Minigel Protein Electrophoresis System

Model P82

Operating and Maintenance Manual 7007342 Rev. 0

Owl Separation Systems

MANUAL NUMBER 7007342

REV	ECR/ECN	DATE	DESCRIPTION	By
0	--	4/27/12	Transfer to Marietta (was Wolverine)	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. ▲

Warning To avoid the risk of personal shock, always disconnect the gel box from the power supply. The unit must be powered from a European certified DC power supply with a current disconnect (no load) detecting shutdown circuit. Running conditions for this unit should not exceed the name plate readings found on the side wall. 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 use as described in this manual. Do not use this product if the power leads are damaged or any of its surfaces are cracked.

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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. Unit must be powered from a European certified DC power supply with a current disconnect (no load) detecting shutdown circuit.

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.

This product is required to comply with the European Union's Waste Electrical & Electronic Equipment (WEEE) Directive 2002/96/EC. It is marked with the WEEE symbol. Thermo Fisher Scientific has contracted with one or more recycling/disposal companies in each EU Member State European Country, and this product should be disposed of or recycled through them. Further information on Thermo Fisher Scientific's compliance with this directive, the recyclers in your country and information on these products will be available at www.thermofisher.com.

- ✔ 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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Regardless of your needs, our professional telephone technicians are available to assist you Monday through Friday from 8:00 a.m. to 6:00 p.m. Eastern Time. Please contact us by telephone or fax. If you wish to write, our mailing address is:

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Section 1 Introduction

The Minigel Protein Electrophoresis System is one of the easiest systems to run for rapid analysis of protein samples in a miniature polyacrylamide gel. The model P82 is designed to provide flat, even banding with unmatched separation. The gels are easily placed in the device using an innovative wedge design, which produces a leak-resistant seal without awkward clamps or grease. The unique fabricated wedge design means no mess, no aggravation and consistent performance. Tough fabricated acrylic gives you durability that you can count on day after day. The two wedges consistently align the cassettes in the proper position. The system can run two gels or one using the blocking plate provided with the unit.

Unpacking and Check Order

Before getting started, unpack the unit and inventory your order. Please save all packing material until you verify that your order is correct and the product performs to your satisfaction. If any parts are missing, contact Technical Services immediately.

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

Model P82

2 wedges for use with thin pre-cast gels

2 wedges for use with hand-cast gels

Buffer tank with upper buffer chamber assembly and electrode wires

Safety lid with red and black power supply leads

2 blocking plates for single gel operation

Specifications and Running Conditions

MODEL P82

Buffer capacity . . . . . .1030ml maximum
Glass size . . . . . .10cm x 10cm L
Footprint . . . . . .6.89in W x 5.36in H x 4.39in D
. . . . . .(17.51cm W x 13.61cm H x 11.14cm D)
Materials . . . . . .Clean and white opaque acrylic
Voltage . . . . . .330V maximum
Current, constant 15/50mA/gel
Time requirements** . . . . . .30-90 min.
Upper chamber 130ml
Lower chamber . . . . . .250ml* (minimum)
. . . . . .900ml* (maximum)

** Minimum is amount of buffer needed in order to make contact, maximum is amount of buffer required for cooling of gel during run.*

***Running conditions and times are for standard denaturing dicontinuous polyacrylamide gels. Running conditions and times will vary with application and buffers.*

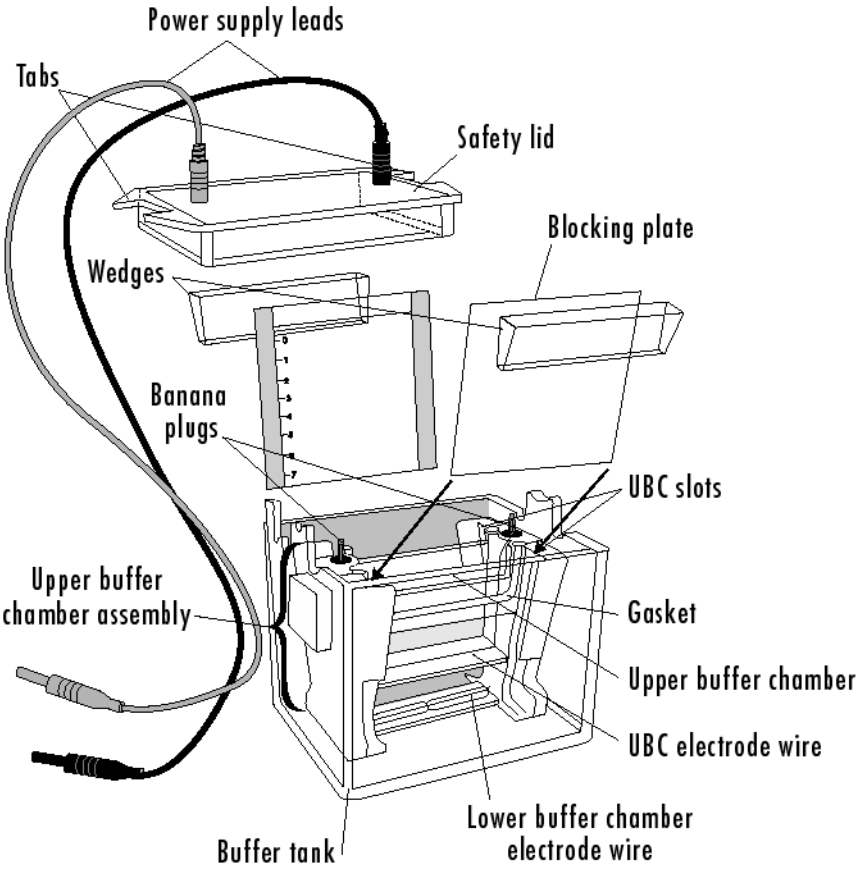

Figure 1-1. Exploded View

Section 2 Operating

1. Remove the lid from the unit by pushing upwards on the tabs and pulling the lid upwards. Remove the clear wedges from the upper buffer chamber slots. The blocking plates can then be removed.

2. Prepare your precast gel according to the manufacturer's instructions. Clean the cassette thoroughly by washing with deionized, distilled water.

3. Inserting gels into the unit:

   - For 2-10 x 10cm precast gels – Gently lower the gel into the slot with the bottom of the gel resting on the white foot of the bottom of the upper buffer chamber assembly. Choose the thicker gels meant for thin precast gel operation. Place the wedges into the upper buffer chamber slot in the orientation shown in the previous diagram. Wedges should be placed with the sloped side facing away from the gel. The gels should be closest to the gasket and the wedges should be closest to the wall of the buffer tank.

   - For single gel operation – Follow the directions above, replacing one of the gels with a blocking plate.

   - For 2- 8 x 10cm precast gels – These shorter gels cannot be placed into the unit by resting on the white foot. Outside of the unit, line the top of the wedge up with the top of the gel. Place this assembly in the upper buffer chamber slot together so that the shorter gel is held suspended in place.

   - For NOVEX™ gels – Gently lower the gel into the slot so that the bottom of the gel rests on the white foot at the bottom of the upper buffer chamber assembly. Choose the thinner wedges meant for thicker precast and hand-cast gel operation. Place the wedges into the upper buffer chamber slot in the orientation shown in the previous diagram. Wedges should be positioned with the sloped side facing away from the gel. The gels should be closest to the gasket and the wedges should be closest to the wall of the buffer tank.

   - For hand-cast gels – Follow the instructions for the Novex gels, using the thinner wedges.

**NOVEX is a registered trademark of Novel Experimental Technology.*

4. Push downward on the wedges until they are firmly in place. There is no need to force the wedge down. This could cause the gel plates to crack.

5. Add running buffer to the upper buffer chamber. See the section on gel solutions and running buffers for recipes. Check for buffer leakage from the upper buffer chamber.

6. Load samples onto the gel. Because of the visual distortion caused by the wedge, it may be easier to load the gel while looking at the notched side of the glass facing the upper buffer chamber. Elevating the tank to eye level for loading purposes can also make this process easier.

7. Add running buffer to the lower outer buffer chamber. Do not fill above the fill line. Filling the buffer chamber below the fill line is also acceptable but not recommended as this smaller volume does not provide as much heat dissipation, possibly causing smiling and other temperature problems to occur.

8. Place cover onto the unit attaching the power leads to the appropriately colored banana plug. The cutouts on the side of the lid should fit easily into sides of the buffer tank.

9. Plug the cords into an appropriate power supply. Precast gels should be run according to the manufacturer's instructions. The run time depends upon the type of gel run and the buffer used. Consult the instructions for your precast gels for run time.

These running conditions are for SDS-PAGE.

Gel Type	**Running conditions**
PAGE One	10 x 10cm - 40mA per gel
	8 x 10cm - 60mA per gel
SepraGel	25-30mA per gel
Handcast gels	0.8cm - 15-40mA per gel, 1.5cm - 40-80mA per gel
Novex	40mA per gel

Casting Your Own Gels

Casting acrylamide gels using the Vertical Gel Caster Model P82 is recommended. This caster allows for easy, leak-proof gel casting in individual pouches of up to four gels at one time. Refer to the instructions for the caster available separately. See the Gel Solutions and Running Buffers Section of this manual for instructions on casting without a casting device.

Finishing Up

1. Turn off power supply.
2. Pull the power supply leads from the power supply.
3. Remove the lid from the unit.
4. Pull the wedges upward, remove them and then pull the gel up and out of the slot.
5. Rinse the glass and proceed with staining, blotting, or other analysis.

Care and Cleaning

Caution Do not use ethanol or organic solvents to cleanacrylic products. Organic solvents cause acrylic to “craze” or crack. Some solvents will dissolve or melt the acrylic. The unit may be disassembled for easy cleaning. Clean all acrylic systems by rinsing with warm water, or clean with warm water and a mild detergent to get rid of any debris. It is important to rinse the gaskets after each use to avoid any salt build-up in the gasket material from the running buffer. This will extend the gasket life, and ensure leak-free runs. ▲

Section 3 Accessories

Description	Part #
Glass plate, blank, 10cm W x 10cm L	P7-10G
Glass plate, notched, 10cm W x 8cm L	P7-10R
Spacer sets (2 side, 1 bottom)	
0.5mm	P7-SA
0.8mm	P7-SC
1.5mm	P7-SD
0.5mm Combs	
6 wells	MP-6A
8 wells	MP-8A
10 wells	MP-10A
12 wells	MP-12A
15 wells	MP-15A
20 wells	MP-20A
0.8mm Combs	
6 wells	MP-6C
8 wells	MP-8C
10 wells	MP-10C
12 wells	MP-12C
1.5mm Combs	
6 wells	MP-6D
8 wells	MP-8D
10 wells	MP-10D
12 wells	MP-12D
Gasket set	P82DS-GK
Wedges, thin, pair	P82DS-013
Wedges, thick, pair	P82DS-012

Section 4 Gel Solutions and Running Buffers

30% Acrylamide Stock Solution 30:0.8

29.22g acrylamide

0.78g bisacrylamide

Bring to100ml with water.

Resolving Gel Buffer Stock – 8X – Tris-HCl – pH 8.8

36.3g Tris

48.0ml 1M HCl

Bring to 100ml with water.

Filter through Whatman No. 1 filter paper and store at 4°C.

Stacking Gel Buffer Stock – 4X – Tris-HCl – pH 6.8

6.0g Tris – dissolve in 40ml water

Titrate to pH 6.8 with 1M HCl

Bring to 100ml with water.

Filter through Whatman No.1 filter paper and store at 4°C.

Making Resolving Gel Solution

Note All volumes are in milliliters. These volumes will make 30ml of solution ▲

Acrylamide %	**20%**	**17.5%**	**15%**	**12.5%**	**10%**	**7.5%**	**5%**
Acrylamide solution	20.0	17.5	15.0	12.5	10.0	7.5	5.0
Resolving gel buffer stock	3.75	3.75	3.75	3.75	3.75	3.75	3.75
10% SDS*	0.3	0.3	0.3	0.3	0.3	0.3	0.3
1.5% Ammonium persulfate	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Water	4.45	6.95	9.45	11.95	14.45	16.95	19.45
TEMED	0.015	0.015	0.015	0.015	0.015	0.015	0.015

**If desired. Substitute water for SDS solution if a non-dissociating system is required.*

2.5% Stacking Gel Solution

2.5ml 30% acrylamide –bisacrylamide solution (30:0.8)
5.0ml 4X stacking gel buffer stock (see previous recipe)
0.2ml 10% SDS solution
1.0ml 10% ammonium persulfate
11.3ml water
15µl TEMED
Will make 20ml of solution

Casting Your Own Gels

For casting single percentage (not gradient) gels without a casting device, you will need the following items:

- Binder clamps
- 1 blank piece of glass, catalog # P7-10G
- 1 notched piece of glass, catalog # P7-10R
- 2 side spacers and 1 bottom spacer (Accessories section of this manual)
- Something to prop your gels up on such as a test tube rack
- Gel solution(s)
- 1 gel casting comb (Accessories section of this manual)
- Ammonium persulfate and TEMED

1. Make a sandwich of the blank and notched pieces of glass with the side and bottom spacers in between. The thickness of the spacers determines the thickness of the gel. The comb must be of the same thickness.

2. Clamp this sandwich tightly using binder clamps along the spacers. Do not clamp beyond the spacers. This assembly can be sealed by using agarose around the edges, grease on the spacers, or casting tape around the edges.

3. Prop this assembly up to allow for pouring of the acrylamide solution. Once everything is assembled and you are ready to pour the ammonium persulfate and TEMED can be added and mixed into the solution.

4. Carefully pipet the resolving gel solution in between the glass plates, allowing room for any stacking gel. Carefully layer n-butanol over this resolving gel and allow it to polymerize for 15 to 30 minutes.

5. After polymerization, pour out the n-butanol and rinse the surface of the resolving gel with distilled water.

6. Add the comb and the stacking gel and allow to polymerize.

Volumes Of Solution Required For Gels

These volumes have been calculated using the glass available for the unit, available spacers, and subtracting the volume of the spacers and the notch. These are approximate volumes. See the list of available accessories in Section 3.

Unit	P82
0.5mm spacer	.4ml
0.8mm spacer	.6ml
1.5mm spacer	.11ml

Sample Buffers

Non-denaturing protein sample solubilization buffer

Tris-HCl – 2X concentration (250mM Tris-HCl, pH 6.8, 30% glycerol, 0.0050% Bromophenol blue)

Dissolve 1.51g Tris base in 10 ml of water and pH to 6.8 with concentrated HCl dropwise. Add 2.5ml of a 0.1% bromophenol blue solution and 15ml of glycerol. Bring to 50ml with water.

Denaturing protein sample solubilization buffer

Tris-SDS – 2X concentration (250mM Tris-HCl, pH 6.8, 2% SDS, 30% glycerol, 0.0050% Bromophenol blue)

Dissolve 1.51g Tris base in 10 ml of water and pH to 6.8 with concentrated HCl dropwise. Add 2.5ml of a 0.1% bromophenol blue solution, 15ml of glycerol, and 2ml of a 10%(w/v) SDS solution. Bring to 50ml with water.

Denaturing reducing protein sample solubilization buffer

Tris-SDS-BME – 2X concentration (250mM Tris-HCl, pH 6.8, 2% SDS, 10% BME, 30% glycerol, 0.0050% Bromophenol blue)

Dissolve 1.51g Tris base in 10ml of water and pH to 6.8 with concentrated HCl dropwise. Add 2.5ml of a 0.1% bromophenol blue solution, 15 ml of glycerol, and 2ml of a 10%(w/v) SDS solution. Add 5mls of ß-mercaptoethanol in a fume hood and bring the solution to 50ml with water.

Running Buffers

Tris-Glycine Buffer for non-denaturing native protein gels

25mM Tris, 192mM Glycine, pH 8.5 final 1X concentration

29.0g Tris

144.0g Glycine

Bring to 1L with water for a 10X buffer.

Tris-Glycine-SDS for denaturing protein gels

25mM Tris, 192mM Glycine, 0.1% SDS, pH 8.5 final 1X concentration

29.0g Tris

144.0g Glycine

10.0g SDS

Bring to 1L with water for a 10X buffer.

Tris-Borate-EDTA for nucleic acids

89mM Tris, 89mM Boric acid, 2.6mM EDTA, pH 8.3 final 1X concentration

54.0g Tris

27.5g Boric acid

2.9g EDTA

Bring to 1L with water for a 10X buffer.

Section 5 Troubleshooting

Below are some possible solutions to potential problems. If these suggestions are unsuccessful, call Technical Services or your local distributor..

Problem	Cause	Solution
1) Longer run time	Buffer is too diluted	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. Be sure that the correct wedges are being used; thick wedges for thin precast gels and thin wedges for thicker hand-cast gels.
	Running at too low a current	Make sure you are running using the suggested running conditions for this unit. When running at constant current, the current value is per gel.
2) 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 diluted.
		Turn down current setting.
3) Smiling of dye front	Center of gel is running hotter than the ends	Turn down current setting.
4) Bands spreading outward	Diffusion of sample when loading	Make sure the samples are loaded quickly and the power is applied as soon as possible after loading.
	Diffusion of sample during run in 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

Problem	Cause	Solution
5) Bands are narrower than the sample wells	Ionic strength of sample is higher than that of the gel	Desalt the sample or use sample buffer of the same strength as the gel
6) Broad lanes at bottom of gel	Will occur when adjoining lanes are loaded with dissimilar samples	Make sure that the salt concentration in all samples is similar
	Normal in gradient gels	
7) Skewed bands	Gel has not polymerized properly at wells	Degas gel solution before casting and increase APS and TEMED concentration. 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
	Resolving gel is uneven at top	Overlay carefully using water saturated n-butanol and make sure casting stand is level
	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 seal with the gasket
8) Streaked bands	Overloading of sample	Use less protein or sample when next loading
	Sample has precipitated	Centrifuge sample before adding sample buffer or use a lower % acrylamide gel.
9) 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 the gasket is seated firmly against the glass
10) 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 sample
11) Glass cracks when putting gel assembly into unit	Wedges are being put in too forcefully	The wedges will seal the upper buffer chamber by pressing the gasket against the gel cassette. If they are forced downward, the glass may crack.
	Wrong wedges are being used	Be sure to choose the correct wedges for your gel; thick wedges for thinner precast gels and thin wedges for thicker hand cast gels.

Section 6 Care and Cleaning

A few tips about caring for your system follow.

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

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The Warranty Period starts two weeks from the date your equipment is shipped from our facility. This allows shipping time so the warranty will go into effect at approximately the same time your equipment is delivered. The warranty protection extends to any subsequent owner.

During the first thirty-six (36) months, component parts proven to be non-conforming in material or workmanship will be replaced at Thermo's expense, including labor. Installation, calibration and certification is not covered by this warranty agreement. The Technical Services Department must be contacted for warranty determination and direction prior to performance of any repairs. Expendable items, glass, filters and gaskets are excluded from this warranty.

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