Horizontal Electrophoresis System

Model C2

Operating and Installation Manual 7217451 Rev. 0

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Owl
Separation Systems

MANUAL NUMBER 7217451

REV	ECR/ECN	DATE	DESCRIPTION	By
0	--	4/9/12	Transferred to Marietta (was 11/2002)	ccs

Important Read this instruction manual. Failure to read, understand and follow the instructions in this manual may result in damage to the unit, injury to operating personnel, and poor equipment performance. ▲

Caution All internal adjustments and maintenance must be performed by qualified service personnel. ▲

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Important operating and/or maintenance instructions. Read the accompanying text carefully.

Potential electrical hazards. Only qualified persons should perform procedures associated with this symbol.

Equipment being maintained or serviced must be turned off and locked off to prevent possible injury.

Hot surface(s) present which may cause burns to unprotected skin, or to materials which may be damaged by elevated temperatures.

Marking of electrical and electronic equipment, which applies to electrical and electronic equipment falling under the Directive 2002/96/EC (WEEE) and the equipment that has been put on the market after 13 August 2005.

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- ✔ Always use the proper protective equipment (clothing, gloves, goggles, etc.)
- ✔ Always dissipate extreme cold or heat and wear protective clothing.
- ✔ Always follow good hygiene practices.
- ✔ Each individual is responsible for his or her own safety.

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Section 1 Safety Information/ Introduction

Please read carefully before operating!

Warning 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-downon- disconnect circuit. Do not move the unit unless the power source to the unit has been disconnected.

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.

Introduction

The Microgel Electrophoresis System is designed for quick restriction fragment analysis. Glass slides with dimensions of 2 x 3" are used as the gel tray. Thin gels may be poured and formed by surface tension. A caster is also available for thicker individual gels. The system also comes with a 7-tooth comb and the power cords necessary for running the unit.

Section 2 Unpack and Check Your Order

Before getting started, unpack the unit and inventory your order. If any parts are missing, refer to the enclosed information sheet regarding returns and exchanges and contact Technical Services immediately.

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

Part No.	Description
C2-S	Buffer Chamber
PSL-5	Power Supply Leads
C2-CST	Casting Chamber
C-GS	Casting Slides, pk of 10
C2-7D	Comb:7 well, 1.5mm thick

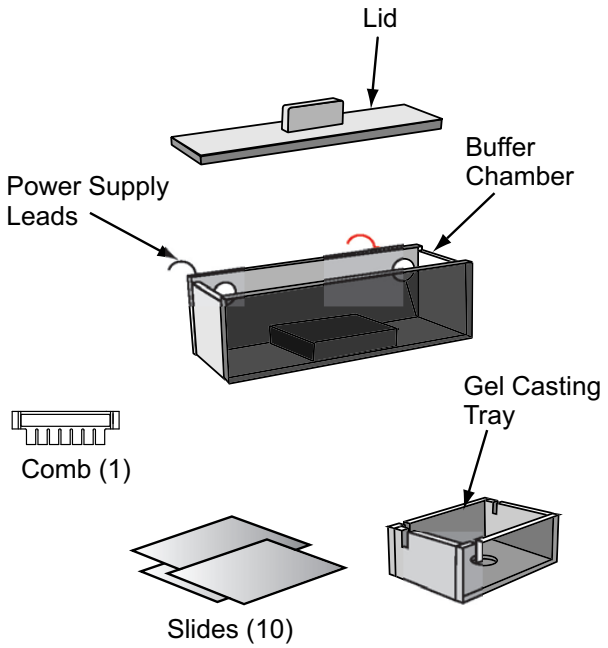


Figure 2-1. Exploded Parts Drawing

Basic Specifications and Recommended Running Conditions - Model C2

Gel size (W x L cm): . . . . . .2” W x 3” L
Buffer Capacity (ml): 100
Voltage Requirements (V): . . . . . .20-70, 30 average

Section 3 Setting Up

Casting Gels

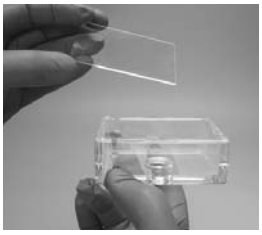


Figure 3-1. Slide

1. A 2 x 3” slide fits into the casting system. A package of 10 is included in the complete system. These slides are also available from Technical Services, part # C-GS.

2. Prepare agarose solution. Pour the gel.

    a. 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 (see Section 6). 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° before casting to avoid warping the caster 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° in a water bath. This provides a ready gel supply in a warm liquid form that will solidify quickly when gels are cast. For further tips on sample preparation and visualization, see Section 6.

3. Pour 12ml of gel solution into the casting unit.

4. Place comb into slot provided on walls of caster. A 7-tooth comb for 2” wide gels is available from Technical Services, part # C2-7D.

5. Allow gel 15 minutes to solidify and remove comb.

6. To remove glass slide and gel from caster, simply place finger into hole.

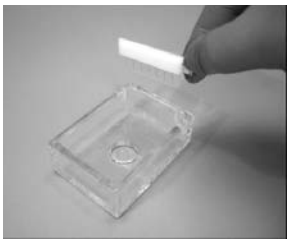


Figure 3-2. Comb

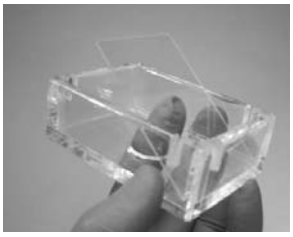


Figure 3-3. Finger Hole

Casting Gels (continued)

7. Place the glass slide on the platform of the Microgel unit. Add running buffer and load samples into wells.

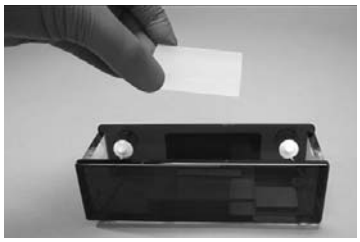


Figure 3-4. Slide on Platform

Section 4 Using the System

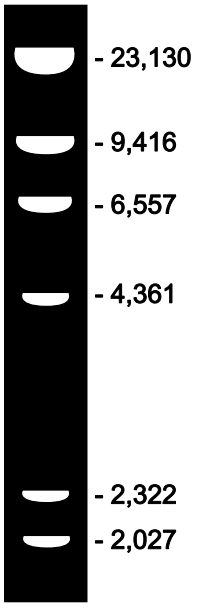
Running the Gel

The Microgel System can be run at voltages in the range of 20 - 70 volts. Our suggested running condition is 30V. However, this can be adjusted to speed up a run or take into consideration the ionic strengths of different running buffers.

Loading the Sample in Gel

1. Remove the gel from the casting chamber.
2. Place the gel tray into the buffer chamber.
3. Pour running buffer into the unit to fill chamber and completely cover and submerge the gel. See Recommended Running Conditions (page 2-2), 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 slow DNA migration in the gel.
4. 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.

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 (λ DNA width Hind III endonulease).



Finishing Up

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 disconnect the power cords from the power source. Carefully remove the tray containing the gel (wear gloves if ethidium bromide is present). The glass slide makes visualization and photography with a UV light source easy without the need to remove the gel from the tray. The gel tray may be placed back into the casting chamber for convenient transport to the darkroom to avoid damage to the gel.

Section 5 Care and Cleaning

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

The unit may be rinsed with warm water, or cleaned with warm water and a mild detergent to get rid of any debris.

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 Technical Services:

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

**Rnase Away® is a registered trademark of Molecular BioProducts, Inc.*

Section 6 Reagents Information

Selection Of Reagents For Gel Electrophoresis

Agarose Gel Casting

There are various types of agarose commercially available that may be used. In addition to standard ultra pure electrophoresis grade agarose, low melting point agarose is also available for easy sample recovery, as well as specialty 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 0.5ug/ml.

Warning Ethidium bromide is a potential carcinogen. Be careful in handling the powder and stock solution. Always wear gloves when handling the powder, solutions and all gels that contain any amount of ethidium bromide. ▲

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

Ethidium Bromide

For photodocumentation of samples, 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 visualize and photograph the separated fragments following the gel run without the need for an additonal staining step. The ethidium bromide is added to both the gel (after heating) and the electrophoresis buffer at a concentration of 0.5ug./ml. Conversely, the gel may be stained in a concentrated ethidium bromide solution after the gel run and rinsed for visualization.

Warning Ethidium bromide is a potential carcinogen. Be careful when handling the powder and stock solution. Always wear gloves when handling powder, solutions and all gels that contain ethidium bromide. ▲

Preparation and 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 EDTA " 2H2O (MW 372.24)

Distilled H2O 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 Na2EDTA " 2H2O (MW 372.24) (or 40ml 0.5M EDTA, pH 8.0) . . . . . .2mM EDTA

Choose the buffer best suited to the experiment. Each buffer has different properties providing the necessary ions for electophoretic migration.

Buffer:	Suggested Use:
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	• General Purpose Buffer • Can be re-used • For electrophoresis of small (<1kb) DNA • Better resolution of small (<1kb) DNA • Decreased DNA mobility • High ionic strength and high buffering capacity - recirculation may not be required for extended run times • Reacts with the agarose making smaller pores and a tighter matrix. This reduces broadening of the DNA bands for sharper resolution.

Sample Buffer

Samples are prepared and mixed with sample buffer before being applied to the prepared gel. Sample buffers contain similar components to the running buffer, dyes for visibility, and glycerol to provide weight to the samples. This increased sample density 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.

DNA Markers

Markers are run on each gel to monitor sample separation and to provide an accurate size estimation of the samples. By running a known marker of a specific concentration, the amount of the DNA can be estimated. These size markers are a suitable restriction digest of commonly available DNA.

Section 7 Troubleshooting

Problem	Solution
Bands seem to be running at an angle.	Check to be sure the casting is being done on a level surface. A leveling platform may be required. Make sure the gel tray is pressed all the way down and rests level on the casting chamber platform.
Samples seem to be running unevenly in certain areas.	Check to be sure the platinum electrode wire is intact and running evenly across the base of the chamber and up the side to the junction of the banana plug. If there appears to be a break in the electrode connection, contact Technical Services immediately. This problem may also be caused by regular casting with very hot agarose gel (>60°F) which may damage the gel tray over time. Always cool the melted agarose to below 60°F before casting to avoid warping the UVT gel tray. Warping the gel tray will cause all subsequent gels to be cast unevenly.
Samples do not band sharply and appear diffused in the gel.	Gels should be no more than 5mm thick and 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.
Samples are not moving as expected through gel, remaining in the wells, running "backwards" or diffusing into the gel.	Check to be sure 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 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 little to no bubbles. Contact Technical Services to schedule a repair. Samples that appear to run backwards through the gel is caused by the tray being placed in the chamber in the reverse direction. The tray should be placed in the chamber with the comb at the edge of the tray closest to the cathode side of the chamber.

Problem (continued)	Solution (continued)
When the comb is removed from the gel, the sample well is ripped and damaged.	Always make sure to allow the gel to solidify completely before moving the casting chamber, gel tray, or removing the combs. After placing the gel tray into the unit in the running position, submerge the gel in 3-5mm of running buffer. Lightly tap each comb gently back and forth to loosen, then slowly pull the comb straight up out of the gel tray. This will break any suction that may exist between the gel and comb. When using all four combs a higher percentage of agarose (>0.5%) may be wise to avoid damage to the sample wells. Low percentage gel and the small sample wells may cause the sides of the wells to collapse when the comb is removed. A higher percentage of agarose forms a tighter gel matrix. Casting a slightly thicker gel may also remedy this problem.
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. The gel should be completely submerged to avoid the gel from drying out, which can smear the bands and possibly melt the gel 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*

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