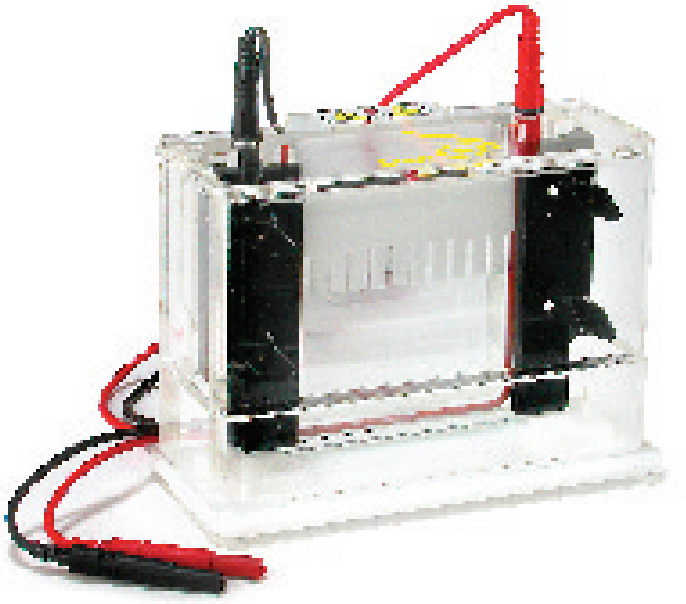
Owner's Manual



The Puffin™ Single Sided, Vertical Electrophoresis System

Model P81

Rev. Date: 11/2002

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).

Table of Contents

The Puffin™ SIngle Sided Vertical Electrophoresis System

General Information

SECTION 1

INTRODUCTION

The Puffin™ Single Sided Vertical Electrophoresis System is simple rugged and provides excellent results. THe upper buffer chamber (UBC) of the single sided vertical system extends the length of the gel to distribute heat evenly across the entire gel surface. Side clamps press glass plates against asilicome gasket to provide univlorm pressure and form a leak proof seal. This heat and pressure distribution system includes a notched alumina ceramic plate which may be used in front of the notched glass for cooler, faster runs.

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.

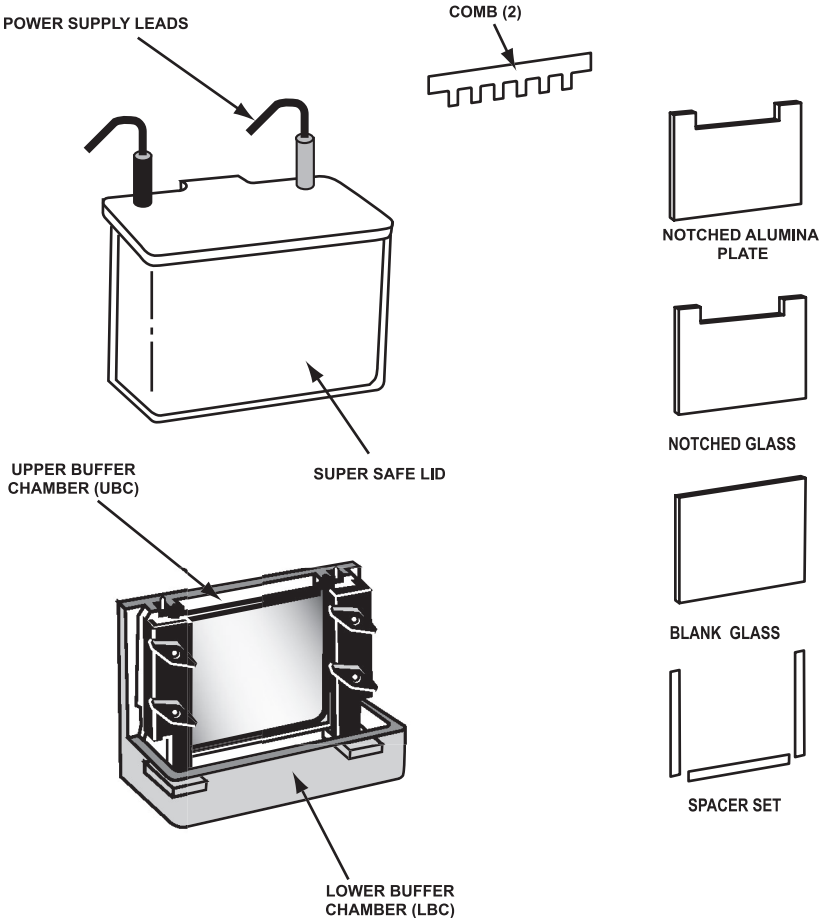
Table 1-1 Parts List

Lid with attached Power Supply Leads	-
Upper Buffer Chamber	-
Lower Buffer Chamber	-
Blank glass plates 3/32" Thick	P7-10G
Size	10cmW x 10cmL
Notched glass plates 3/32" Thick	P7-10R
Size	10cmW x 10cmL
Notched Alumina Plates 1.0mm Thick	P7-10RA
Size	10cmW x 10cmL
Spacers, 0.8mm Thick	P7-SC
Well Comb, 10 teeth, 0.8mm thick	MP-10C

Table 1-2 Specifications

Unit/Model Number	P81
Gel size	10cmW x 10cmL & 10cmW x 8cmL
Upper Buffer Chamber Capicity	100ml
Lower Buffer Chamber Capicity	50ml
Total Running Buffer	150ml
Total Buffer Capacity	150ml
Current, Constant	15-35mA/gel
Time Requirements	30-90 min.
Sample Capacity	10
Dimensions (cm) H x W x D	12.7 x 18.4 x 8.3
Glass Size (cm)W x L	10 x 10

Figure 1-1 Parts Diagram



Running the Gel

NOTE: Owl offers a Rabbit™ multiple gradient caster and a Joey™ gel caster. See page 16 for details.

STEP 1

After the gel is cast, place the gel with the notched or offset glass plate facing the inside of the upper buffer chamber. The gel cassette must be placed squarely on the corners in order to provide a good seal with the gasket and avoid leakage of buffer from the upper buffer chamber to the lower buffer chamber.

STEP 2

Add running buffer to the upper buffer chamber making sure the running buffer is 3mm below the top of the blank glass, ensuring sufficient contact with the top of the gel surface. Be sure that the running buffer is not leaking from the upper buffer chamber to the lower buffer chamber. If buffer is leaking you will need to drain the UBC and reset the cassettes.

STEP 3

Remove combs by gently pulling straight up from the gel. Carefully load samples into the wells formed by the comb. Rinse wells with water then buffer.

STEP 4

Add buffer to the lower buffer chamber to approximately 2-3mm above the base of the gel using the fill line as a guide (max. fill). The bottom end of the gel/agarose assembly should be in contact with the running buffer.

STEP 5

Set the safety lid onto the unit so that the power supply leads are connected. Begin the gel run.

Table 3-1 Maximum Buffer Volumes

Unit	P81
Upper Buffer Chamber	100ml
Lower Buffer Chamber	50ml

Running Conditions

Running conditions depend on several parameters:

- Buffer system used
- Whether or not heating would affect subsequent processing of the proteins or gel
- Thickness of the gel
- How fast the gel will be run – for example, set it up in the late afternoon and have the gel done the next morning, or have it done in 45 minutes or less

A guideline for 2nd dimension gels; the range would be 30-80mA constant current.

Example: For an SDS-PAGE gel in the P8DS that is 0.8mm thick and temperatures over 37°C are not an issue, 40mA per gel is appropriate. If the gel were 1.5mm thick the setting could be 60mA or higher.

Table 3-2 Recommended Running Conditions

Unit/Model Number	P81
Current, Constant	15-35mA/gel
Time Requirements	30-90 min.

After the Gel Run

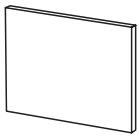
1. Turn off power supply.

2. Remove the lid. Slide and lift the upper buffer chamber from the lower buffer chamber and drain buffer chambers separately.

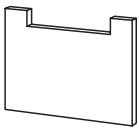
3. Loosen wing knobs and slide side clamps to remove gel cassettes. The gel is ready for staining and blotting. Contact Owl at 800-242-5560 or see the Owl product catalog for additional staining and blotting accessories.

Glass

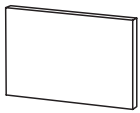
BLANK
The plate which faces you during electrophoresis. All gel sandwiches require one piece of blank glass.


BLANK

NOTCHED
The plate which faces the chamber during electrophoresis. Spacers are placed over the "ears" of the plate when casting vertical gels. Buffer accesses the gel between the ears.


NOTCHED

OFFSET
Offset plates may be used in place of notched plates. They require sponge tips mounted on the spacers. Sponge tips take the place of the "ears", and prevent buffer from running out of the upper buffer chamber from the sides.


OFFSET

FROSTED
Frosted plates are used for vertical agarose electrophoresis. One side of the plate has a rough surface to prevent agarose from sliding down.

Spacers

STANDARD
Protein spacer sets include two side spacers and one bottom spacer. Spacers and combs must be of identical thickness to be used together.

Reagent Information

RUNNING BUFFER

TGS

Tris - 3.0285g/L
Glycine - 14.4g/L
SDS - 1.0g/L
pH 8.3 (Laemmli, 1970)
q.s. to 1L
Note: For Native Protein Electrophoresis do not add SDS.

Table 5-1 Sample Buffer

2X Concentration Stock		/L	/10 mL	Final Concentration With Sample*
2%	SDS	20g	0.2	1%
10%	BME	10mL	0.1	5%
250mM	Tris	6.057g	.0606g	125mM
30%	Glycerol	300 mL	3 mL	15%
0.002%	Bromo Phenol Blue	.02g	.0002g	0.001%

** add sample buffer 1:1 with sample solution.*
Caution: 2X Sample Buffer containing 2-mercaptoethanol should be prepared in a fume hood. 0.2M (final concentration) Dithiothreitol (DTT) may be used in place of 2-mercaptoethanol. DDT should be added before use and made fresh.

ACRYLAMIDE SOLUTION

Stock acrylamide solution for table 5-2:
= 29.2g Acrylamide and .8g bis-Acrylamide, q.s. 100mL H_2O

Table 5-2 Gel Prepartion (SDS-Page continuous buffer system)

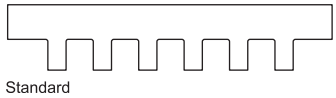
	% Acrylamide*				
Stock Solution	20.0	15.0	12.5	10.0	5.0
Acrylamide-Bisacrylamide (30:0.8)	20.0	15.0	12.5	10.0	5.0
0.5 M Sodium Phosphate Buffer pH 7.2	6.0	6.0	6.0	6.0	6.0
10% (w/v) SDS	0.3	0.3	0.3	0.3	0.3
Water	2.2	7.2	9.7	12.2	17.2
1.5% (w/v) APS	1.5	1.5	1.5	1.5	1.5
TEMED	0.015	0.015	0.015	0.015	0.015

** The columns represent volumes (ml) of stock solutions required to prepare 30ml of gel mixture.*

Combs

STANDARD

• 0.5mm(A), 0.8mm (C) and 1.5mm (D) thicknesses



PREPARATIVE

• One long well and one marker lane

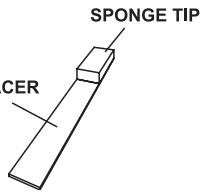
CUSTOM COMBS

Call Owl Customer Service for more information, 800-242-5560.

Notched Alumina Plates - For P8DS Models Only

Notched Alumina plates can take the place of the notched glass when casting and allows for better heat exchange than glass. This is important when the protein is heat sensitive or if a gel needs to be run a little faster without the negative effects of heating. Heating the gel during the run can cause smiling and other distortions of the gel.

Offset vs. Notched Glass



All units require a blank piece of glass and an offset or notched piece of glass. Offset glass is glass that is about 2cm shorter than the blank piece without "ears" on the sides. Notched glass has two "ears" that are left behind when a cut is made in the middle of the top of the glass. Both offset glass and notched glass allow the gel and samples to make contact with the upper buffer chamber. Offset glass has to be used with sponge tips, which take the place of the notches on the glass. The advantage of offset glass is that this glass is more rigid. Notched glass is easier to use and does not require the addition of sponge tips.

Troubleshooting

PROBLEM	CAUSE	SOLUTION
Broad lanes at bottom of gel	• Will occur when adjoining lanes are loaded with dissimilar samples.	• Normal in gradient gels.
Skewed bands	• Gel has not polymerized properly at wells.	• Degas gel solution before casting and increase APS and TEMED concentrations. The comb can be wiped with TEMED just prior to casting to improve polymerization.
	• Salt concentration is too high in sample.	• Dialyze sample or use desalting column.
	• The upper buffer chamber is leaking either through the gel or along the sides.	• Check gel to make sure that it is a solid slab inside the glass and check the setup of the apparatus to ensure a secure seal with the gasket.
Streaked bands	• Overloading of sample.	• Use less protein or sample when loading.
	• Sample has precipitated.	• Centrifuge sample before adding sample buffer or use a lower % acrylamide gel.
Frowning of outside lanes	• Leakage of buffer along sides or along spacers inside the gel assembly.	• Do not move spacers after polymerization and make sure that gasket is seated firmly against the glass. Always load your samples with an empty lower buffer chamber so that leaks are caught before you begin the run.
Double bands ("doublets")	• Due to reoxidation or insufficient reduction of the sample.	• If using a reducing agent, prepare fresh sample buffer every 30 days. Increase the concentration of 2-mercaptoethanol or dithiothreitol in the sample.
Glass cracks when putting gel assembly in unit	• Gel is too thin for the clamping system.	• Use glass appropriate for the unit. If this is not possible, use an extra piece of blank glass to take up the space. If the clamps are used with their flat side against the glass, thinner glass may often be used.
	• Gasket is old or flattened making it impossible to make a good seal.	• Wash gasket after each use to remove salts. If gasket is old and has lost its flexibility, it may need to be replaced. If unit has been previously overtightened, the gasket may need to be removed and reseated. A cracked and dry gel often is an indicator of overheating.

Troubleshooting

PROBLEM	CAUSE	SOLUTION
Longer run time	• Buffer is too dilute	• Check buffer recipe; remake buffer and try again. See if voltage produced by the current you are running at is the same. If it differs significantly, your buffer may not have been made up correctly.
	• Upper buffer chamber is leaking	• Make sure that the gel assembly is seated firmly against the gasket. Remove gasket, wash in warm water to remove excess salts, and place the gasket back in the groove. If the clamps have been overtightened in the past, the gasket can be pushed too far into the gasket groove and will not make a seal.
	• Running at too low a current	• Use running conditions as stated in table 3-2. When running at constant current, the current value is per gel.
Running too fast	• Buffers are too concentrated	• Check buffer recipe; remake and try again. If voltage is lower than usual when running at constant current, the buffer is probably too dilute.
	• Voltage or current set too high	• Turn down current setting
Smiling of dye front	• Center of gel is running hotter than the ends	• Turn down current setting
Bands spreading outwards	• Diffusion of sample when loading.	• Make sure that the samples are loaded quickly and the power is applied as soon as possible after loading.
	• Diffusion of sample during run in the stacking gel.	• Increase % of stacking gel or increase current by 25% when stacking.
	• Lower ionic strength of sample.	• Match the ionic strength of the sample with that of the gel.
Bands are narrower than sample wells	• Ionic strength of sample is higher than of the gel.	• Desalt the sample or use sample buffer of the same strength as the gel.

Care & Cleaning

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 & Cleaning

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	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
Dioctyl 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

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

Table 8-1 Replacement Parts

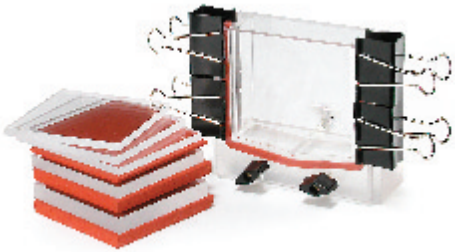
Description	P81
Power Supply Leads	PSL-5
Clamp Assemblies	P8-CL
Replacement Gaskets	R12009
Blank glass plates 3/32" Thick	P7-10G, 10cmW x 10cmL
Notched glass plates 3/32" Thick	P7-10R, 10cmW x 10cmL
Frosted Notched glass plates 3/32" Thick	P7-10FR, 10cmW x 10cmL
Frosted Blank Glass Plates 3/32" Thick	P7-10FG, 10cmW x 10cmL
Notched Alumina Plates 1.0mm Thick	P7-10RA, 10cmW x 10cmL
Spacers, 0.5mm Thick	P7-SA
Spacers, 0.8mm Thick	P7-SC
Spacers, 1.5mm Thick	P7-SD
Blocking Plate for Single Gel Operation	P8DS-016
Spacer Placer (pkg of 3)	JG4-PL

Table 8-2 Combs Options

Model P81 Catalog Number	Comb Type	Number of Teeth	Thickness of Tooth (mm)	Width of Teeth (mm)	EST Well Volume (ul)
MP-6A	Well	6	0.5	11.1	89
MP-6C	Well	6	0.8	11.1	142
MP-6D	Well	6	1.5	11.1	266
MP-8A	Well	8	0.5	7.7	62
MP-8C	Well	8	0.8	7.7	99
MP-8D	Well	8	1.5	7.7	185
MP-10A	Well	10	0.5	5.7	46
MP-10C	Well	10	0.8	5.7	73
MP-10D	Well	10	1.5	5.6	134
MP-12A	Well	12	0.5	4.3	34
MP-12C	Well	12	0.8	4.3	55
MP-12D	Well	12	1.5	4.3	103
MP-15A	Well	15	0.5	2.9	23
MP-20A	Well	20	0.5	1.6	13
XCM	Custom		0.5, 0.8 1.5, 2.0, 3.0		

Optional Equipment

RABBIT™ MULTIPLE GRADIENT CASTER



The Multiple Gradient Caster System features an easy-to-use casting base and specially designed spacer plates for quick casting of high quality linear or gradient gels. Using the acrylic and foam spacer plates allows you to cast from one to five gels simultaneously. A silicone gasket provides a leak proof seal and the casting port allows the casting of gradient gels from the base of the caster.

Rabbit	P7-CST
Gel Size	10cmWX 10cmL

THE JOEY™ GEL CASTER

The patented Joey Gel Casting System provides a simple method of casting acrylamide gels without taping or special sealing of the gel plates. Plastic pouches hold glass plates and spacers snugly together in the casting stand while pouring. These pouches meet specific measurement tolerances to allow for a tight fit around glass plates. Gels may be cast ahead of time and sealed inside the plastic pouch, creating your own pre-cast gels. Up to four gels may be cast at one time using the Joey.

Joey	JGC-4
Gel Size	10cmWX 10cmL

RELATED OWL PRODUCTS

- Panther Semi-dry Electroblotter
- Puffin Single Sided Vertical Gel Electrophoresis System
- Bandit Tank Style Electroblotter
- Powdered Buffers
- Silver Stain
- Pro Blue

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.: _____

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.

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Separation Systems

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