Friedrich, "Structural characterization of blotting membranes and the influence of membrane parameters for electroblothing and subsequent amino acid sequence analysis of proteins", *Electrophoresis* 14: 831-838, 1993. A useful reference if you plan to do protein sequencing of samples transferred samples.
5. LeGenre, Nancy "Immobilon-P Transfer Membrane: Applications and Utility in Protein Biochemical Analysis", *BioTechniques* suppl to vol 9: 788-805 (1990). This references deals specifically with transfer conditions using Immobilon-P type membranes.
6. Tovey, E.R. and B.A. Baldo. 1987. Comparison of semidry and conventional tank-buffer electrotransfer of proteins from polyacrylamide gels to nitrocellulose membranes. *Electrophoresis* 8: 384-387. This paper discusses quantitative yields of proteins of different molecular weights using different transfer conditions.
7. Dunbar, B.S., Ed. 1994. *Protein Blotting: A Practical Approach*. IRL Press at Oxford University Press, Oxford, England. A great guide to blotting techniques, including visualization, immunological techniques, and sequence analysis.

Nucleic Acid Blotting References

8. Trnovsky, Jan, "Semidry Electroblothing of DNA and RNA from Agarose and Polyacrylamide Gels", *BioTechniques* 13: 800-804 (1992).
9. "Blotting, Hybridization & Detection: An S&S Laboratory Manual", a publication of Schleicher and Schuell, c. 1995. This publication, put out by a leading manufacturer of blotting membranes, gives a good set of protocols for transfer of both proteins and nucleic acids.
10. "Hybond Blotting Guide: The direct route to excellent blotting results", Amersham Life Science. This publication gives very helpful hints and tips for producing good Western, Northern and Southern blots, along with a useful reference list. It also includes a very useful troubleshooting guide for nucleic acid and protein blots with pictures of the problems, description of symptoms, and proposed solutions.

How long will it take to blot the proteins from my gel?

Transfer times have to be determined experimentally. This is because transfer time is dependent upon:

- Percentage of gel
- Type and amount of cross linking in the gel
- Type of protein: cytoplasmic, membrane, nuclear
- Size of protein

There is no formula for determining transfer time. There are too many variables involved to give specific transfer conditions that will work for every protocol.

Guidelines are:

- HEP-1 and HEP-3: 2mA/cm² of gel for 1 hour

These guidelines are just a starting point and exact conditions have to be determined

Different kinds of blotting

Western Blotting is a blotting method for proteins that use specific antibodies attached to particular protein to help identify it. It is often performed after SDS-PAGE or some other form of polyacrylamide gel electrophoresis.

Southern Blotting is a method sometimes called hybridization because a radioactive probe is “hybridized” or attached to specific pieces of DNA.

Northern Blotting is a similar method but the molecules involved are RNA.

Both Southern and Northern blotting generally require the DNA or RNA to first be separated out on an agarose gel.

side with the inserted spatula and gradually increasing the angle until the plate is completely separated from the gel.

If the gel sticks to the top plate in an isolated spot, a stream of water from a squirt bottle can be sprayed at the spot to aid separation.

Remove the gel from the remaining plate. Tip the plate up side down, and start one edge, and allow it to roll off into transfer buffer. Alternatively, place the plate with the gel attached, into transfer buffer.

Incubate the gel in transfer buffer for 15 min. with gentle agitation. If the gel is on the plate, it will become loose during this step.

DNA gels- Preparing a sequencing gel for transfer on the HEP-3

2. Remove the gel cassette from the gel box and place under cold, running tap water until the surfaces of both glass plates are cool. This facilitates handling of the gel and prevents the gel from “curling” as it cools once it is removed from between the glass plates.
3. Place the glass plate sandwich, with the notched or shorter plate facing up, flat on paper towels on the lab bench and remove any excess liquid and remaining tape (or binder clips).
4. Remove one side spacer (wearing protective gloves) and insert a long metal spatula between the glass plates where the spacer had been present. Gently rock the spatula, forcing separation of the plate from the gel. The gel will normally remain affixed to the bottom (not notched) plate. Remove the top (notched) plate by slowly lifting it from the side with the inserted spatula and gradually increasing the angle until the top plate is completely separated from the gel. If the gel sticks to the top plate in an isolated spot, a stream of water from a wash bottle can be sprayed at the spot to aid separation. Occasionally, a gel sticks to the top plate instead of the bottom plate. In this case, flip the gel sandwich over and follow the same procedure.
5. Once the plates are separated, remove the second side spacer along with any extraneous bits of acrylamide around the gel. If excess water was utilized to aid in the separation of the gel from the glass plate, use a paper towel to absorb the excess liquid. Using a piece of dry blotting paper

(Whatman 3MM chromatography paper, 46 x 57cm), gently roll it onto the gel beginning at one end and working slowly towards the other end. Care should be taken to prevent air bubbles from forming between the paper and the gel. Once the blotting paper is in position, set another glass plate on top of the paper. Flip the entire sandwich over. The gel should now be resting on top of the filter paper between the two pieces of glass. Position the sandwich with one long side of the glass hanging about one-third of the way off of the counter top. Slowly push the bottom plate back toward the counter top, holding the top plate in place, which will allow the blotting paper and gel to peel away from the top piece of glass. The weight of the gel and blotting paper causes the gel to slowly peel away from the top plate. Continue this process until the gel is almost entirely peeled away from the glass. A small portion of the gel may still be attached to the glass; in this case, a small stream of water from a wash bottle can be used to aid in removal of the gel.

6. An alternative procedure at this step is to peel the paper, with the gel attached, from the glass plate upon which it rests. Beginning at one end of the gel, slowly lift and peel back the paper with the gel attached. As before, a stream of water from a wash bottle can be sprayed at any spot to aid separation of the gel from the glass plate.

7. Place the gel resting on the Whatman, 3MM paper flat on a lab bench.

8. Wearing gloves cut a piece of nylon membrane the size of the gel and blotting paper. Briefly (but thoroughly) wet the membrane in 0.5X TBE in a shallow tray, being careful not to crease the membrane. Holding the membrane by two corners, allow the excess liquid to drip from the membrane into the tray.

Gel Sandwich Assembly

Step 1 - Wearing gloves, cut the membrane to the size of the gel and blotting paper.

Step 2 - Mark the membrane, to indicate the side to which the samples will be on. This is important in the event that any successive probe is negative, and to indicate sample orientation. This can be done by either clipping a corner of the membrane or using a ball point pen. Clip the same corner until you retire.

PROBLEM	CAUSE	SOLUTION
<p>Nitrocellulose</p> <p>Insufficient binding of proteins to the membrane</p>	<p>membranes</p> <ul style="list-style-type: none"> • Over-transfer through the membrane • Not enough methanol in the transfer buffer • Low MW proteins are not binding well or are being washed away • SDS is preventing binding 	<ul style="list-style-type: none"> • Use 0.2micron pore size nitrocellulose instead of 0.45micron, or use PVDF with a higher binding capacity. • Nitrocellulose binds proteins best when 20% methanol is used in the transfer buffer. • Use glutaraldehyde to crosslink the proteins to the membrane and use Tween-20 in the wash steps. • Eliminate SDS in the transfer buffer.
<p>PVDF</p> <p>Smearred or swirled transfer and missing bands</p>	<ul style="list-style-type: none"> • Membrane was dried out before it was added to the transfer sandwich • Alcohol was not used to prewet the membrane • Air spaces are interfering with contact between the gel and the membrane • Electrophoretic conditions were incorrect or not ideal • Transferring at too high a current • Membrane was not thoroughly wetted. • Too much current 	<ul style="list-style-type: none"> • Membrane should be completely gray and slightly translucent when added to the sandwich. If it has dried out, rewet in methanol and equilibrate in transfer buffer. • PVDF is hydrophobic and requires a short soak in methanol prior to transfer. • Roll a test tube or pipet over the membrane (make sure it is clean) before putting the rest of the filter paper on the sandwich. Transfer will not occur where the gel is not in contact with the membrane. • Running conditions, sample preparation, percentage acrylamide, and many other variables can affect the migration and resolution of proteins. Please review your electrophoresis conditions. • Please refer to the running conditions in Table 1. • Always pre-wet the membrane according to the manufacturer's instructions. White spots indicate dry areas of the membrane. • Running at constant voltage can cause power fluctuations that will cause overheating. A buffer that has not been made correctly or that has too high in ionic strength can also burn a gel by overheating. A cracked and dry gel often is an indicator of overheating.

PROBLEM	CAUSE	SOLUTION
<p>Transfer efficiency is poor</p>	<ul style="list-style-type: none"> • Current is too low • Power supply is inappropriate for semidry transfer • Transfer performed for too short a time • Transfer sandwich was assembled in the wrong order • The pH of the transfer buffer is too close to the isoelectric point of the protein • Too much methanol in the transfer buffer • High percentage gels restrict transfer • Puddles of buffer were present on the cathode allowing the current to bypass the stack • The filter paper was too dry 	<ul style="list-style-type: none"> • Semidry transfer should be performed at constant current. Current density should be between 0.5 and 3mA/cm² of stack surface area. • Many power supplies will shut off or blow a fuse when run at the conditions required for semidry transfer. Semidry transfer requires low voltage (often less than 10V) and high current. Check with the manufacturer of the power supply to determine whether it is appropriate for semidry transfer. • Increase the amount of time for transfer. • The Panther semi-dry electroblotter is configured with the cathode on the bottom, and anode on top. This means that an upward transfer is being performed rather than downward. Follow the instructions carefully when assembling the transfer sandwich. • Try a more acidic or basic transfer buffer. • Reducing methanol can help elute proteins from the gel, but can reduce binding to nitrocellulose membranes. • Higher percentage acrylamide or crosslinker can restrict elution of proteins. Use the lowest percentage acrylamide possible to separate your proteins. • Always clean up the lower plate before closing the lid of the transfer apparatus. Do not squeeze the stack excessively as this also creates puddles that the current can pass through. • Filter paper should be saturated with transfer buffer before adding them to the sandwich.

Step 3 - Wet the membrane according to its manufactures recommendations, followed by a quick equilibration in transfer buffer. It is often helpful to have all the filter paper and membranes sitting in transfer buffer as you start to build the blotting sandwich.

Step 4 - With this device, the transfer is in the upward direction as shown in the figure below, and the blotting sandwich is built upward, upon the mirrored stainless steel plate of the bottom half of the blotter.

Step 5 - Lay three filter pads, one after the other down. Each should be soaking wet with buffer. In fact, the lower chamber may filled with buffer as you build the blotting sandwich.

Step 6 - Add a few mL of buffer to the filter pad, and gently layer the gel. Beginning at one end of the filter paper, align the gel with the paper's edge, and slowly lower the other end, driving out any bubble.

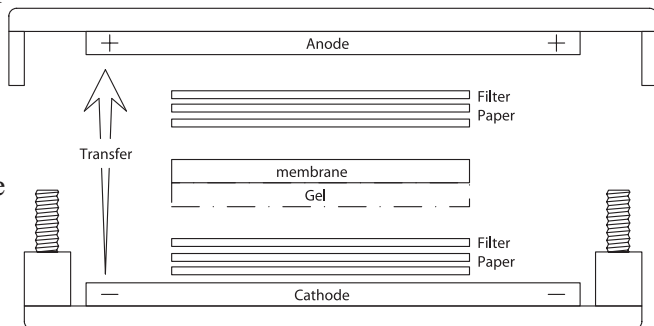
Step 7 - Wearing gloves, gently smooth out any bubbles by forcing them to the closest edge of the gel. Test tubes and pipettes many also be used for this purpose.

Step 8 - Alternatively, you can place the filter paper in a box with buffer, toss in the gel, and drain the box until the gel falls to the pad. Add a few mL of buffer to the filter pads already in the bottom chamber. Then pick up the paper and gel and layer them on the filter stack.

Step 9 - Add a few mL of buffer to the gel, and gently layer the membrane, as you did the gel.

Step 10 - Repeat with three more pieces of filter paper.

Step 11 - Holding the stack, drain off all excess buffer from the plate. Wipe away any droplets around the edge of the stack.



Running the Blot

Step 1 - Place and loosely tighten down the lid with the supplied black knobs. The weight of the lid is usually enough, and the screws are not required. Note: Do Not Use the Screws for gels thicker than 1.5mm, or when using more than 6 filter pads (total). If the screws are used, tip the unit on an angle, to drain off any excess buffer that may have squeezed out. This will safely remain in the mote around the electrode plate.

Step 2- Attach the power leads (red to red, black to black) to an appropriate power supply. The red lead has a shroud, that will stop it from attaching to the black cathode.

Step 3 - Run the blot.

Transfer settings

Blotting takes place at a given migration rate for a specified time. The units are mA times hrs. If you need to slow the transfer down, to say coincide with the setting up of a probe, simply decrease the current (mA) to match the added time you require

$$(\text{mA})(\text{hr}) \text{ Std setting} = (\text{mA})(\text{hr}) \text{ New setting}$$

alternatively, one can increase the current to decrease the time. This assume that you have determined an initial mAh value that works well for the molecules you are interested in.

A current to use for a 45 min time period is based on the area (cm²) of your gel (which is the resistor in this system). A range of 0.8 to 2 mA per square centimeter of gel.

For example, if you had a 10x10 cm gel, the area would be 100 cm², so the current range would be 80 mA (0.8mAcm⁻² x 100cm²) to 200 mA (2mAcm⁻¹ x 100cm²).

Blots may also be run at constant voltage. Some power supplies have difficulty sustaining steady voltages at these low voltage settings. If you

find that voltages are fluctuating, or that the power supply shuts itself off when set on constant voltage, use constant current settings instead. Read your power supply's instructions to ensure that the power supply will work at a voltage lower than 10V. These voltages often occur in semidry blotting. Contact the manufacturer regarding the unit's performance under high current, low voltage conditions if you have any questions.

Factors That Affect Transfer Efficiency

While general conditions can be described which will result in successful transfer of most molecules, it should be noted that optimal transfer conditions will vary based on the characteristics of the molecule you are working with. Some factors that affect transfer rate and efficiency include molecule size, charge, gel thickness and percentage, and hydrophobicity. The reference list at the end of this booklet provides useful information that can help you choose optimal conditions for efficient transfer of a specific molecule.

RUNNING CONDITIONS

	<i>Protein</i>	<i>HEP-3/ Sequencing gel – see HEP-1 settings for other size gels on this unit</i>	<i>DNA/RNA</i>
Filter Paper	Owl FP-1, 20 x 20cm, FP-6, 9 x 9cm, FP-4, 10 x 10cm, FP-7, 12 x 16cm, or FP-5, 18 x 20cm	Owl FP-2, 35 x 45cm, or FP-3, 46 x 57cm	Owl FP-1, FP-4, FP-5, FP-6, or FP-7
Membrane	Nitrocellulose .45 or 0.2 μ , PVDF 0.45 μ or 0.2 μ	Nylon	Nylon
Transfer Buffer	Towbin Buffer, Owl Electroblot Buffer Kit, ER-35, 3 Buffer System Bjerrum and Schafer-Nielsen	0.5X-1X TBE	0.5X-1XTBE, TAE, NAQ
Power Supply	Owl OSP-135 or equivalent	High current/low voltage power supply	OSP-135 or equivalent
Power Settings	Constant current 0.8-3mA per cm ² gel surface area 10-14 Volts maximum	For entire gel, 1200-1400 mA approximately 0.8mA/cm ² / produces ~5-8V	Constant Current 0.5-3mA/cm ² gel surface area 10-14 Volts maximum
Running Time	30 minutes to 2 hours Needs to be experimentally determined (large molecules need longer transfer times)	15-20 minutes at this setting	30 minutes to 2 hours, generally in the lower range