

Instruction Manual

**Model H4 and Model H5
Horizontal Gel
Electrophoresis Apparatus**

CAT. SERIES 11025 and 21087



Essential Technologies for the Science of Life™

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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.

1.2 Warnings

1. **Caution! Electrical Hazard!** This equipment is designed for use with a DC power supply providing up to 250 VDC. Although equipped with a safety interlock system, this apparatus should always be operated with extreme caution. Careless handling can result in electrical shock.
 2. Never operate damaged or leaking equipment.
 3. Always turn off the DC power source prior to disconnecting the power cords from the apparatus. Disconnect power cords from the power source first, and then from the apparatus.
 4. Certain reagents indicated for use in this manual are of a hazardous nature (*e.g.*, ethidium bromide, acetic acid, and boric acid, among others). The researcher is cautioned to exercise care when handling these reagents. The equipment used in these procedures (*e.g.*, ultraviolet lamps, electrophoresis apparatus, and high voltage power supplies) should be used following the manufacturer's safety recommendations.
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Overview

2.1 Description

The Model H4 and H5 Horizontal Gel Electrophoresis Apparatus are designed for separation of preparative and analytical quantities of nucleic acids. They are suitable for agarose gel electrophoresis procedures in which buffer recirculation may be required. This instruction manual provides operating procedures for the use of both the Model H5 Apparatus (11 × 14-cm gel bed) and the Model H4 Apparatus (20 × 25-cm gel bed).

2.2 Components

The Model H4 and Model H5 Apparatus and their components are engineered for durable performance and easy storage. Each apparatus includes the following components:

- One ABS electrophoresis tank with threaded ports for buffer circulation
- One clear acrylic safety lid with permanently attached 122-cm (48-in.) DC power cords
- One UVT tray with multiple positioning slots for well-forming combs
 - Model H4 Apparatus: 20 × 25-cm gel bed
 - Model H5 Apparatus: 11 × 14-cm gel bed
- Well-forming combs
 - Model H4 Apparatus: 20-tooth, 1-, 2-, and 3-mm thick
 - Model H5 Apparatus: 14-tooth, 1- and 2-mm thick
- Two threaded 6-mm (0.25-in.) O.D. tubing adaptors
- Two threaded plugs
- Two plastic siderail clips
- One spool of Teflon® thread tape
- One instruction manual

Additional combs and UVT trays are available separately. Consult Chapter 5 for ordering information.

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Operating Instructions

3.1 Apparatus Setup

1. Remove the safety lid by sliding it to the side and lifting it free of the shielded banana plugs, one side at a time.
2. If you plan to use the buffer circulation feature, install the threaded tubing adaptors into the buffer ports on the side of the apparatus; otherwise, install the threaded plugs. Before insertion, wrap a short piece of TEFLON® thread tape around the adaptor or plug threads to prevent buffer leakage.

Note: Do not overtighten adaptors or plugs in the buffer ports.

3.2 Gel Casting

1. Seal both ends of the UVT tray with plastic, mylar-based, or autoclave tape.

Note: If you are using a HORIZON® Gel Casting System (available separately), sealing tape will not be needed.

2. Place the tray on a flat, level surface, whether on the lab bench, in the electrophoresis tank, or in the appropriate HORIZON Gel Casting System. Be sure that the tray is level.
3. Insert a comb or combs into the desired alignment slots of the UVT tray. Ensure that each

comb is unobstructed and rests squarely in its slots. The apparatus is now ready for gel casting.

4. Prepare the desired volume of molten agarose in electrophoresis buffer in a loosely capped bottle or Erlenmeyer flask. The Model H4 requires 50 ml of agarose gel solution per 1 mm of gel thickness; the Model H5 requires 15.5 ml/mm.
5. Allow the molten agarose to cool to 50°C to 60°C.

Caution: Casting gels with agarose above 60°C may cause the bottom of the UVT tray to bow.

6. Pour the measured volume of molten agarose into the center of the UVT tray. Use a pipette tip to distribute the agarose evenly over the surface of the UVT tray and to remove any air bubbles, particularly from around comb teeth.
7. Allow the agarose to cool until thoroughly solidified, usually 15 to 30 min.

3.3 Electrophoresis

1. Carefully remove the gel sealing tape (or HORIZON Gel Casting System dams) from the ends of the UVT tray. Place the UVT tray and gel in the electrophoresis tank, and verify that the tray is positioned with the sample wells at the proper end. Ensure that the electrophoresis tank is level and that the UVT tray is seated flush and centered on the flat tray support area.

Note: Nucleic acids will migrate toward the positive electrode.

Operating Instructions

2. Press the plastic siderail clips onto one side wall of the electrophoresis tank, one at each end of the UVT tray. Slide the clips against the ends of the UVT tray to hold it in position during electrophoresis.
3. To operate the buffer circulation system, connect a circulation pump to two lengths of 6-mm (0.25-in.) O.D. tubing, and fit the free ends of the tubing over the adaptors on the side of the apparatus.
4. Pour sufficient electrophoresis buffer into the electrophoresis tank to cover the gel to a depth of 1 to 2 mm. This requires ~750 ml for the Model H5 Apparatus and ~1.5 L for the Model H4 Apparatus.
5. Gently remove the comb(s). To avoid tearing the bottom of the wells, gently wiggle each comb to free the teeth from the gel. Slightly lift up one side of the comb, then the other. Rinse each comb with deionized water and wipe dry before storing.
6. Remove any trapped air bubbles to ensure that the wells fill completely with buffer.
7. Use a micropipette to load the samples on the floor of the wells. Samples should contain sufficient glycerol or sucrose to be denser than the electrophoresis buffer. See tables 1 and 2 for sample loading capacities for each comb at various gel thicknesses.

Table 1. Sample Volumes for Model H4 Apparatus Combs as a Function of Gel Thickness.

Comb type	Tooth width (mm)	Comb thickness (mm)	Gel thickness ^a (mm)	Capacity/well (μl)		
Prep ^b	165	3	3	1,100		
			4	1,600		
			5	2,100		
12-tooth	12.7	1	3	28		
			4	41		
			5	54		
		2	3	57		
			4	82		
			5	108		
15-tooth	9.5	1	3	21		
			4	31		
			5	40		
		2	3	42		
			4	62		
			5	80		
20-tooth	6.4	1	3	14		
			4	21		
			5	27		
		2	3	28		
			4	42		
			5	54		
		3	3	42		
			4	63		
			5	81		
		30-tooth	4.7	1	3	10
					4	15
					5	20
2	3			21		
	4			30		
	5			40		
3	3			31		
	4			45		
	5			60		

Note: Volumes given are approximate. Low-percentage gels (<0.6%) and low-melting-point agarose gels may have lower sample well volumes.

^aVolume of agarose required: 3 mm, 150 ml; 4 mm, 200 ml; 5 mm, 250 ml.

^bTooth width and capacity values are for the central, preparative well.

Operating Instructions

Table 2. Sample Volumes for Model H5 Apparatus Combs as a Function of Gel Thickness.

Comb type	Tooth width (mm)	Comb thickness (mm)	Gel thickness ^a (mm)	Capacity/well (μl)
Prep ^b	92	2	3	410
			4	600
			5	780
10-tooth	7.9	1	3	17
			4	25
			5	33
		2	3	34
			4	50
			5	66
14-tooth	4.7	1	3	10
			4	15
			5	20
		2	3	20
			4	30
			5	40
20-tooth	3.8	1	3	8
			4	12
			5	16
		2	3	16
			4	24
			5	32

Note: Volumes given are approximate. Low-percentage gels (<0.6%) and low-melting-point agarose gels may have lower sample well volumes.

^aVolume of agarose required: 3 mm, 45 ml; 4 mm, 60 ml; 5 mm, 75 ml.

^bTooth width and capacity values are for the central, preparative well.

- Place the safety lid on the unit.

Caution: *Do not* attempt to run the unit without the lid in place.

- Connect the power cords to the electrophoresis tank and a 250-V DC power supply.

Note: Nucleic acids will migrate toward the positive electrode.

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10. If you are using the buffer circulation feature, start the pump.
 11. Turn on the power supply and select the desired voltage. Small bubbles will rise from the electrodes when the unit is properly connected.
 12. When electrophoresis is complete, turn off the power supply. Disconnect the DC power cords at the power supply first, and then at the apparatus. Turn off the circulation pump.

3.4 Post-Electrophoresis

1. Remove the safety lid. Lift out the UVT tray and gel.
2. Carefully slide the gel out of the UVT tray for staining or subsequent analysis.
3. Properly discard the electrophoresis buffer. Do not reuse the buffer. Disconnect the buffer circulation tubing from the adaptors on the side of the apparatus.
4. Thoroughly rinse the electrophoresis tank, tubing adaptors, and tubing with deionized water.
5. Remove any residual agarose from the UVT tray by rinsing with deionized water. Wipe dry or allow to air dry before storing.

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Troubleshooting Guide

Many procedural and operational problems can be solved by carefully following the instructions in this manual. Some suggestions for equipment and procedural troubleshooting are given below. Should these suggestions not resolve the problem, or if you have questions regarding procedures, please call the TECH-LINE™ (numbers listed on the back cover of this manual). If you need to return the unit for repair, contact the Customer Service Department or your local distributor for shipping instructions. Please include a full description of the problem.

Problem	Comments
<i>Equipment:</i>	
Bubbles do not appear on the electrodes when DC voltage is connected	Verify that the DC power supply is operating properly. Verify continuity of the power cords with an ohmmeter. Verify continuity of the electrodes with an ohmmeter.
Electrodes turn gray	This occurs under normal operating conditions. Performance is not affected.
Agarose solution leaks during casting	Verify the edge of the UVT tray is clean of debris and dry before applying tape. Cool agarose to 50 to 60°C before pouring
<i>Electrophoresis:</i>	
BPB dye turns yellow (pH change) during electrophoresis	Check the pH of the electrophoresis buffer (refer to tables 1 and 2). Be sure to use Tris Base and not Tris-HCl. Mix the buffer periodically during electrophoresis. Connect a pump to circulate the buffer.
Samples leak underneath the gel upon loading	The bottom of the wells were torn when the comb was removed. See Chapter 3 for recommended comb removal procedure.

Problem	Comments
Gel melts or becomes soft near sample wells	This is due to the combination of pH drift and high temperature. Circulate or remix buffer periodically. Reduce the electrophoretic voltage.
Pronounced “smiling” along one edge of the gel occurs (corresponding bands in different lanes migrate slower toward one edge of the gel)	Gel was cast or electrophoresed out of level. Use the “bull’s eye” level to verify that the apparatus is level prior to gel casting and electrophoresis
S-shaped lanes (anomalous migration-front results in lanes that are not all running at a uniform speed)	Mix the buffer periodically during electrophoresis. Switch to a lower conductivity/higher buffering capacity buffer, <i>i.e.</i> , from 1X TAE or 1X TBE to 0.5X TBE. Reduce the salt concentration of the sample. Connect a pump to circulate the buffer.
“Flaming” bands (excessive fluorescence appearing as a trail above the band)	Reduce the amount of DNA in the sample. Reduce the amount of protein and/or glycerol in the sample.
“Wiggly” or “slanting” bands (bands are not straight lines or parallel to the top edges of the gel)	Verify that the wells are free of particles and bubbles before and after loading samples. Verify that the agarose is completely dissolved before casting gels. Remove any particulate matter from the agarose before casting gels. Be sure that bubbles are not trapped against the comb during gel casting.
All bands appear as “doublets” (each band is represented twice within the same lane)	Concentrate the sample and use a thin (2- to 3-mm) gel with a thin (1-mm) comb. Prevent gel movement during photography. Reduce voltage. Band doublets may result due to denaturation from excess heat from running gel at high voltage.

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Related Products

The following accessories and replacement parts are available, separately, for use with the Model H4 and Model H5 Apparatus:

Product	Size	Cat. No.
Model H4 Apparatus Accessories:		
HORIZON 20•25 Gel Casting System	----	21069-067
Blot Transfer System 20•25		
Apparatus Only	----	11056-017
Blotting Materials	----	11056-033
Preparative DELRIN® Comb with marker lanes: 3.0-mm thick	----	61010-021
Analytical DELRIN Combs		
12-tooth: 1.0-mm thick	----	11953-064
2.0-mm thick	----	11953-080
15-tooth: 1.0-mm thick	----	11953-072
2.0-mm thick	----	11953-098
20-tooth: 1.0-mm thick	----	41007-014
2.0-mm thick	----	41007-022
3.0-mm thick	----	41007-030
30-tooth: 1.0-mm thick	----	11951-043
2.0-mm thick	----	11951-019
3.0-mm thick	----	11951-050
Custom DELRIN Combs	----	11953-031*
UVT Tray (20.0 × 25.0 × 3.5-cm)	----	31006-026
Custom-Length UVT Tray	----	11961-059*
Bull's-Eye Level	----	11957-016
Tygon® Tubing (50 ft)	----	31048-010

Product	Size	Cat. No.
Model H4 Apparatus Replacement Parts:		
Electrode Replacement	----	11025-038
Electrode Hardware Repair Kit (see figure 1)(Includes 2 rubber washers, 2 hex nuts, 1 red plug block, 1 black plug block, 1 red cap nut, 1 black cap nut, 2 banana plugs, 2 electrode boots)	----	11987-013
Rail Clips (pair)	----	11964-053
H4 Safety Lid Replacement (new style) (with attached power cords)	----	11025-079
Buffer Recirculation Kit (Includes 2 threaded plugs, 2 threaded 6-mm O.D. tubing adapters, and 1 spool of Teflon tape)	----	11961-075
Model H5 Apparatus Accessories:		
HORIZON 11•14 Gel Casting System	----	11068-046
Blot Transfer System 11•14		
Apparatus Only	----	21054-010
Blotting Materials	----	21054-036
Preparative DELRIN Comb with marker lanes: 2.0-mm thick	----	41086-018
Analytical DELRIN Combs		
10-tooth: 1.0-mm thick	----	11951-068
2.0-mm thick	----	11951-084
14-tooth: 1.0-mm thick	----	31081-011
2.0-mm thick	----	31081-029
20-tooth: 1.0-mm thick	----	11951-076
2.0-mm thick	----	11951-092
Custom DELRIN Combs	----	11951-035*
UVT Tray (11.0 × 14.0 × 2.0-cm)	----	11084-019
Custom-Length UVT Tray	----	11961-042*
Bull's-Eye Level	----	11957-016
TYGON Tubing (50 ft)	----	31048-010

Related Products

Product	Size	Cat. No.
Model H5 Apparatus Replacement Parts:		
Electrode Replacement	----	21087-036
Electrode Hardware Repair Kit (see figure 1) (Includes 2 rubber washers, 2 hex nuts, 1 red plug block, 1 black plug block, 1 red cap nut, 1 black cap nut, 2 banana plugs, 2 electrode boots)	----	11987-013
H5 Safety Lid Replacement (new style) (with attached power cords)	----	21087-127
Buffer Recirculation Kit (Includes 2 threaded plugs, 2 threaded 6-mm O.D. tubing adapters, and 1 spool of Teflon tape)	----	11961-075
Rail Clips (pair)	----	11964-053
Purification:		
CONCERT™ Rapid Gel Extraction System	50 reactions	11456-019
	250 reactions	11456-027
CONCERT™ Matrix Gel Extraction System	150 reactions	11457-017

*Order forms for custom combs and UVT trays can be found in the catalogue or on our web site (www.lifetech.com).

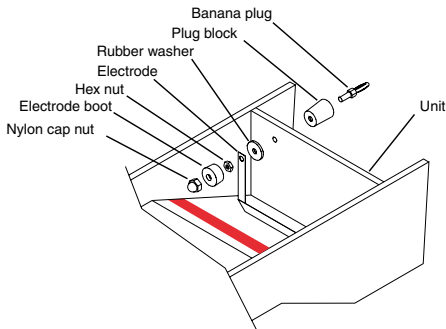


Figure 1. Electrode Hardware Replacement Kit

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Additional Information

6.1 Care and Handling

The components of the Model H4 and Model H5 Apparatus are fabricated from ABS and acrylic. As with any laboratory instrument, adequate care ensures consistent and reliable performance.

After each use, wash all components gently with water and nonabrasive soap or detergent, and rinse well in deionized water. Wipe dry with a soft cloth or paper towel, or allow to air dry. To remove grease and oils, use a light application of hexane, kerosene, or aliphatic naphtha. *Never* use abrasive cleaners, window sprays, or rough cloths 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 Specifications

Model H4 Apparatus:

Weight	2.36 kg (5.19 lb)
Dimensions (W × L × H).....	25.4 × 42.2 × 14.0 cm (10.0 × 16.6 × 5.5 in.)
Construction	ABS, acrylic
Gel dimensions (W × L)	20 × 25 cm (7.9 × 9.8 in.)
Maximum gel thickness	12 mm
Working buffer volume.....	1.5 L
Electrode material.....	platinum/niobium laminate
Combs (included).....	20-tooth, 1.0-mm thick 20-tooth, 2.0-mm thick 20-tooth, 3.0-mm thick

Model H5 Apparatus:

Weight	1.19 kg (2.62 lb)
Dimensions (W × L × H).....	16.5 × 33.0 × 11.3 cm (6.5 × 13.0 × 4.4 in.)
Construction	ABS, acrylic
Gel dimensions (W × L)	11 × 14 cm (4.3 × 5.5 in.)
Maximum gel thickness	12 mm
Working buffer volume.....	650 ml
Electrode material.....	platinum/niobium laminate
Combs (included).....	14-tooth, 1.0-mm thick 14-tooth, 2.0-mm thick

6.3 Warranty

Life Technologies, Inc. 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.

Life Technologies reserves the right to make improvements in design, construction, and appearance without notice.

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