Owner's Manual

The Opossum™ Horizontal System

Model A2-OK

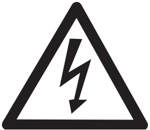
Apogent™

Rev. Date: 3/2003

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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General Information

INTRODUCTION

The Opossum™ Horizontal Multiple Gel, Model A2-OK system is ideal for teaching labs or labs that need to run multiple gels simultaneously. Up to six minigels (7cmW x 8cmL) can be run on this versatile system. Along with optional combs the Opossum™ can screen up to 144 samples at once (120 with supplied combs). EasyCast™ casting design allows the casting of gels without tape or plugs. U.V. Transmissible (UVT) gel trays may be placed directly on the transilluminator for photodocumentation.

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 within 7 days of purchase at 800-242-5560.

Reference the order or catalog number on your invoice and check the corresponding parts list on page 2.

Table 1-1 Specifications

Gel size	7cmW x 8cmL (x6)
Running Buffer Volume	2.3L
Footprint	28cmW x 37.5cmL x 10cmH

General Information

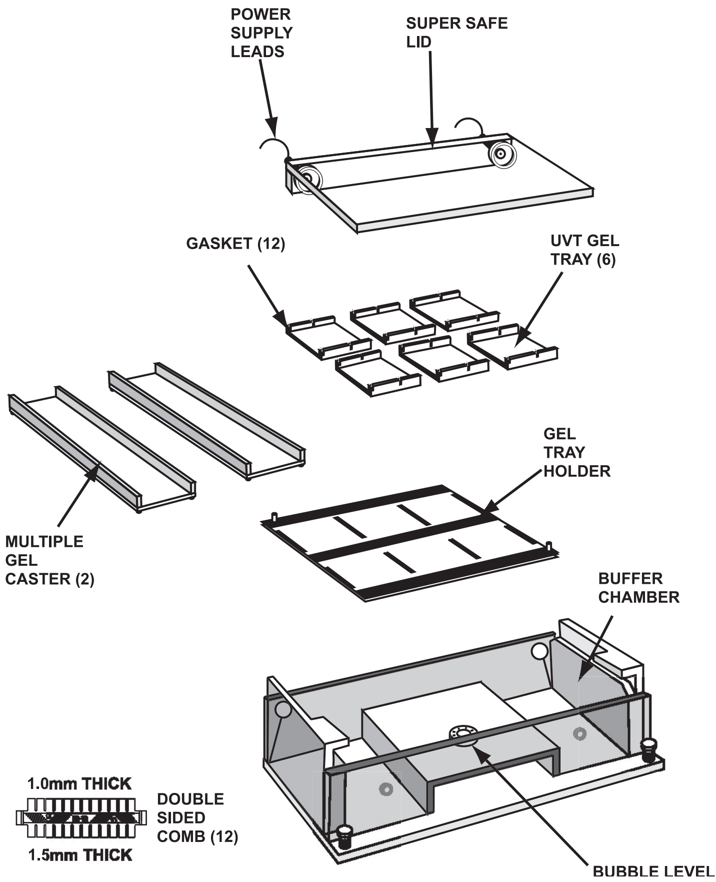


Figure 1-1 Parts Diagram

Table 1-2 Parts List

Buffer Chamber	1
Combs 10 well comb, Double Sided, 1.0/1.5mm Thick	12
Super Safe Lid with attached power supply leads	1
Multiple Gel Caster	2
Gel Tray Holder	1
UV Transmissible gel trays	6

Setting Up

STEP 1

Remove the SuperSafe™ lid from the buffer chamber. The SuperSafe™ Lid is attached to the back of the unit at the junction of the lid's attached power supply leads to the banana plugs located on the unit. To remove hold the front of the buffer chamber with one hand and pull the lid off; sliding it off evenly by holding the center of the back of the lid.

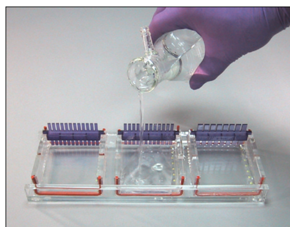
STEP 2

To cast gels, place the UVT gel tray(s) into the external multiple gel caster with the gaskets facing the inside of the gel caster. (up to 3 UVT gel trays may fit in one external multiple gel caster). The gasketed sides of the UVT gel tray form a leak proof seal along the inside of the gel caster. Owl offers a leveling platform, catalog no. B-LP, page 17, if needed.

STEP 3

Preparing the gel

Using electrophoresis-grade agarose and compatible electrophoresis buffer the gel may be prepared in various ways. The percentage of agarose and the electrophoretic buffer used is determined by the size of the samples to be separated and further recovery of the samples. The agarose and buffer are mixed and heated over a heat source, in a microwave oven, or in an autoclave until the agarose is completely dissolved. The prepared gel then must be cooled to below 60˚C before casting to avoid warping the UVT gel tray due to excessive heat. If numerous gels are to be run in one day, a large volume of gel may be prepared and placed in a covered bottle stored between 40˚- 60˚C in a water bath. This provides a ready gel supply in a warm liquid form that will solidify quickly when gels are cast.



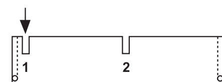
STEP 4

Insert the desired comb or combs into the comb slots to form the sample wells. Pour or pipet warm agarose into the UVT gel tray (s) (< 60C˚). If only a small portion of gel is required for proper sample separation, multiple combs may be used to run 1 or 2 sets of equal distance samples simultaneously expanding the number of samples per gel that may be run. Repeat for up to 6 gels (3 UVT gel trays per external multiple gel caster).

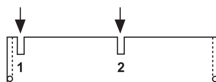
Migration Distance

• Run one sample set on a gel in each tray

• Run two sample sets on a gel of equal length (comb slots) in each tray

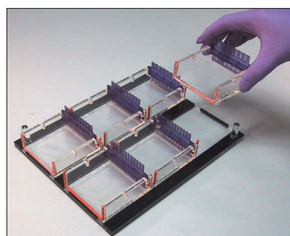


One comb for a 7.3cm run length

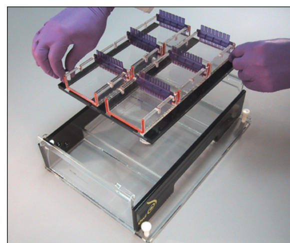


Two combs for two 3.6cm run lengths

STEP 1
Once the gel is completely solidified, lift the UVT gel tray out of the external gel caster and place it onto the gel tray holder, in the running position. Once all UVT gel trays are placed onto the gel tray holder, place the gel tray holder into the buffer chamber with the first comb of all UVT gel trays closest to the cathode side of the chamber. This running position exposes the open ends of the agarose to the buffer. Standard agarose should solidify completely in about 30 minutes. If low melting point or a speciality agarose is used, consult the instructions that came with the product.



STEP 2
Pour enough compatible running buffer into the unit to fill the buffer chamber and completely cover and submerge the gel(s). Correct buffer level is clearly marked on the units side wall as “FILL LINE”. See Recommended Running Conditions for approximate buffer volumes needed for your unit. Too little buffer may cause the gel to dry out during the run, while excess buffer may decrease DNA mobility and cause band distortion.



STEP 3

Carefully remove the comb (or combs) from each UVT gel tray by tapping lightly to loosen, and slowly lifting straight up out of the UVT gel tray(s) to avoid damage to the wells.

STEP 4

Wet loading - loading the sample in the gel when it is submerged in buffer.

a. Place the gel tray holder with the UVT gel trays into the buffer chamber in the running position.

b. Pour running buffer into the unit to fill chamber and completely cover and submerge the gel.

c. Load prepared samples into the wells. Samples should be mixed with a sample loading buffer; giving weight to the samples so that they drop evenly into the wells, and contain tracking dye to monitor the gel run.

NOTE: Load prepared samples into the wells. Samples should be mixed with a sample loading buffer; giving weight to the samples so that they drop evenly into the wells and containing tracking dyes to monitor the gel run.
NOTE: It is wise to always run a sample lane of a known "standard ladder", see page 7, to determine concentration and size of separated fragments after the gel run, and to aid in photo documentation and analysis.

STEP 5

Carefully slide the SuperSafe™ lid with attached power supply leads onto the unit. This will connect the power cords to the banana plug electrodes. Plug the other end of the power supply leads into an appropriate power supply, completing the circuit. The gel is now a resister in the circuit.

STEP 6

Turn on power supply. Refer to Table 3-1, for running conditions. Carefully monitor the gel run to avoid samples running into the path of another set of samples.

Table 3-1, Specifications and Recommended Running Conditions

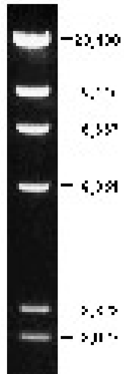
Model	A2-OK
Gel Size (w x L in cm.)	7cmW X8cmL (6)
Buffer Capacity	2.3L
Voltage Requirements (V)	20-150
Time Requirements (min.)	30-60

Finishing Up

STEP 1
When the gel run is complete and tracking dye has migrated as far through the gel as desired, or to the end of the gel, turn off the power supply and slide off the SuperSafe™ lid to disconnect from the power source. Carefully remove the gel tray holder containing the UVT gel trays (wear gloves if ethidium bromide is present). The UV Transmissible (UVT) gel tray makes visualization and photography with a UV light source easy without the need to remove the gel from the tray.

STEP 2
The buffer chamber should be rinsed under warm running water after each use, including the UVT gel tray (s). An RNase/DNase decontaminate may be used. This electrophoresis system must never be autoclaved, baked, or microwaved!

Running a Standard Ladder



It is recommended to always run a sample lane of a known “standard ladder” or “marker” to determine concentration and size of separated fragments after the gel run, and to aid in photodocumentation and analysis. Migration patterns and fragment sizes for commonly used DNA molecular weight markers are shown in this figure.

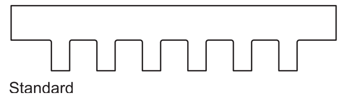
Loading Samples

It is sometimes easier to load the sample wells dry before placing buffer into the buffer chamber. After the gel solidifies, remove the UVT gel tray from the external gel caster and place the UVT gel tray on the lab bench. Carefully remove the sample combs by tapping and lifting straight up. Samples mixed with loading buffer that does not contain dye may be easier to load dry, especially in larger gel units to avoid cross contamination. After loading all sample lanes, place the gel tray into the buffer chamber on the gel tray holder in the running position with the gel edges facing out toward the buffer chambers and slowly fill the chamber with buffer.

Comb Options

Standard

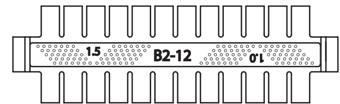
1.0mm and 1.5mm thickness: for all models.
Combs are hand fabricated for high quality precision in low volumes. Each comb has an acrylic spine with Lexan® teeth.



Standard

Double Sided

Double Sided Molded Combs combine 1.0mm and 1.5mm tooth thickness on one comb. Double sided combs provide greater precision due to the exact manufacturing technique which provides greater control over tooth size and spacing than the traditional machining methods. The number of teeth and arrows that point to the designated thickness is molded onto the spine for easy identification. A raised section of the spine helps the user grip the comb when removing it from the gel. Owl’s combs are molded from durable polycarbonate that holds up through years of use.



Double Sided

Preparative

Preparative combs are manufactured with an acrylic spine and Lexan® teeth. Used for extremely large samples.

Preparative

Custom Combs

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

Ethidium Bromide -

Ethidium bromide is ideal for the flurometric detection of nucleic acids in gel electrophoresis. The addition of ethidium bromide to both the prepared gel and running buffer is a convenient way to monitor separation and keep a photographic log of gel runs. Ethidium Bromide is prepared as 10mg/ml in distilled water and used as a stock working solution of 5.0µg/ml in the electrophoresis buffer and gel. Mix ethidium bromide powder or tablet thoroughly into solution checking for any precipitate and store at room temperature protected from light.

Amount of Agarose to prepare:

Gel volume is determined by the following formula and may be adjusted according to need or preference:

Table 5-2, Amount of Agarose

gel width(cm) X gel length (cm) X gel thickness (cm) = ml of agarose

		Agarose Volume in ml per gel thickness in cm.			
Model #	**Gel size(cm)**	**0.25cm**	**0.5cm**	**0.75cm**	**1.0cm**
A2-OK	7 X 8	14ml	28ml	42ml	56ml

Agarose Gel Loading Buffer

Samples are prepared and combined with gel loading buffer before being applied to the prepared gel. Sample buffer usually contains similar components to the running buffer, dyes for visibility, and glycerol to provide some weight to the samples. This increased sample density and color allows easy visualization of the samples and ensures samples load evenly into the wells and do not float out during loading. Dyes also migrate toward the anode end of the electrophoresis chamber at predictable rates allowing the gel run to be monitored.

The most commonly used loading buffer is glycerol, bromophenol blue, and xylene cyanol.

Reagent Information

There are various types of agarose commercially available that may be used. Besides standard ultra pure electrophoresis grade agarose, there are also numerous low melting point products for easy sample recovery, as well as speciality products formulated for specific uses to separate/recover very small or very large fragments etc.

To visualize and photograph the samples after the gel run for a permanent record, the gel may be stained during or following the run with a variety of stains. The most common stain for DNA is ethidium bromide. Ethidium bromide may be added directly to the gel and running buffer to quickly and easily visualize and photograph the separated fragments following the gel run without the need for additional staining. If this is not added, then following the gel run the gel may also be soaked in a concentrated ethidium bromide solution and rinsed for the same visualization. The ethidium bromide is added to both the gel (after heating) and the electrophoresis buffer at a concentration of 0.5µg/ml.

Ethidium bromide is a potential carcinogen. Care in handling the powder and stock solution must be taken. Always wear gloves when handling the powder, solutions and all gels that contain any amount of ethidium bromide.

Table 5-1, Mobility range of DNA in different percentage agarose gels

Agarose % (w/v)	Approximate range of separated DNA fragments (kb)
0.3	60 to 5
0.5	30 to 1
0.7	12 to 0.8
1.0	10 to 0.5
1.2	7 to 0.3
1.5	4 to 0.2
2.0	3 to 0.1
3.0	< 0.1

It should be noted, an increased agarose % gives better separation of small fragments and also bands very close together that tend to be more difficult to separate, visualize and photograph. A specialty agarose product formulated to increase resolution of low molecular mass samples may also be used.

Example: A good mid range gel percentage would be 0.7%, or 0.7g agarose in 100mls electrophoresis buffer (TBE or TAE), following heating and dissolving the agarose, 10µl of ethidium bromide stock solution (5mg/ml) is added. The gel would be run with compatible electrophoretic running buffer (1X TBE or 1X TAE) that also contained ethidium bromide 1 liter of the running buffer would contain 100µl of this 5mg/ml ethidium bromide stock solution.

Preparation & Properties of TAE and TBE Electrophoresis Buffer Systems:

These buffers are used because they both have a basic pH which gives the phosphate group of the DNA a net negative charge allowing migration of the DNA toward the positive anode in the electrophoresis chamber.

TAE - Tris acetate with EDTA (40mM Tris base 40mM acetic acid,1mM EDTA)

50X stock solution, pH ~8.5:	1X working solution:
242g Tris base	40mM Tris acetate
57.1ml glacial acetic acid	1mM EDTA
18.61g Na_2EDTA - $2H_2O$ (MW 372.24)	
Distilled H_2O to 1 liter final volume	

TBE - Tris borate with EDTA (89mM Tris base, 89mM boric acid, 2mM EDTA)

10X stock solution:	1X working solution:
108g Tris base	89mM Tris base
55g boric acid	89mM boric acid
7.44g Na_2EDTA - $2H_2O$ (MW 372.24)	2mM EDTA
(or 40 ml 0.5 M EDTA, pH 8.0)	
Distilled H_2O to 1 liter final volume	
Do not adjust pH	

Buffer:	**Suggested Uses and Comments:**
TAE Buffer	Use when DNA is to be recovered For electrophoresis of large (>20kb) DNA Applications requiring high resolution Has low ionic strength and low buffering capacity - recirculation may be necessary for long runs (>4hrs.)
TBE Buffer	For electrophoresis of small (<1kb) DNA Better resolution of small (<1kb) DNA Decreased DNA mobility High ionic strength and high buffering capacity - no recirculation needed for extended run times

TBE buffer reacts with the agarose making smaller pores and a tighter matrix. This reduces broadening of the DNA bands for sharper resolution.

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.

Troubleshooting

PROBLEM	SOLUTION
Agarose leaks when casting the gel	Check to see if the gasket is correctly seated in groove and even all the way around. Remove gasket and reseat by smoothing it out gently with your thumb from one end to the other. After each use rinse the gel tray under warm running water. Please contact Owl to purchase replacement gaskets. Check to make sure gasketed end of the gel tray have been placed with the gaskets facing the sides of the external gel caster
Bands seem to be running at an angle.	Check to be sure that the unit is properly leveled for casting and running the gel. Owl offer a leveling platform, BP-LP, see page 25. Always center the UVT gel tray in the buffer chamber and cool the agarose to below 60°C before pouring to avoid warping the UVT gel tray (s).
Samples seem to be running unevenly in certain areas.	Check that the platinum electrode wire is intact running flat and evenly across the outer corners and up the side to the junction of the banana plug area. This problem could also be caused by regular casting with very hot agarose gel (>60°C) which may damage the UVT gel tray over time. Always cool the melted agarose to below 60°C before casting to avoid warping the UVT gel tray. Warping the UVT gel tray will cause all subsequent gels to be cast unevenly.

Troubleshooting

PROBLEM	SOLUTION
Samples do not band sharply and appear diffuse in the gel.	Gels should be allowed to solidify completely before running. For standard agarose this would be about 30 minutes, if low melting point agarose is used it may be necessary to completely solidify gels at a cooler temperature in the refrigerator or cold room. Gels should be submerged in 3-5mm of buffer to avoid gel dry out, but excess buffer >5mm can cause decreased DNA mobility and band distortion.
Bands are not sharp, clear, and even.	Always follow the proper procedure for preparing the agarose product according to the manufacturers instructions. When preparing the agarose be sure all the agarose powder is in solution before heating. In general, add powdered agarose to distilled water and swirl to mix. Make sure all the powder is equally wet to ensure proper melting. Heat in a microwave oven, boiling water bath, or hot plate with occasional swirling to melt and mix completely. Cool agarose liquid to below 60°C and cast. Note: High percentage gels may thicken and solidify rapidly and should be cast while still a liquid.
Samples are not moving as expected through the gel, remaining in the wells, or diffusing into the gel.	Check that a complete power circuit is achieved between the unit and the power supply. Platinum wire and banana plugs should be intact. To test, simply fill the unit with running buffer and attach to the power supply without a gel or UVT gel tray in the unit. The platinum wires on both sides of the unit should produce small bubbles as the current passes through. If a complete circuit does not exist there will be few to no bubbles. Contact Owl's Customer Service Dept. to schedule a repair.

Troubleshooting

PROBLEM	SOLUTION
When the comb is removed from the gel some sample wells are ripped and damaged.	Always make sure to allow the gel to solidify completely before moving the gel tray, unit, or removing the comb. To avoid damage to the sample wells, gently rock the comb back and forth lightly to loosen, then slowly pull the comb straight up out of the gel tray. This rocking helps to avoid suction as the comb is removed. Alternatively, once casting is complete, simply submerging the gel with running buffer will help loosen the comb. Using a higher percentage of agarose that forms a tighter gel matrix may remedy this problem as well.
The gel seems to run slower under the usual running conditions.	The volume of running buffer used to submerge the gel should only be between 3-5mm over the gel surface. Gel should be completely submerged to avoid the gel from drying out, which can smear the bands and possibly melt the gel (s) due to overheating. If excessive running buffer is added the mobility of the DNA decreases and band distortion may result. Excess buffer causes heat to build up and buffer condensation inside the unit may result.

Additional Sources for Reference

Maniatis T., E. F. Fritsch and J. Sambrook. ***Molecular Cloning: A Laboratory Manual***, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Short Protocols in Molecular Biology, - A Compendium of Methods from Current Protocols in Molecular Biology, Edited by Fredrick M. Ausubel, et. al.

Adams, D., and R. Ogden, ***Electrophoresis in Agarose and Acrylamide Gels, Methods in Enzymology***, Vol. 152 (1987) Academic Press, Inc.

Fotador, U.. ***Simultaneous Use of Standard and Low-Melting Agarose for the Separation and Isolation of DNA by Electrophoresis***, BioTechniques, Vol. 10, No. 2, (1991)

Boots, S. ***Gel Electrophoresis of DNA*** ; Analytical Chemistry, Vol. 61, No. 8, April 15, 1989

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®, contact Molecular BioProducts at 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*

Optional Equipment

Leveling Platform

The three point leveling platform, Model B-LP, ensures a flat casting and running surface. the platform is 46cm x 36cm and is large enough to fit most applications. One bubble level (BBL-1) is included.

Optional Equipment

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

Replacement Parts

Cat. No.	Description
PSL-5	Power Supply Leads
B1A-UVT	Gasketed UVT gel tray
B1A-CST	Multiple Gel Caster
A2-OT	Gel Tray Holder
B1A-GK	Replacement Gaskets (1 pair)

Combs

Catalog #	# of teeth	Tooth Width (mm)	Thickness (mm)
B1A-5C	5	11	1.0
B1A-5D	5	11	1.5
B1A-6	6	9	1.5/1.0
B1A-8	8	6	1.5 /1.0
B1A-10	10	5	1.5 /1.0
B1A-12	12	3.5	1.5 /1.0
B1A-PREP	2	57/4.8	

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.

55 Heritage Avenue
Portsmouth, NH 03801

T. (603) 559-9297
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F. (603) 559-9258

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