GIBCO BRL®

Instruction Manual

Mini-V8·10 Vertical Gel Electrophoresis System

CAT. SERIES 21078

LIFE TECHNOLOGIES®

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Table of Contents

Table of Contents

IMPORTANT:

1. **Read and carefully follow manual instructions.**
2. **Do not alter equipment. Failure to adhere to these directions could result in personal and/or laboratory hazards, as well as invalidate equipment warranty.**

1 Notices to Customer

1.1 Important Information

This product is authorized for laboratory research use only. The product has not been qualified or found safe and effective for any human or animal diagnostic or therapeutic application. Uses for other than the labeled intended use may be a violation of applicable law.

If the product is used in a manner not specified by the manufacturer, the protection provided by the product may be impaired.

1.2 Warnings

1. **DANGER! HIGH VOLTAGE!** This system requires a 100 to 250-V DC power supply for operation and is therefore a potential shock hazard. Although equipped with a safety lid, this equipment should always be operated with caution. Careless handling can result in electrical shock. The system should be operated by trained personnel only. This manual should be readily accessible to all users.
2. Never operate damaged or leaking equipment.
3. Always turn off the DC power source prior to removing the lid from the apparatus. Disconnect power cords from the power supply first, and then remove the lid.

4. For maximum safety, always operate this system in an isolated, low-traffic area that is not accessible to unauthorized personnel.
5. Before operating this equipment, make sure that all electrical connections are secure and that the power cords show no signs of damage. See section 6.2, for additional information.
6. Certain reagents indicated for use in this manual are of a hazardous nature. The researcher is cautioned to exercise care when handling these reagents. The equipment used in these procedures (*e.g.,* high voltage power supplies) should be used following the manufacturer's safety recommendations.

2 Overview

2.1 Description

This manual provides operating instructions for the Mini-V 8•10 Vertical Gel Electrophoresis System in both electrophoresis and electrophoretic blotting applications. The electrophoresis apparatus and blotting modules are available both together as a system and separately.

The Mini-V 8•10 System is designed for vertical polyacrylamide gel separations of proteins and nucleic acids and for electrophoretic blotting of the separated molecules from the gel to a membrane. Both the electrophoresis and blotting modules accept one or two 8 × 10-cm, 0.5, 0.75, or 1.5-mm thick gels, in any combination. Glass gel plates can range from 7 to 8.2-cm long and from 1 to 2-mm thick, and can be of different or equal length. The maximum blotting area is 7.5 × 9 cm. No “blank gels” or sealing plates are required for single gel operation.

The unique gel-support system simplifies gel handling by eliminating the need for clamps or gaskets. The rim-and-ledge design effectively separates the buffer tank into an upper and a lower reservoir, and also allows a single set of electrodes to serve for both gel electrophoresis and electrophoretic blot transfer. The electrode placement and reservoir shape yield electrophoretic

separations with minimal “smiling” and uniform, efficient blotting. Because sealing between the rim and ledge and between the gel wedge block and gel plate assembly is not liquid-tight, a portion of the electrical current bypasses the gel during operation, similar to the excess current resulting from “submarine” operation of horizontal gels.

Although this unit may, as a result, have a substantially different current requirement than a conventional apparatus at a comparable voltage setting, performance in gel electrophoresis or electrophoretic blotting is not affected. The Mini-V 8•10 System is not appropriate for use in isoelectric focusing or other procedures in which different buffers are required for the anode and the cathode.

Note: The standard Mini-V 8•10 System is provided with a platinum/niobium lower electrode and a type 316 stainless steel upper electrode. In the standard configuration, the unit must not be operated in a reverse polarity [upper electrode as anode (+)] or used for field inversion gel electrophoresis (FIGE). A replacement platinum/niobium upper electrode is available for reverse polarity applications; however, it may not be used for FIGE (see Chapter 5, Related Products).

2.2 Components

The Mini-V 8•10 Vertical Gel Electrophoresis System is designed for easy assembly and reliable performance. The electrophoresis system is composed of a buffer tank unit and both the electrophoresis module and the blot module. The electrophoresis apparatus (buffer tank unit with electrophoresis module) and blot module are available separately. Refer to figure 1 to identify the following principal components:

Buffer Tank Unit

- Electrophoresis/blotting buffer tank and safety lid with integral power cords.

Electrophoresis Module

- Gel support frame and gel wedge block
- Glass plates [two short (7.25 × 10.25-cm) and two long (8.25 × 10.25-cm)] with spacers and combs*
- Gel loading template for 6 and 10-toothed combs (four provided)*

Blot Module

- Blot support frame, blot restrainer, and blot clamping knob
- Transfer pressure pads*
- Blotting membranes and filter paper sheets*

Many of these components are available separately. For ordering information, refer to Chapter 5.

* Not shown.

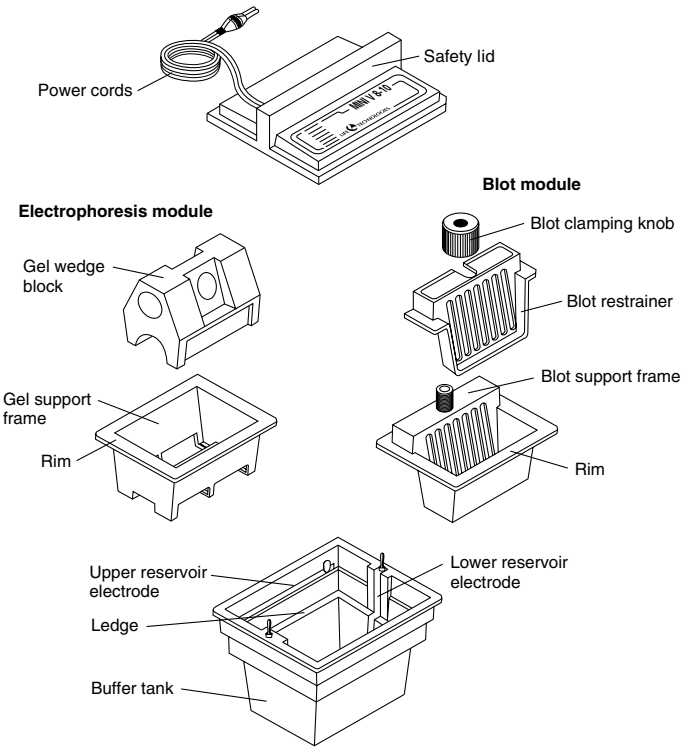

Figure 1. Mini-V 8•10 Vertical Gel Electrophoresis System.

3 Operating Instructions

This chapter provides operating instructions for the Mini-V 8•10 Vertical Gel Electrophoresis System in both electrophoresis and electrophoretic blotting applications. Please read all of the instructions before using the Mini-V 8•10 System. Review figure 1, as needed, to identify features and components discussed in these instructions.

3.1 Gel Casting

Individual gels can be cast after sealing the glass plate and spacer assembly with tape, with agarose, or with the Mini-V Casting Clamp as described in the following protocols. These techniques are appropriate for casting either polyacrylamide or agarose gels. For casting multiple gels at one time, or for polyacrylamide gradient or buffer-gradient gels, the Mini-V 8•10 Gel Casting System can be used. Consult the Operating Instructions for the Mini-V 8•10 Gel Casting System for more information on these procedures.

3.1.1 Preparing an Individual Gel Plate Assembly

1. Make sure the glass plates, spacers, and combs are clean and free of dried gel fragments or dust.
2. Lay a long plate on a clean surface. Place one spacer along each short edge. Next, place the short plate on top of the spacers so that the

sides and bottoms of both plates and the spacers are approximately even. Stand the plates up on a flat surface to align the bottom edges.

Note: For gels 1 cm longer, use two long plates instead of one long plate and one short plate.

3. Clamp the assembly together with a spring clip (available separately) at each short edge over the spacers (figure 2). Push the spring clips to the bottom of the assembly so it will stand upright.

   Note: Spring clips should grip the glass plates over the side spacers *only*. Clamping unsupported glass will distort the thickness of the gel.

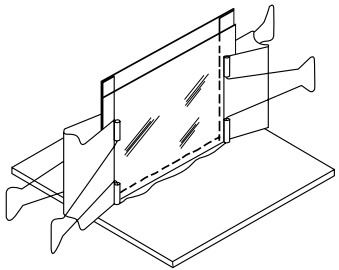

Figure 2. Sealing the Gel Plate Assembly with Agarose.

4. If discontinuous polyacrylamide gels are being prepared, in which case a stacking gel will be cast later, insert a comb and mark the short plate 0.5 to 1 cm below the bottom edge of the teeth to allow space for the stacking gel. Remove the comb before casting the resolving gel.

5. Prepare 5 to 10 ml of molten 1% (w/v) agarose in electrophoresis or resolving gel buffer. Allow it cool to 50°C to 60°C.
6. On the surface of a glass support plate, apply a straight line of molten agarose 10 cm long. Immediately place the glass plate assembly upright on the line of agarose (figure 2). By capillary action, the agarose will form a plug to seal the bottom of the glass plate assembly. Allow the agarose to solidify for 5 to 10 min before proceeding.

   Note: The agarose plug can be dislodged very easily, resulting in leaks. Carefully handle the assembly in subsequent steps.

3.1.2 Preparing an Individual Gel Plate Assembly Using the Mini-V Gel Casting Clamp

1. Place the short glass plate into the casting clamp making sure that it sits firmly against the bottom of the casting clamp.
2. Place the side spacers on top of the glass plate at the outside edges. The casting clamp can be used with any of the available side spacers.
3. While holding the short glass plate in place, slide the long glass plate down into the casting clamp on top of the side spacers.
4. Make sure that both glass plates and the side spacers are properly positioned and firmly seated in the casting clamp. The sides of the casting clamp will extend above the top of the glass plates when casting 8 × 10 cm gels.
5. Before casting a gel, make sure that the bottom fill port is sealed.

3.1.3 Preparing Discontinuous Buffer System Gels

Warning: Acrylamide is a known neurotoxin. Consult the Material Safety Data Sheet for more information.

1. Prepare the acrylamide solutions (see section 6.3.1 for a formulation for Tris-glycine discontinuous gels for protein analysis). Each 1.5-mm thick gel requires approximately 10 ml of resolving gel solution; each 0.5 or 0.75-mm thick gel requires approximately 5 ml. A stacking gel of either thickness can be cast with 5 ml of solution.

   Note: A gel loading template is provided to aid in locating wells for sample loading. Wells may also be visualized by tinting the gel: to give the stacking gel a pale blue color, add 5 µl of a saturated solution of bromophenol blue in water per 10 ml of stacking gel solution.

2. Carefully pipet the resolving gel acrylamide solution into the gel plate assembly, filling to 1 to 2 mm above the desired level. Overlay the solution with a few drops of water-saturated n-butanol to keep the gel surface flat and the interface sharp during polymerization. Keep the assembly vertical while the acrylamide polymerizes.

3. After the resolving gel has polymerized (15 to 30 min), pour off any unpolymerized solution and butanol. Rinse the top of the gel with deionized water and drain thoroughly. Move the clips up to grip the glass over the spacers at the top of the short plate.

4. Add stacking gel solution to within 2 mm of the top of the short plate. Break any small bubbles by touching them with 1 to 2 µl of n-butanol. Insert the comb fully. Be careful not to trap bubbles under the comb teeth.

5. Tilt the plate assembly back to a 20° to 30° angle (figure 3), and add 100 to 200 µl of additional stacking gel solution to the top of the gel. This will improve polymerization at the outside edges of the comb. Allow the acrylamide solution to polymerize completely before removing the comb.

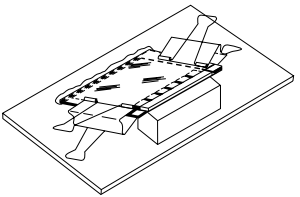

Figure 3. Tilting the Gel During Well Polymerization.

3.1.4 Preparing Continuous Buffer System Gels

Warning: Acrylamide is a known neurotoxin. Consult the Material Safety Data Sheet for more information.

1. Prepare the acrylamide solution (see section 6.3.2 for a formulation for TBE gels for DNA analysis). Each 1.5-mm thick gel requires approximately 10 ml of resolving gel solution; each 0.5 or 0.75-mm thick gel requires approximately 5 ml.

   Note: A gel loading template is provided to aid in locating wells for sample loading. Wells may also be visualized by tinting the gel: to give the gel a pale blue color, add 5 µl of a saturated solution of bromophenol blue in water per 10 ml of stacking gel solution.

2. Taking care not to disturb or dislodge the agarose plug, carefully move the clips up to grip the glass over the spacers at the top of the short plate.

3. Fill the plate assembly with acrylamide solution to within 2 to 3 mm of the top of the short glass plate. Break any small bubbles by touching them with 1 to 2 µl of n-butanol. Insert a well forming comb, taking care not to trap bubbles under teeth.

4. Carefully, so as not to dislodge the agarose plug, tilt the glass plate assembly back to a 20° to 30° angle (figure 3). Add 100 to 200 µl of additional acrylamide solution to the top of the gel. This will improve polymerization at the edges of the comb. Allow the gel solution to polymerize completely before removing the comb.

3.2 Assembling the Gel Electrophoresis Apparatus

1. Carefully remove the comb. Rinse the comb with deionized water. Rinse off any thin sheets of polyacrylamide or agarose on the outside of the gel plate assembly. Keep the agarose plug in place to prevent trapping bubbles under the gel. Remove the spring clips and, if used, all gel sealing tape.

2. Place the buffer tank on a level surface and set the gel support frame into the empty tank. Make sure the rim of the gel support frame is evenly seated completely around the ledge of the tank.

3. Place a gel loading template against the long glass plate, aligning the well outline with the sample wells of the gel. Use a few drops of buffer on the glass to hold the template in place. Place the gel plate and template assem-

bly into the gel support frame with the long plate and gel loading template resting vertically against the frame (figure 4).

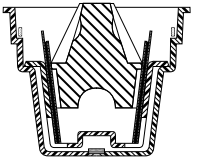

Figure 4. Cross Section of the Electrophoresis Apparatus.

Note: Make sure that the plates lie flat against the long sides of the frame, and the bottoms of the plates touch the support frame. Also, check that the wells and template remain aligned. If only one gel is being used, no "blank" glass is required on the opposite side.

4. Add 600 to 650 ml of electrophoresis buffer to the tank. To reduce the temperature during electrophoresis, this buffer may be chilled to 4°C in advance.

5. Gently place the gel wedge block into the gel support frame until it is pressed firmly against the glass plates (figure 4). When the wedge block is fully seated, the electrophoresis buffer should cover the upper electrode by 3 to 5 mm, but be 8 to 10 mm below the top edge of the tank. Add more buffer if needed. Make sure that no bubbles are trapped under the bottom edge of the gel. Bubbles trapped under the rim or between the gel and the wedge block will not affect performance.

   Caution: *Do not* overfill the buffer tank. Damage to the apparatus may result.

3.3 Electrophoresis

3.3.1 Loading Samples

1. Carefully rinse the wells with electrophoresis buffer to remove bubbles, polyacrylamide fragments, and any residual unpolymerized acrylamide. Straighten the well walls with a fine-tipped pipette.

2. Load the samples into the wells with a syringe or fine-tipped micropipette. Samples should contain 10% (w/v) sucrose, ficoll, or glycerol to increase sample density and reduce mixing during loading. When the gel loading template is used or the gel is tinted with bromophenol blue, the wells can be located easily. Sample loading volumes are given in table 1.

   Note: If the gel loading template is not used, locating wells in an untinted gel can be made more convenient by either of the two following methods:

   Method A. Outline the wells with a water-resistant marking pen on the long glass plate before assembling the apparatus.

   Method B. Remove the gel wedge block temporarily and view the wells of the nearer gel through the front side of the buffer tank. Removing the gel wedge block will lower the buffer level below the edge of the short glass plate; samples can then be loaded under the buffer remaining in the wells. If the tank must be turned to load the second gel, make sure that the gels do not shift position. Replace the gel wedge block carefully.

Table 1. Sample Loading Volumes for the Mini-V 8•10 Apparatus Combs as a Function of Gel Thickness.

Number of Teeth	Tooth Width (mm)	Gel Thickness (mm)	Nominal Capacity/Well (µl)
1 (center)	63.4	1.5	475
6	10.7	0.5 0.75 1.5	25 40 80
10	5.6	0.5 0.75 1.5	12 20 40

Note: All loading volumes are calculated for a sample depth of 5 mm.

3.3.2 Electrophoresis

1. Place the safety lid on the buffer tank and attach the power cords [red to (+), black to (–)] to the power supply.

   Note: Make sure the safety lid sits securely on the buffer tank. The safety lid and buffer tank are indexed on one corner so the lid will seat properly in only one orientation.

   The red (+) lead is connected to the lower (red) electrode, which is the anode during electrophoresis. In the standard configuration, *do not* reverse connections at the power supply to use the upper (black) electrode as the anode.

This electrode is made of stainless steel and will corrode if used as an anode. If the unit must be used in this reverse orientation, you must first install a platinum/niobium replacement upper reservoir electrode, available separately.

2. Turn on the power supply and adjust the settings to the desired voltage or current. Standard SDS gel electrophoresis is conveniently performed at 125 to 200 V (constant voltage) for 45 min to 1 h. Typical operating currents and temperatures for both conventional apparatus and the Mini-V 8•10 System are shown in table 2. A 5% polyacrylamide DNA gel in TBE buffer requires ~1 h at 100 V.

   Note: The first time the apparatus is used for a particular type of gel, monitor the progress of electrophoresis frequently. At the same voltage or current settings, electrophoresis times may differ from other brands of apparatus due to differences in electrode placement and bypass currents.

   Note: During the course of a separation at constant voltage, current will drop as bubbles collect under the rim and effectively improve the seal. With either constant voltage or constant current, the temperature will rise due to ohmic heating of the solution. The extent of the rise will depend on the buffer composition, voltage, and current settings. To reduce the final temperature, begin with chilled electrophoresis buffer, reduce the voltage or current settings, or work in a cold room.

Operating Instructions

Table 2. Typical Operating Parameters for Protein Electrophoresis.

Apparatus Type	Voltage (V)	Current (mA)	Temperature[a] (°C)
Conventional	200	84–47	44–47
Mini-V 8•10	200	200–150	40–48

[a]Upper buffer chamber at completion, starting with room temperature buffer, and with two 0.75-mm thick discontinuous buffer system protein gels (formulations in Chapter 6).

3.3.3 Post-Electrophoresis

1. When the marker dye front has reached the bottom of the gel, turn off the power supply, disconnect the leads, and remove the safety lid.
2. Grasp the gel wedge block and carefully lift it out of the apparatus. It may be slippery in detergent-containing solutions. If the gel support frame lifts out with the gel wedge block, allow the assembly to drain into the tank before setting it on the bench; then gently rock the wedge block along its long axis to work it loose and remove it from the gel support frame.
3. Remove the gel plate assembly from the gel frame and lay it on a paper towel. Use a thin spatula to carefully pry the upper glass plate away from the gel.
4. Transfer the gel to a container of stain, fixative, or transfer buffer for further processing.
5. Rinse all apparatus components thoroughly in deionized water and wipe or air dry.

3.4 Electrophoretic Blotting

The following protocols have been found to yield reliable transfer of proteins to nitrocellulose membranes and DNA to nylon membranes. However, these protocols should be used *only* as a starting point for developing the most efficient procedure for the particular proteins or nucleic acids under investigation. Suggestions for troubleshooting some common problems are given in Chapter 4. For more detailed information on protein blotting techniques, see *Antibodies: A Laboratory Manual* by E. Harlow and D. Lane (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988)

Note: Wear gloves while handling gels, membranes, filter paper, or transfer pressure pads to avoid fingerprint artifacts.

3.4.1 Preparing a Protein Gel for Blotting

1. For each transfer, prepare in advance 1.5 L of transfer buffer and store it at 4°C. The formulation for a transfer buffer suitable for many proteins is given in section 6.3.1.

   Note: Variations in the pH, the concentration of methanol, or the presence or absence of detergents may improve the efficiency of transfer and membrane binding of specific proteins.

2. After electrophoresis, equilibrate the gel in 50 ml of cold transfer buffer for at least 15 min for 0.75-mm thick gels, longer for thicker gels. Proceed to Section 3.4.3.

Note: Gels must be thoroughly equilibrated to remove the salts and detergents present in the electrophoresis buffer. If gels are not equilibrated, blotting will generate excess heat during the transfer, and gels of less than 12% polyacrylamide will shrink due to the methanol in the transfer buffer.

3.4.2 Preparing a Nucleic Acid Gel for Blotting

1. For each transfer, prepare in advance 1.5 L of transfer buffer and store it at 4°C. The formulation for 0.5X TBE, a transfer buffer suitable for most DNA transfers, is given in section 6.3.2.

   Note: This transfer buffer is for use *only* with nylon membranes.

2. If the samples are double stranded DNA, the DNA must be denatured in the gel after electrophoresis before blotting. An effective glyoxal/dimethylsulfoxide method is described in section 5.3, but alkaline denaturation techniques may also be used.

3.4.3 Blot Transfer

1. Cut a piece of blotting membrane to dimensions slightly larger than the gel. Immerse the membrane in transfer buffer and allow it to wet completely. There should be no dry spots in the membrane.

   Note: Handle membranes and filter paper only with gloved hands or clean forceps. Membranes and filter paper are also available precut. See Chapter 5 for ordering information.

2. Cut two pieces of heavy filter paper to the same dimensions as the transfer pressure pads. Saturate both pieces and three pads in transfer buffer. For blotting two gels, saturate four pieces of filter paper and three pads.
3. Place the blot restrainer in a suitable container, such as a glass baking dish [20-cm (8-in.) square], containing 1 to 2 cm of cold transfer buffer. The wide, slotted face of the restrainer forms a tray on which the transfer stack is assembled (figure 5).
4. Place one transfer pressure pad on the restrainer. Cover it with one piece of wet filter paper. If the stack is not under the surface of the transfer buffer, use a Pasteur pipette to saturate the filter paper thoroughly.
5. Place the gel in the center of the filter paper, and then wet the surface of the gel with additional buffer. Make sure no bubbles are trapped under the gel.
6. Holding the blotting membrane at both ends in a “U” shape, lower it onto the gel, making contact first at the center. Carefully lower one end, then the other, taking care not to trap any bubbles between the membrane and the gel. Saturate the surface of the membrane with more buffer, and use a test tube or gloved finger to smooth the membrane into close contact with the gel and to force out any small bubbles.

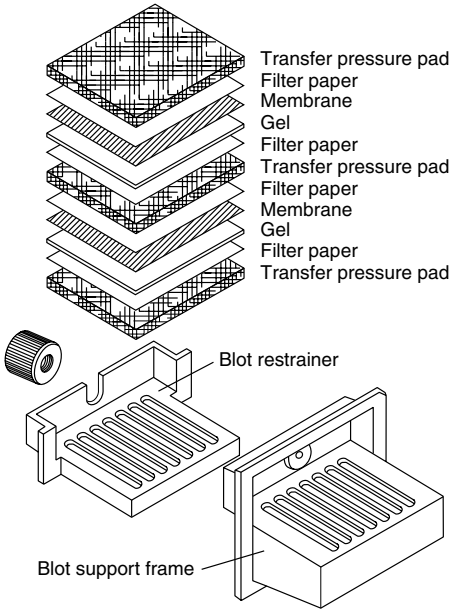

Figure 5. Blotting Stack Assembly.

Note: Once the membrane makes contact with the gel, do *not* move it. Moving the membrane may cause spurious “shadow” bands due to a small amount of rapid capillary transfer.

7. Place a piece of wetted filter paper on top of the membrane, followed by another transfer pressure pad.

8. If a second gel is to be blotted simultaneously, repeat steps 4 through 7, using the second pad as a base for the second gel. If only one gel is to be blotted, place the third pressure pad on top of the stack for proper clamping thickness.

   Note: Be careful not to overload samples in the first gel in the stack. Overloaded bands may transfer through the first membrane, through the second gel, and to the second membrane, where they will bind in a smeared patch. To avoid this problem, blot gels singly, or include a second backup membrane for the first gel to reduce the chance of overloaded bands reaching the second gel membrane.

9. Fill the buffer tank with 1 L of chilled (4°C) transfer buffer.

10. Slide the assembled blotting stack into the blot support frame. This is best done by holding the blot restrainer with one gloved hand, with your thumb pressing down gently on the pad and your fingers under the slotted panel. With the other hand holding the blot support frame with its slotted panel up, slide the blot restrainer and blotting stack into the blot support frame. Once the blot restrainer is fully in the frame, squeeze the blot restrainer firmly and evenly: with a blotting stack consisting of one or two gels and three pressure pads, the top edge of the restrainer should align with the top back edge of the clamping surface of the support frame. Secure the blot restrainer in position by tightening the blot clamping knob.

Note: After hand pressure is released, the lower edge of the restrainer will spring out slightly to its proper position.

11. Place the assembled blot module in the buffer tank in either orientation (figure 6). Be sure the buffer covers the upper electrodes by 3 to 5 mm, but is 8 to 10 mm below the top edge of the tank.

    Caution: Do not overfill the buffer tank. Damage to the apparatus may result.

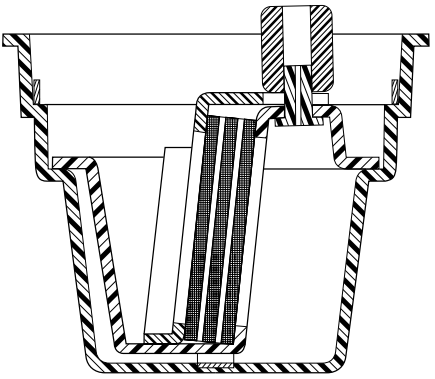

Figure 6. Cross Section of the Blot Module.

12. Place the safety lid on the buffer tank, and connect the power cords [red to (+), black to (–)] to a power supply.

Note: Make sure the safety lid sits securely on the buffer tank. The safety lid and buffer tank are indexed on one corner so the lid will seat properly in only one orientation. The red (+) lead is connected to the lower (red) electrode, which is the anode during electrophoresis. In the standard configuration, *do not* reverse connections at the power supply to use the upper (black) electrode as the anode. This electrode is made of stainless steel and will corrode if used as an anode. If the unit must be used in this reverse orientation, you must first install a platinum/niobium replacement upper reservoir electrode, available separately.

13. Turn on the power supply and adjust it to the proper settings. The optimum time and voltage will vary, depending on the nature and size of the molecules being transferred.

    For protein blots, a starting point is 150 V (constant voltage). Transfer from a 0.75-mm thick, 12% polyacrylamide gel requires ~1 h in the transfer buffer given in section 6.3.1; the current will start at ~85 to 120 mA, increasing to ~130 to 160 mA during the transfer. Typical operating currents and temperatures for electrophoretic protein blotting, for both conventional apparatus and for the Mini-V 8•10 System, are shown in table 3.

    For nucleic acid gels, use 300 mA (constant current) for 1 to 2 h as the starting point. In this case, voltage will drop during the transfer.

    To check for completeness or transfer with either type of gel, stain the gel afterwards.

Operating Instructions

Table 3. Typical Operating Parameters for Protein Blotting.

Apparatus Type	Voltage (V)	Current (mA)	Temperature[a] (°C)
Conventional	100	220–400	28–35
Mini-V 8•10	150	85–160	23–35

[a]Final temperature, starting with buffer at 4°C.

14. When transfer is complete, turn off the power supply and disconnect the leads. Remove the lid and lift the blot module from the tank. Discard the transfer buffer; *do not* reuse it.

15. Disassemble the blot module and stack, using gloved hands and forceps. Discard the filter paper. Process the membranes as desired. Rinse all apparatus components, including pressure pads, in deionized water after each use. Pressure pads will become compressed after several uses and should be replaced.

4 Troubleshooting Guide

Many instrument problems can be solved by carefully following the instructions in this manual. Some suggestions for troubleshooting are given below. Should these suggestions not resolve the problem, call the the Life Technologies TECH-LINE℠ at one of the numbers listed on the back cover of this manual. If the unit must be returned for repair, please contact the Customer Service Department or your local distributor for shipping instructions. Please include a full description of the problem.

Problem	Comment
Electrophoresis:	
The glass plates crack	The gel wedge block may be too tight. Insert it just enough to secure the gel assembly. It will expand slightly as the buffer warms during electrophoresis. The voltage or current setting may be too high, causing overheating. The electrophoresis buffer may be too concentrated, leading to too high a current and overheating at constant voltage.
The gel dye front is not straight	Verify that the system is level. Verify that the buffer depth is sufficient to cover the upper electrode by 3 to 5 mm. The gel may be overheating. Reduce the voltage or current. Use chilled electrophoresis buffer.

Problem	Comment
Bands are distorted or streaked	Samples may contain excess salt. Dialyze or desalt before loading on gel. Samples may be too concentrated. Dilute samples or reduce voltage. Samples may contain precipitated material. Centrifuge or filter before loading on gel. The upper surface of the resolving gel may not be flat. Use water-saturated n-butanol to overlay the gel solution, rather than water or buffer. The sample well may contain small fragments or films of polyacrylamide. Rinse wells thoroughly before loading samples.
Lanes are broader at the bottom than the top	This is normal if adjoining lanes are not loaded with similar samples. It is also normal for polyacrylamide gradient gels.
Dye migration is slower than expected	The electrophoresis buffer may be too concentrated. Check buffer preparation procedure. If the concentration is high at constant current, the voltage will be lower than usual. Check voltage or current settings on power supply. Check for secure connections of lid and power cords.
Dye migration is faster than expected	The electrophoresis buffer may be too diluted. Check buffer preparation procedure. Check voltage or current settings on power supply.

Problem	Comment
The upper electrode and/or buffer becomes brown or rusty	The standard stainless steel upper (black) electrode may be connected to the power supply in the wrong polarity. It must be used as the (–) electrode (cathode) only. The safety lid may not be on in the proper orientation. Check that the indexed corners of the lid and the tank match.
The red electrode banana plug rusts, or red insulation blackens	The buffer tank may be overfilled. The buffer level should be kept at least 8 to 10 mm below the top edge of the tank.
The safety lid rocks or does not seat evenly	The safety lid may not be on in the seat proper orientation. Check that the indexed corners of the lid and the tank match.
Electrophoretic Blotting:	
The blot transfer is uneven or has spots	The blotting membrane must make full, even contact with the gel in the blot stack. There should be no bubbles between the layers of filter paper and gel or membrane in the blot stack. The gel must be fully equilibrated in transfer buffer.
Bands streak on the gel contact side of the filter	Clamping pressure on the blotting stack must be firm. With one or two gels and three blot pressure pads, the top edge of the blot restrainer should be approximately even with the top back edge of the clamping surface of the blot support frame.

Problem	Comment
	The gel may be overloaded. Samples may electrophorese through one gel and membrane and bind to the second membrane in the stack. Check for overload by placing two membranes on the same gel. If unsure about overload, blot only one gel at a time to avoid transfer through the second gel.
	Some proteins may not bind well in certain transfer buffers. Try other transfer buffer compositions.
Sample detection is missing or weak	Verify proper order of gel and membrane in the blotting stack.
	For larger molecules, increase the transfer time.
	Decrease the acrylamide or crosslinker concentration to increase large molecule transfer mobility.
	Do not transfer small molecules for as long or use a higher acrylamide concentration.
	The wrong type of membrane was used. Some types of nylon do not bind proteins, and nitrocellulose will not bind nucleic acids in transfer buffers used for electrophoretic blotting.
	For proteins, include some SDS in the transfer buffer to ensure that the protein has a net negative charge.
	For smaller proteins, use smaller pore (0.22 µm) membranes.
	For proteins, include a lane of prestained protein molecular weight standards for a visual check on the transfer procedure.

5 Related Products

The following accessories and replacement parts are available separately for use with the Mini-V 8•10 Vertical Gel Electrophoresis System:

Product	Cat. No.
Spacer Set complete with two sets of side spacers	
0.5-mm thick	11958-139
0.75-mm thick	11958-147
1.5-mm thick	11958-154
Analytical Teflon® Combs	
6-tooth: 0.5-mm thick	11958-071
0.75-mm thick	11958-089
1.5-mm thick	11958-097
10-tooth: 0.5-mm thick	11958-105
0.75-mm thick	11958-113
1.5-mm thick	11958-121
Preparative Teflon Comb with marker lanes: 1.5-mm thick	11958-063
Custom Teflon Combs	*
Glass Plates 8.25 × 10.25 cm and 7.25 × 10.25 cm (pkg. of 10 pairs)	21078-035
Mini-V Casting Clamp	
Pkg of 1	21078-241
Pkg of 2	21078-233
Spring Clips (pkg. of 12)	11098-019

Related Products

Product	Cat. No.
Blot Module complete with 3 transfer pressure pads, 6 membranes, and 12 filter paper sheets	21078-019
Transfer Pressure Pads 7.6 × 9.1 cm (pkg. of 6)	11958-048
Blotting Membranes and Filter Paper Sheets 7.6 × 9.1 cm (pkg. of 12 sets)	
Nitrocellulose-1	11467-016**
Supported Nitrocellulose-1	11465-093**
Replacement Parts:	
Mini-V 8•10 Gel Casting System	21078-027
Platinum/Niobium Upper Electrode	11958-162
Lower Electrode Replacement Kit	11958-261
Gel Wedge Replacement	11958-287
Safety Lid Replacement (with power cords)	11958-279

* An order form for custom combs can be found in the catalogue or on our web site (www.lifetech.com).

** Not all products are available in all markets. Please contact your local Life Technologies representative for further information.

Power supplies, prepared acrylamide solutions, buffers, and other reagents suitable for use with the Mini-V 8•10 System are also available. Please consult the *Life Technologies Catalogue and Reference Guide* or contact the TECH-LINE for additional information.

6 Additional Information

6.1 Care and Handling

The principal components of the Mini-V 8•10 Vertical Gel Electrophoresis System are made from polycarbonate, ABS, and polyurethane. As with any laboratory instrument, adequate care ensures consistent and reliable performance.

After each use, rinse all components with deionized water. Wipe dry with a soft cloth or paper towel, or allow to air dry. Whenever necessary, all components may be washed gently with water and a nonabrasive detergent, and rinsed and dried as above. To remove grease and oils, use a light application of hexane, kerosene, or aliphatic naphtha. *Never* use abrasive cleaners, window sprays, or scouring pads to clean the components, as these can cause surface damage.

Additional cautions:

- Do not autoclave or dry-heat sterilize the apparatus or components.
- Do not expose the apparatus or components to phenol, acetone, benzene, halogenated hydrocarbon solvents, or undiluted laboratory alcohols.
- Avoid prolonged exposure of the apparatus or components to UV light.

Additional Information

6.2 Maintenance

The following simple, routine inspection and maintenance procedures will help ensure both the safety and the performance of the Mini-V 8•10 System. For ordering information on replacement parts, please call the TECH-LINE.

- Inspect electrical connections and power cords regularly. If the banana plug connectors to either the apparatus or the power supply do not exhibit reasonable friction or can be rocked easily, replace the banana plugs or safety lid assembly as appropriate.
- If power cords show any signs of wear or damage (*e.g.,* cracks, nicks, abrasions, or melted insulation), replace the safety lid assembly immediately.
- Examine the electrode banana plugs on the buffer tank to ensure that they are free of corrosion. If the plugs are corroded, they should be replaced.

6.3 Gel and Buffer Formulations

6.3.1 Protein Gels (Discontinuous Denaturing Gels)

Resolving Gel

To prepare 10 ml of acrylamide solution of various percentages, sufficient for two 0.75 or 0.5-mm thick gels:

	Volume (ml) by Gel Percentage			
Component	**7.5%**	**10%**	**12.5%**	**15%**
Acrylamide/bis stock solution [29% (w/v) acrylamide, 1% (w/v) bisacrylamide in deionized water]	2.5	3.3	4.2	5.0
1.5 M Tris-HCl (pH 8.8)	2.5	2.5	2.5	2.5
10% (w/v) SDS	0.1	0.1	0.1	0.1
Deionized water	4.8	4.0	3.1	2.3
10% (w/v) APS	0.1	0.1	0.1	0.1
TEMED	0.01	0.01	0.01	0.01

Stacking Gel

To prepare 10 ml of 4% acrylamide solution, sufficient for two gels:

Component	**Volume (ml)**
Acrylamide/bis stock solution [29% (w/v) acrylamide, 1% (w/v) bisacrylamide in deionized water]	1.3
0.5 M Tris-HCl (pH 6.8)	2.5
10% (w/v) SDS	0.1
Deionized water	6.0
Saturated bromophenol blue (optional)	0.005
10% (w/v) APS	0.1
TEMED	0.01

Additional Information

Electrophoresis Buffer

24.8 mM Tris base

192 mM glycine

0.1% (w/v) SDS

Final pH 8.3

At 200 V (constant voltage), bromophenol blue will move ~5 cm through a 0.75-mm thick, 10% gel in 45 to 50 min. During electrophoresis, current will start at ~200 mA and drop to ~150 mA. For buffers with twice this concentration of Tris and glycine, reduce the voltage to 125 to 150 V.

Protein Transfer Buffer

24.8 mM Tris base

192 mM glycine

10% (v/v) methanol

Final pH 8.3

Chill to 4°C before use.

At 200 V (constant voltage), ~1 h is required for transfer of proteins from a 0.75-mm thick, 12% resolving gel. During transfer, current will start at ~85 to 120 mA and increase to ~130 to 160 mA. The temperature will rise from 4°C to ~20°C to 35°C.

6.3.2 DNA Gels

TBE Buffer

100 mM Tris base

90 mM boric acid

1 mM EDTA

Final pH 8.3

For tinted gels, add 5 µl saturated bromophenol blue per 10 ml of gel solution.

At 100 V (constant voltage), ~1 h is required for bromophenol blue to move 6 cm through a 0.75-mm thick, 5% polyacrylamide gel. During electrophoresis, the current will drop from ~100 mA to ~40 mA.

DNA Denaturation Buffer

1 M glyoxal

25 mM $NaPO_4$ (pH 6.5)

50% (v/v) dimethylsulfoxide

Heat polyacrylamide gels for 60 min at 50°C in a covered container.

DNA Transfer Buffer

(0.5X TBE, for transfer to nylon membranes)

50 mM Tris base

49 mM boric acid

0.5 mM EDTA

Final pH 8.3

Chill to 4°C before use.

Transfer requires at least 2.5 h at 300 mA (constant current) for a 0.75-mm thick, 5% polyacrylamide gel. Initial voltage will be ~330 V, dropping to ~200 V during the transfer. The transfer should be performed at 4°C and started with chilled transfer buffer. The final temperature will be 30°C to 35°C.

Additional Information

6.4 Specifications

"Installation Category I"

Weight
- Buffer tank and safety lid..... 0.39 kg (0.86 lb.)
- Electrophoresis module..... 0.40 kg (0.89 lb.)
- Blot module .....0.10 kg (0.23 lb.)

Dimensions
(tank and lid, W × L × H).....16.7 × 14.0 × 15 cm (6.5 × 5.5 × 5.9 in.)

Gel dimensions (H × W).....6.5–7.2 × 8.7 cm (2.6–2.8 × 3.4 in.)

Construction .....polycarbonate, Flame retardant ABS, polyurethane

Electrode materials.....platinum-niobium laminate type 316 stainless steel

Maximum gel thickness .....1.5 mm

Working buffer volume
- Electrophoresis.....600 to 650 ml
- Electrophoretic blotting.....1 L

Combs and spacers included.....2 sets, 10-tooth, 0.75-mm thick

Voltage Range.....500 VDC Max

Current Range .....0.5 A Max

Operating Temperature Range.....4-30°C (non-condensing atmosphere)

6.5 Warranty

Life Technologies, a Division of Invitrogen Corporation, warrants apparatus of its manufacture against defects in materials and workmanship, under normal service, for one year from the date of receipt by the purchaser. This warranty excludes damages resulting from shipping, misuse, carelessness, or neglect. Life Technologies' liability under the warranty is limited to the repair of such defects or the replacement of the product, at its option, and is subject to receipt of reasonable proof by the customer that the defect is embraced within the terms of the warranty. All claims made under this warranty must be presented to Life Technologies within one year following the date of delivery of the product to the customer.

This warranty is in lieu of any other warranties or guarantees, expressed or implied, arising by law or otherwise. Life Technologies makes no other warranty, expressed or implied, including warranties or merchantability or fitness for a particular purpose. Under no circumstances shall Life Technologies be liable for damages either consequential, compensatory, incidental, or special, sounding in negligence, strict liability, breach of warranty, or any other theory, arising out of the use of the product listed herein.

Additional Information

Life Technologies reserves the right to make improvements in the design, construction, or appearance of this product without notice.

6.6 Declaration of Conformity and CE Mark

Note: The information outlined in this section applies only to customers located in the European Union (EU). The EU is currently comprised of 15 member countries.

This laboratory apparatus is identified with the **CE** mark. This mark indicates that the product complies to the following EU Directives and Standards:

Application of Council Directive(s):
73/23/EEC Low Voltage Directive

Standards:
EN 61010-1:1993 Product Safety

EU Representative:
Life Technologies Ltd.

EU Address:
3 Fountain Dr.
Inchinnan Business Park
Paisley, PA49RF Scotland

A copy of the Declaration of Conformity certificate is available upon request.

Part No. 14257
Lot No. 1086763