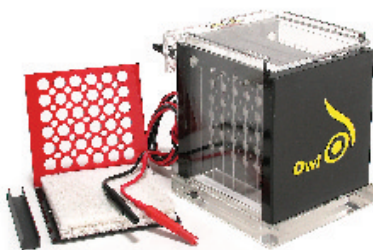


Owner's Manual



VEP-3



VEP-2

The Bandit™ Tank Electroblotting System

Model VEP-2 and VEP-3

Apogent.

Rev. Date: 3/2003

owI 
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).*



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The Bandit™ Tank Electroblotting System

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INTRODUCTION

The Owl **Bandit™ VEP-2** tank style electroblotting system is designed to provide uniform, reproducible protein transfers over a wide molecular weight range. Tank style blotting is highly efficient. Efficient transfers of up to four polyacrylamide mini protein gels are possible simultaneously in this robust system. The integrated cooling base allows temperature controlled runs. Stir bars may be added to the bottom of the buffer chamber to allow for increased buffer circulation and heat exchange. This is the perfect addition to any mini protein system.

The Owl **Bandit™ VEP-3** tank style electroblotting system is designed to provide uniform, reproducible protein transfers for larger sized gels or multiple mini gels. This system provides efficient transfers of polyacrylamide protein gels. A removable cooling block allows for efficient heat transfer. Stir bars may be added to the bottom of the buffer chamber to circulate buffer and provide consistent cooling. The cassettes are coded to ensure proper anode/cathode orientation. The cassette is designed to allow for easy loading of the gel sandwich and locks securely for loading into the buffer chamber. These features make this the preferred system for large gel blots. Tank systems are also recommended for large molecular weight proteins (>150KD).

UNPACK & CHECK YOUR ORDER

Before starting, unpack the unit and inventory your order. If any parts are missing, refer to the warranty section on the back cover of this manual and contact Owl immediately at 800-242-5560.

Reference the order or catalog number on your invoice and check the corresponding parts list.

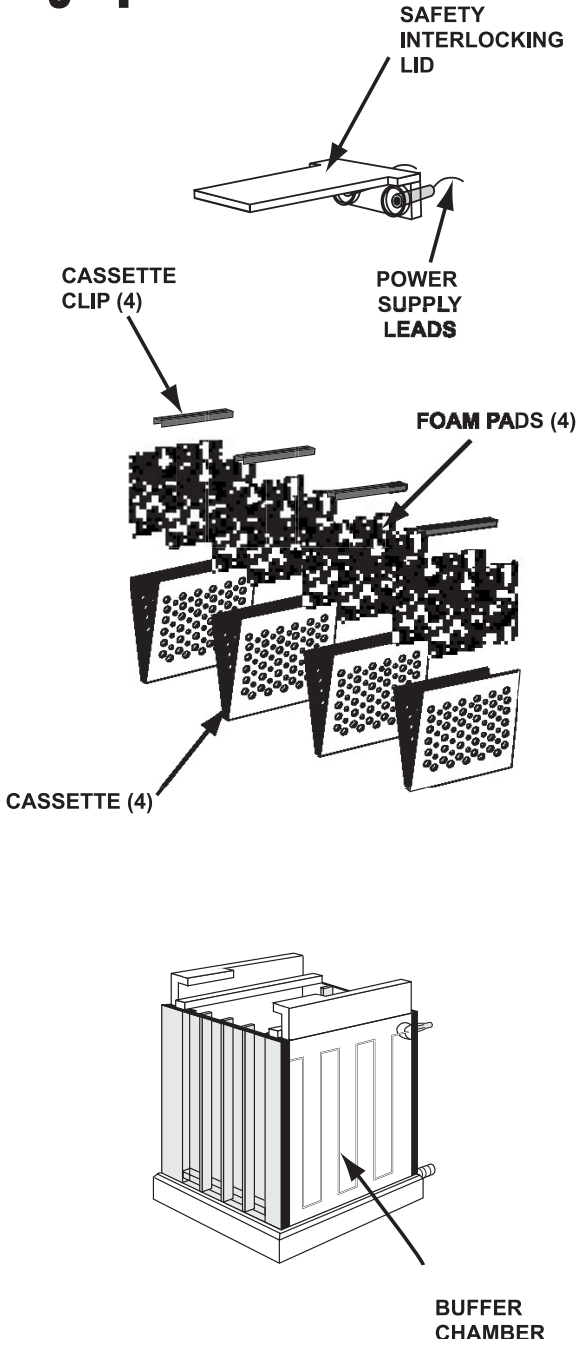


Figure 1-1 VEP-2 Parts Diagram

SPECIFICATIONS AND RECOMMENDED RUNNING CONDITIONS

Model VEP-2

Dimensions (Footprint):
5.125in W x 6.44in H x 7.3in D
13cm W x 16.3cm H x 18.5cm D

Buffer required: 1300ml

Gel size(maximum): 10cm x 10cm

Current requirements: 200-500mA

Time required: 45 minutes to 2 hours

Table 1-1 Parts List

Description	Qty.
Lid with attached Power Supply Leads	1
Buffer Chamber with Platinum Wire Electrode Panels and Cooling Base	1
Blotting Cassettes and clips with Foam Pads	4
Blotting Filter Paper, 9cm x 9cm	16
Blotting Cassette Clips	4

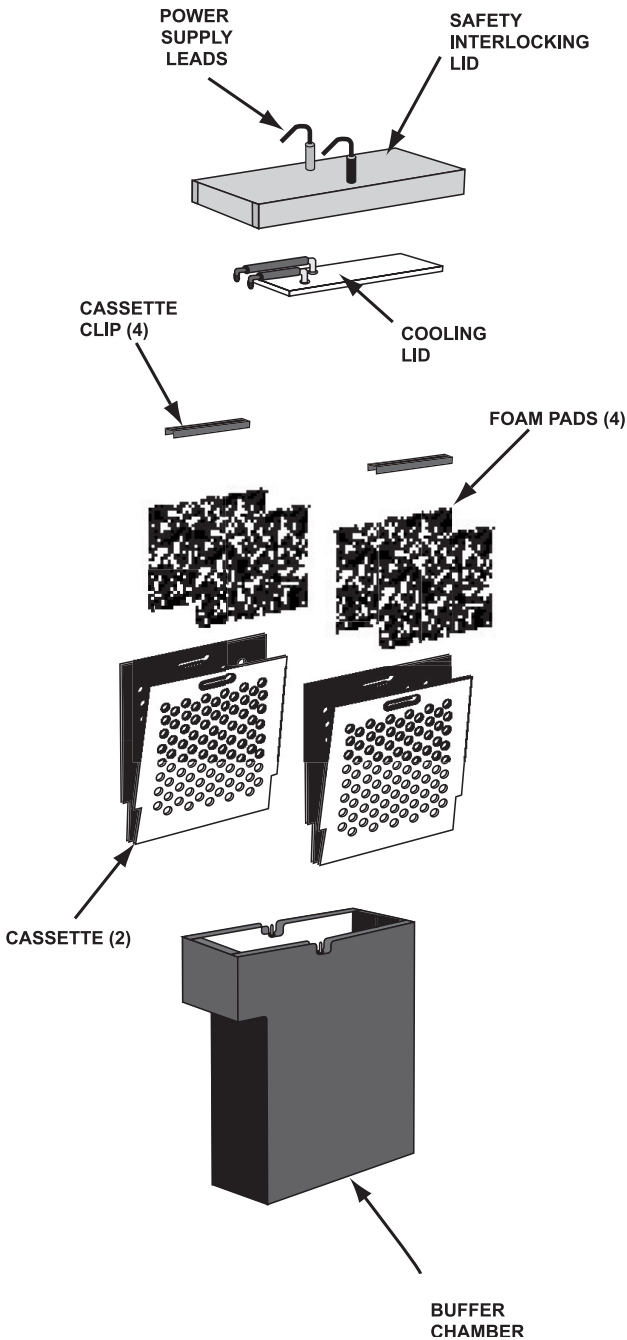


Figure 1-2 VEP-3 Parts Diagram

SPECIFICATIONS AND RECOMMENDED RUNNING CONDITIONS

Model VEP-3

Dimensions (Footprint):
30cm W x 30cm H x 9cm D

Buffer required: 4.0L

Gel size(maximum):
20cm x 30cm

Current requirements: 1.5 A

Time required: 60-120 min.

Table 1-2 Parts List

Description	Qty.
Lid with attached Power Supply Leads	1
Buffer Chamber with Stainless Steel Cathode Plate and Titanium Anode Plate	1
Blotting Cassettes with Foam Pads	2
Blotting Filter Paper, 20cmW x 18.5cmL	25
Cooling Lid	1

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 protein 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 electroblotting, 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 the Buffers section, page 14 of this manual for buffer recipes. 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. Buffer should be cooled to 4°C.

Filter Paper:

Sometimes called blotting paper, it is 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

STEP 1

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

STEP 2

Open the gel cassette by gently rocking a spatula between the plates, 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 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.

STEP 3

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.

STEP 4

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.

STEP 5

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

STEP 6

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.

STEP 7

Wet the membrane according to its manufacturer's 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 8

The red and black sides of the transfer cassette are hinged, and open up to a convenient angle for building a gel sandwich. This is best done in a dish or shallow plastic box. Place the cassette with the black side down.

STEP 9

Lay a Scotch Bright® pad on the black half of the cassette, followed by a filter pad. Both should be soaking wet with buffer. In fact, the tray may end up filled with buffer as you build the blotting sandwich.

STEP 10

Add a few mL of buffer to the filter pad, and gently layer the gel. Beginning at one end of the gel align the filter pad with the gel edge, and slowly lower the other end, driving out any bubbles.

STEP 11

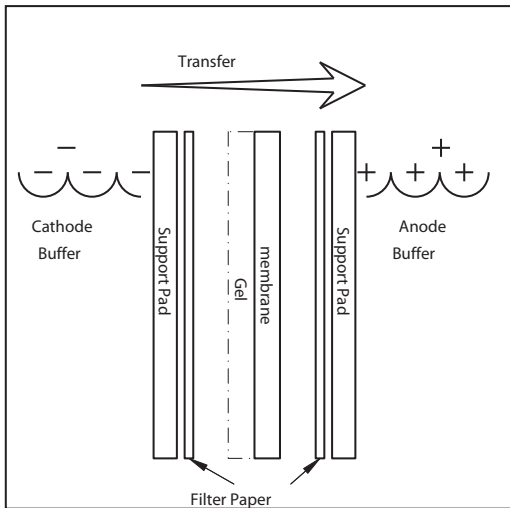
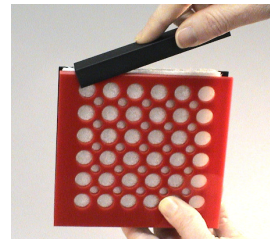
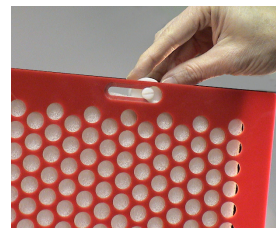
Wearing gloves, gently smooth out any bubbles by forcing them to the closest edge of the gel. Test tubes and pipettes may also be used for this purpose.

STEP 12

Alternatively, you can place the filter pad in a box with buffer, toss in the gel, and drain the box until the gel falls to the pad. Then pick up the two and layer them on the Scotch Bright® pad.

STEP 13

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

**BLOT ASSEMBLY****VEP-2 CASSETTE****VEP-3 CASSETTE****STEP 14**

Add another filter pad and complete the sandwich with a Scotch Bright[®] pad.

STEP 15

Close and lock the cassette. The VEP-2 uses a black C-clamp, that is pressed over the two open ends of the cassette, opposite the hinge. The VEP-3, has an integral lock, which when the red half of the cassette is lowered into position, the lock is slid to the right, engaging both halves of the cassette.

STEP 16

Add the cassettes to their respective slots in the buffer tank. Place them into the tanks already filled with cold buffer (4°C) with the clamps facing up.

STEP 17

The VEP-3, will fit into the buffer tank one way, to insure that the polarity of the cassette is correct. Match the red cassette side to the white (or red) bars on the inside of the chamber. If the cassette is reversed, it will stick out of the chamber and the lid will not fit.

Running the Blot

STEP 1

Add a stir bar to the buffer chamber.

STEP 2

Place the lid with power leads on the unit.

STEP 3

Attach the power leads (red to red, black to black) to an appropriate power supply.

STEP 4

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

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

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

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.

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:

- VEP-2: 400mA for 2 hours
- VEP-3: 1200mA for 2 hours

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 a 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 of an agarose gel.

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 with PVDF membrane. Towbin buffer (containing 5-20% methanol) is a commonly used buffer for protein transfers to nitrocellulose membrane.

0.025M Tris Base
0.192M Glycine
5-20% 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

PROBLEM	SOLUTION
<p>Smeared or swirled transfer and missing bands</p>	<p>Nitrocellulose membranes:</p> <ul style="list-style-type: none"> - Over-transfer through the membrane - Low MW proteins are not binding well or are being washed away - SDS is preventing binding <p>PVDF:</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 - Use 0.2micron pore size nitrocellulose instead of 0.45micron, or use PVDF with a higher binding capacity. - Use glutaraldehyde to crosslink the proteins to the membrane and use Tween-20 in the wash steps. - Eliminate SDS in the transfer buffer. - 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.
<p>Brown coloration of membrane or cracking of gel after transfer</p>	<p>Electrophoretic conditions were incorrect or not ideal</p> <ul style="list-style-type: none"> - Transferring at too high a current - Membrane was not thoroughly saturated. - 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.

PROBLEM	SOLUTION
Brown coloration of membrane or cracking of gel after transfer, <i>continued</i>	<ul style="list-style-type: none"> -Please refer to the running conditions in Table I. -Always pre-wet the membrane according to the manufacturer's instructions. White spots indicate dry areas of the membrane. -Too much current -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.
Transfer efficiency is poor	<p>Power supply is inappropriate for transfer</p> <ul style="list-style-type: none"> -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 -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 -Filter paper should be saturated with transfer buffer before adding them to the sandwich

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	Lacquer thinner	A	Sulfuric acid concentrate	U
Detergent Solution (Heavy Duty)	S	Methyl alcohol (50%)	D	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
Diethyl 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.

Optional Equipment

SECTION 7

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

Item Description	Catalog No.
Complete System	VEP-2
Accessories	Catalog No.
Power Supply Leads	PSL-5
Blotting Cassettes	VP2-BC
Foam Pads (pkg of 10)	VP2-BP
Blotting Filter Paper, 10cm x 10cm (pkg of 100)	FP-4
Blotting Filter Paper, 9cm x 9cm (pkg of 100)	FP-6

Item Description	Catalog No.
Complete System	VEP-3
Accessories	Catalog No.
Power Supply Leads	PSL-5
Blotting Cassettes	VP3-BC
Foam Pads (pkg of 10)	VP3-BP
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

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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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.