

Instruction Manual

HORIZON 11•14 and 20•25 Gel Electrophoresis Apparatus

CAT. SERIES 11068 & 21069



Essential Technologies for the Science of Life™

Table of Contents

1.	Notices to Customer 1	
	1.1 Important Information 1	
	1.2 Warnings 1	
2.	Overview	2
	2.1 Description 2	2
	2.2 Components	2
3.	Operating Instructions 4	
	3.1 Assembly for Gel Casting 4	
	3.2 Gel Casting 4	
	3.3 Electrophoresis	,
	3.4 Post-Electrophoresis 5	,
4.	Troubleshooting Guide	;
5.	Applications	,
	5.1 Considerations for Agarose Gel Electrophoresis7	
	5.1.1 Selecting Gel Concentration	•
	5.1.2 Preparing Agarose for Gels 7	•
	5.1.3 Preparing Samples and Loading the Gel	;
	5.1.4 Using Multiple Combs 11	
	5.2 Considerations for Electrophoresis Buffers 11	
	5.2.1 Resolution Effects11	
	5.2.2 Heat Effects	
	5.3 Ethidium Bromide Staining of Double-Stranded DNA	
	5.4 Gel Photography 12	
6.	References 13	
7.	Related Products	
8.	Additional Information	;
	8.1 Care and Handling	;
	8.2 Specifications	;
	8.3 Warranty 17	
	8.3 Warranty	
Fig		
Fig 1.	8.4 Declaration of Conformity and CE Mark	,
-	8.4 Declaration of Conformity and CE Mark	,
1. 2. 3.	8.4 Declaration of Conformity and CE Mark 17 ures HORIZON 11•14 Gel Electrophoresis Apparatus 3 HORIZON 20•25 Gel Electrophoresis Apparatus 3 Gel Casting Configuration 4	, ; ;
1. 2. 3. 4.	8.4 Declaration of Conformity and CE Mark 17 ures HORIZON 11•14 Gel Electrophoresis Apparatus 3 HORIZON 20•25 Gel Electrophoresis Apparatus 3 Gel Casting Configuration 4 Buffer Recirculation Connections 5	
1. 2. 3.	8.4 Declaration of Conformity and CE Mark 17 ures HORIZON 11•14 Gel Electrophoresis Apparatus 3 HORIZON 20•25 Gel Electrophoresis Apparatus 3 Gel Casting Configuration 4	
1. 2. 3. 4.	8.4 Declaration of Conformity and CE Mark 17 ures 11.14 Gel Electrophoresis Apparatus 3 HORIZON 11.14 Gel Electrophoresis Apparatus 3 Gel Casting Configuration 4 Buffer Recirculation Connections 5 Horizon 11.14 and 20.25 Replacement Parts 15	
1. 2. 3. 4. 5.	8.4 Declaration of Conformity and CE Mark 17 ures 11.14 Gel Electrophoresis Apparatus 3 HORIZON 11.14 Gel Electrophoresis Apparatus 3 Gel Casting Configuration 4 Buffer Recirculation Connections 5 Horizon 11.14 and 20.25 Replacement Parts 15	
1. 2. 3. 4. 5. Tab 1. 2.	8.4 Declaration of Conformity and CE Mark 17 ures HORIZON 11•14 Gel Electrophoresis Apparatus 3 HORIZON 20•25 Gel Electrophoresis Apparatus 3 Gel Casting Configuration 4 Buffer Recirculation Connections 5 Horizon 11•14 and 20•25 Replacement Parts 15 IOX TAE Electrophoresis Buffer 7 10X TBE Electrophoresis Buffer 7	
1. 2. 3. 4. 5. Tab 1. 2. 3.	8.4 Declaration of Conformity and CE Mark 17 ures HORIZON 11•14 Gel Electrophoresis Apparatus 3 HORIZON 20•25 Gel Electrophoresis Apparatus 3 Gel Casting Configuration 4 Buffer Recirculation Connections 5 Horizon 11•14 and 20•25 Replacement Parts 15 oles 10X TAE Electrophoresis Buffer. 7 10X TBE Electrophoresis Buffer. 7 Agarose Volume Requirement for Different Gel Thicknesses 8	
1. 2. 3. 4. 5. Tab 1. 2. 3. 4.	8.4 Declaration of Conformity and CE Mark 17 ures HORIZON 11•14 Gel Electrophoresis Apparatus 3 HORIZON 20•25 Gel Electrophoresis Apparatus 3 Gel Casting Configuration 4 Buffer Recirculation Connections 5 Horizon 11•14 and 20•25 Replacement Parts 15 oles 10X TAE Electrophoresis Buffer. 7 10X TBE Electrophoresis Buffer. 7 Agarose Volume Requirement for Different Gel Thicknesses 8 10X Sample Loading Buffer 8	
1. 2. 3. 4. 5. Tab 1. 2. 3.	8.4 Declaration of Conformity and CE Mark 17 ures 19 HORIZON 11•14 Gel Electrophoresis Apparatus 3 HORIZON 20•25 Gel Electrophoresis Apparatus 3 Gel Casting Configuration 4 Buffer Recirculation Connections 5 Horizon 11•14 and 20•25 Replacement Parts 15 Des 10X TAE Electrophoresis Buffer. 7 10X TBE Electrophoresis Buffer. 7 Agarose Volume Requirement for Different Gel Thicknesses 8 10X Sample Loading Buffer 8 Sample Volumes for HORIZON 11•14 Apparatus Combs as a 8	
1. 2. 3. 4. 5. Tab 1. 2. 3. 4. 5.	8.4 Declaration of Conformity and CE Mark 17 ures 19 HORIZON 11•14 Gel Electrophoresis Apparatus 3 HORIZON 20•25 Gel Electrophoresis Apparatus 3 Gel Casting Configuration 4 Buffer Recirculation Connections 5 Horizon 11•14 and 20•25 Replacement Parts 15 Des 10X TAE Electrophoresis Buffer. 7 10X TBE Electrophoresis Buffer. 7 Agarose Volume Requirement for Different Gel Thicknesses 8 10X Sample Loading Buffer 8 Sample Volumes for HORIZON 11•14 Apparatus Combs as a 9	
1. 2. 3. 4. 5. Tab 1. 2. 3. 4.	8.4 Declaration of Conformity and CE Mark 17 ures 3 HORIZON 11•14 Gel Electrophoresis Apparatus 3 Gel Casting Configuration 4 Buffer Recirculation Connections 5 Horizon 11•14 and 20•25 Replacement Parts 15 Dies 10X TAE Electrophoresis Buffer 7 10X TBE Electrophoresis Buffer 7 Agarose Volume Requirement for Different Gel Thicknesses 8 10X Sample Loading Buffer 8 Sample Volumes for HORIZON 11•14 Apparatus Combs as a 9 Sample Volumes for HORIZON 20•25 Apparatus Combs as a 9	
1. 2. 3. 4. 5. Tab 1. 2. 3. 4. 5.	8.4 Declaration of Conformity and CE Mark 17 ures 19 HORIZON 11•14 Gel Electrophoresis Apparatus 3 HORIZON 20•25 Gel Electrophoresis Apparatus 3 Gel Casting Configuration 4 Buffer Recirculation Connections 5 Horizon 11•14 and 20•25 Replacement Parts 15 Des 10X TAE Electrophoresis Buffer. 7 10X TBE Electrophoresis Buffer. 7 Agarose Volume Requirement for Different Gel Thicknesses 8 10X Sample Loading Buffer 8 Sample Volumes for HORIZON 11•14 Apparatus Combs as a 9	
1. 2. 3. 4. 5. Tab 1. 2. 3. 4. 5.	8.4 Declaration of Conformity and CE Mark 17 ures 3 HORIZON 11•14 Gel Electrophoresis Apparatus 3 Gel Casting Configuration 4 Buffer Recirculation Connections 5 Horizon 11•14 and 20•25 Replacement Parts 15 Deles 10X TAE Electrophoresis Buffer 7 10X TBE Electrophoresis Buffer 7 Agarose Volume Requirement for Different Gel Thicknesses 8 10X Sample Loading Buffer 8 Sample Volumes for HORIZON 11•14 Apparatus Combs as a 9 Sample Volumes for HORIZON 20•25 Apparatus Combs as a 9 Sample Volumes for HORIZON 20•25 Apparatus Combs as a 9 Sample Volumes for HORIZON 20•25 Apparatus Combs as a 9 Sample Volumes for HORIZON 20•25 Apparatus Combs as a 9 Sample Volumes for HORIZON 20•25 Apparatus Combs as a 9 Supple Volumes for HORIZON 20•25 Apparatus Combs as a 10	
1. 2. 3. 4. 5. Tab 1. 2. 3. 4. 5. 6. 7.	8.4 Declaration of Conformity and CE Mark 17 ures 3 HORIZON 11•14 Gel Electrophoresis Apparatus 3 Gel Casting Configuration 4 Buffer Recirculation Connections 5 Horizon 11•14 and 20•25 Replacement Parts 15 Des 10X TAE Electrophoresis Buffer 7 10X TBE Electrophoresis Buffer 7 Agarose Volume Requirement for Different Gel Thicknesses 8 10X Sample Loading Buffer 8 Sample Volumes for HORIZON 11•14 Apparatus Combs as a 9 Sample Volumes for HORIZON 20•25 Apparatus Combs as a 9 Sample Volumes for HORIZON 11•14 Apparatus Combs as a 9 Sample Volumes for HORIZON 20•25 Apparatus Combs as a 9 Sample Volumes for HORIZON 20•25 Apparatus Combs as a 10 Nominal Electrophoresis Times for 1% Agarose Gels at 10	
 1. 2. 3. 4. 5. Tab 1. 2. 3. 4. 5. 6. 7. Conlinc. 	8.4 Declaration of Conformity and CE Mark 17 ures 19 HORIZON 11•14 Gel Electrophoresis Apparatus 3 HORIZON 20•25 Gel Electrophoresis Apparatus 3 Gel Casting Configuration 4 Buffer Recirculation Connections 5 Horizon 11•14 and 20•25 Replacement Parts 15 oles 10X TAE Electrophoresis Buffer 7 10X TAE Electrophoresis Buffer 7 Agarose Volume Requirement for Different Gel Thicknesses 8 10X Sample Loading Buffer 8 Sample Volumes for HORIZON 11•14 Apparatus Combs as a 9 Sample Volumes for HORIZON 20•25 Apparatus Combs as a 9 Sample Volumes for HORIZON 20•25 Apparatus Combs as a 10 Nominal Electrophoresis Times for 1% Agarose Gels at 12	

i

This page is intentionally left blank.

.

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.

Because of the double-wall construction of the HORIZON 11•14 and the HORIZON 20•25 Apparatus, the heat generated by electrophoresis does not dissipate as quickly as with Models H4 and H5 and discontinued Models H1 and H3. With the HORIZON Apparatus, gels electrophoresed at the same voltage will be warmer, and samples will move faster. These effects are particularly evident at higher voltages, and may necessitate additional buffer circulating or cooling steps to avoid melting the gel. Consult Chapter 5, for further information and a table of nominal electrophoresis times at various voltages.



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!** Although equipped with a safety interlock system, this apparatus should always be operated with extreme caution. Careless handling could result in electrical shock.

- 2. Never operate damaged or leaking equipment.
- 3. Always turn off the power supply before opening 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.*, high voltage power supplies, ultraviolet lamps, and electrophoresis apparatus) should be used following the manufacturer's safety recommendations.

Overview

2.1 Description

The HORIZON[®] 11•14 and the HORIZON 20•25 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 circulation may be required.

2.2 Components

The HORIZON 11•14 and the HORIZON 20•25 Apparatus are designed for simplified gel casting and electrophoresis. These apparatus are engineered for durable performance and easy storage. Refer to figures 1 and 2 to identify the following features and components:

- One ABS electrophoresis tank with clear acrylic safety interlock lid, adjustable leveling feet, self-sealing ports for buffer circulation, molded V-grooves for placement of gel casting dams, and a tray support platform with red wellvisualization strips and black centimeter graduations
- One UVT tray (11 × 14-cm or 20 × 25-cm gel bed) with multiple positioning slots for well-forming combs
- One pair of aluminum gel casting dams
- One well-forming comb:

with HORIZON 11•14 Apparatus: 14-tooth, 1-mm-thick

with HORIZON 20•25 Apparatus: 20-tooth, 1-mm-thick

- One pair of quick-connect fittings for the buffer circulation ports, with 1 m of 6-mm (0.25-in) I.D. circulation tubing
- One bull's eye level
- One pair of 48-in. red and black power cords
- One instruction manual

Many of these components are also available separately. Consult Chapter 7 for ordering information.

Overview

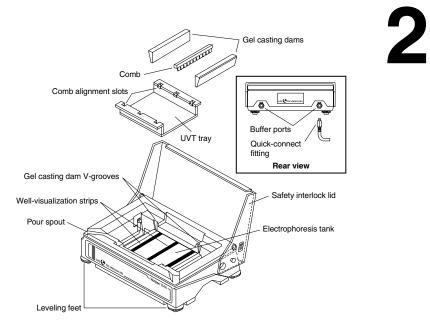


Figure 1. Horizon 11•14 Horizontal Gel Electrophoresis Apparatus.

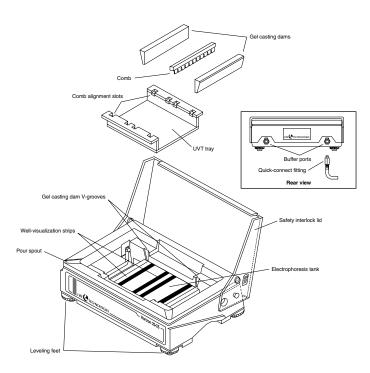


Figure 2. Horizon 20•25 Horizontal Gel Electrophoresis Apparatus.

Operating Instructions

This chapter provides operating instructions for the HORIZON 11•14 and 20•25 Apparatus. Refer to Chapter 5 for information on commonly used buffers, agarose concentrations, sample volumes, and post-electrophoresis handling of the gel. Review figures 1 and 2, as necessary, to identify the features and components discussed in these instructions.

Note: If you are using gels cast in advance with the HORIZON 11•14 or 20•25 Gel Casting System, proceed to Section 3.3.

3.1 Assembly for Gel Casting

- 1. Place the apparatus on a flat, level surface. Open the safety interlock lid. Place the UVT tray in the electrophoresis tank on the tray support platform. The red well-visualization strips should be visible through the tray.
- 2. Slide the gel casting dams simultaneously into the V-grooves in the electrophoresis tank. Make sure that the tops of the dams are level. Apply even, gentle downward pressure to both dams to seat the sealing surfaces of the dams against the ends of the the UVT tray. Do not force the dams down since this can displace the tray out of level.
- 3. Place the bull's-eye level in the center of the UVT tray and turn the adjustable feet, as needed, to level the apparatus.
- 4. Insert a comb or combs into the preferred alignment slots of the UVT tray. The teeth of each comb should line up over one of the well-visualization strips. Ensure that each comb rests unobstructed and squarely in its slots. The apparatus is now ready for gel casting (figure 3).

Note: Nucleic acids will migrate toward the positive (red) electrodes at the right side of the HORIZON Apparatus.

3.2 Gel Casting

- 1. Prepare the desired volume of molten agarose in electrophoresis buffer in a bottle or Erlenmeyer flask. For information on agarose preparation, buffer formulation, and the effects of varying gel concentration and volume, see Chapter 5.
- Loosely cap the container. Allow the molten agarose to cool to 50°C to 60°C.
 Caution: Casting gels with agarose above 60°C will result in poor sealing at the gel casting dams and may cause the bottom of the UVT tray to bow.
- 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.
 Note: If molten agarose leaks from below gel casting dams, be sure dams are seated properly (see Section 3.1).
- 4. Allow the agarose to cool until thoroughly solidified, usually 15 to 30 min.
- 5. To store gels prior to electrophoresis, gently remove gel casting dams and comb(s), wet the gel surface with a small amount of electrophoresis buffer, and wrap the UVT tray (with the gel still in place) with plastic wrap or seal in a plastic bag. Store at 4°C. Gels can be stored for 1 to 2 days or longer, if well sealed.



Figure 3. Gel Casting Configuration.

Operating Instructions

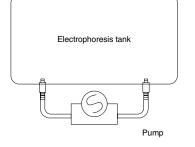


Figure 4. Buffer Recirculation Connections.

3.3 Electrophoresis

- 1. Remove the gel casting dams, rinse them with deionized water, and wipe them dry before storing.
- 2. When transferring a gel from the HORIZON 11•14 or 20•25 Gel Casting System, verify that the UVT tray is oriented so that the sample wells are in the desired alignment. Check that the sample wells line up over one of the well-visualization strips and that the UVT tray is seated flush on the tray support platform.

Note: Nucleic acids will migrate toward the positive (red) electrodes at the right side of the apparatus.

- 3. Pour sufficient electrophoresis buffer into the electrophoresis tank to cover the gel to a depth of 1 to 2 mm. This requires ~700 ml for the HORIZON 11•14 Apparatus and ~1.55 L for the HORIZON 20•25 Apparatus.
- 4. To operate the buffer circulation system (especially important when using TAE electrophoresis buffer see Chapter 5), connect the circulation pump to the tubing and quick-connect fittings, and insert the fittings into the buffer ports on the back of the electrophoresis tank (see figure 4). Listen for a distinct "click" to verify that the fittings are properly seated.
- 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 micropipet or automatic pipet to load the samples on the floor of the wells. Samples should contain sufficient glycerol or sucrose to be denser than the electrophoresis buffer. For sample loading buffer formulation and the loading capacities for each comb relative to various gel thicknesses, see tables 5 and 6 in Chapter 5.
- 8. Close the safety interlock lid.
- 9. Connect the power cords to the electrophoresis tank and a 250-V DC power supply. Connect the positive (red) lead at the right side of the apparatus and the negative (black) lead at the left.
- 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. Nominal electrophoresis times for TAE and TBE buffers are listed in table 7 in Chapter 5.
- 12. Monitor electrophoresis by following the migration of the bromophenol blue (BPB) dye. Movement should be in the direction of the positive electrodes at the right side of the apparatus. Use the black 1-cm graduations visible below the UVT tray to determine approximate migration rates.
- 13. When electrophoresis is complete, turn off the power supply. Disconnect the power cords from the power supply and the HORIZON Apparatus. Turn off the circulation pump.

3.4 Post-Electrophoresis

- 1. Open the safety interlock lid. Lift out the UVT tray and gel.
- Slide the gel out of the UVT tray for staining or subsequent analysis (see Chapter 5 for further information). Remove the gel with care; agarose gels tear easily if not properly supported.
- 3. Properly discard the electrophoresis buffer. Use the pouring spouts at the front corners of the unit to transfer the buffer to a waste receptacle. Do not reuse the buffer. Disconnect the buffer circulation pump by pressing the metal tab on each buffer port to release the quick-connect fittings.
- 4. Thoroughly rinse the electrophoresis tank, quick-connect fittings, 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.

Troubleshooting Guide

4

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-LINESM (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
Equipment:	
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 that the sealing surfaces of the UVT tray and the gel casting dams are clean.
	Verify that the gel casting dams are properly seated.
	Verify that the ends of the UVT tray are flat and free of nicks.
	Cool the agarose to 50°C to 60°C before pouring.
Electrophoresis:	
BPB dye turns yellow (pH change) during electrophoresis. Results are uninterpretable.	Check the pH of the electrophoresis buffer (refer to tables 1 and 2). Be sure to use Tris Base and not Tris-HCI.
	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.
5	··· · · · · · · · · · · · · · · · · ·
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 anes 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	Mix the buffer periodically during electrophoresis.
results in lanes that are not all running at a	Switch to a low conductivity/high buffering capacity buffer (0.5X TBE).
uniform speed)	Reduce the salt concentration of the sample. Connect a pump to circulate the buffer.
'Flaming" bands (excessive fluorescence	Reduce the amount of DNA in the sample.
appearing as a trail above the band)	Reduce the amount of protein and/or glycerol in the sample.
Wiggly" or "slanting" bands (bands are not	Verify that the wells are free of particles and bubbles before and after loading samples.
straight lines or parallel to the top edges of the gel)	Verify that the agarose is completely dissolved before casting gels. Remove any particulate matter from the agarose before casting gels.
and 901)	Be sure that bubbles are not trapped against the comb during gel casting.
All bands appear as "doublets" (each band is	Concentrate the sample and use a thin (2- to 3-mm) gel with a thin (1-mm) comb.
represented twice within the same lane).	Prevent gel movement during photography.

5.1 Considerations for Agarose Gel Electrophoresis

5.1.1 Selecting Gel Concentration

The choice of agarose concentration for a gel depends on the range of fragment sizes to be separated. The typical agarose concentration is 0.3% to 2.0%. Large DNA fragments require low-percentage gels, while small DNA fragments resolve best on high-percentage gels. Gels containing <0.5% agarose are very weak and should be electrophoresed at a low temperature (\sim 4°C). For routine electrophoresis, 0.75% to 1.0% agarose gels provide a wide range of separation (0.15 to 15 kb). For a more complete treatment of factors that affect the separation of nucleic acids in agarose gels, see Chapter 6, references 1 and 2.

Thin (2- to 3-mm thick) and low-percentage agarose gels yield better photographs than thick or high-percentage gels, which exhibit increased opaqueness and autofluorescence.

5.1.2 Preparing Agarose for Gels

The following protocol yields a 1% (w/v) agarose gel. Varying the amount of agarose added in step 1 will produce gels of higher or lower concentration. See Section 5.2 to determine whether Tris-acetate/EDTA (TAE) buffer or Trisborate/EDTA (TBE) buffer (formulas in tables 1 and 2) is preferable for your specific application. To determine the volume of agarose solution required to produce gels of various thicknesses, see table 3.

Table 1. 10X TAE Electrophoresis Buffer.

Component	Amount	Concentration
Tris base	48.4 g	400 mM
Na ₂ EDTA•2H ₂ O	7.4 g	20 mM
Sodium acetate, anhydrous	16.4 g	200 mM
Glacial acetic acid	17.0 ml	296 mM
Deionized water	to 1 L	

Note: This is a 10X concentration solution. Dilute with deionized water prior to use. Final pH should be 7.8 at 25° C.

Table 2. 10X TI	BE Electrophoresis	Buffer.
-----------------	--------------------	---------

Component	Amount	Concentration
Tris base	121.1 g	1 M
Boric acid, anhydrous	55.6 g	0.9 M
Na ₂ EDTA•2H ₂ O	3.7 g	10 mM
Deionized water	to 1 L	

Note: This is a 10X concentration solution. Dilute with deionized water prior to use. Final pH should be 8.3 at 25°C.

Table 3.	Agarose	Volume	Requirement	for	Different	Gel	Thicknesses.
----------	---------	--------	-------------	-----	-----------	-----	--------------

Gel Dimensions (cm)	Gel Thickness (mm)	Agarose Volume (ml)
11 × 14	3	50
	4	65
	5	80
20 × 25	3	150
	4	200
	5	250

Note: Volumes given are approximate.

- 1. Add 1 g of agarose per 100 ml of 1X TAE or 1X TBE electrophoresis buffer (see tables 1 and 2 for buffer formulas) in a bottle or Erlenmeyer flask of at least twice the final volume of solution.
- 2. Loosely cap and weigh the flask.
- 3. Dissolve the agarose in electrophoresis buffer by heating in a microwave oven or boiling water bath with occasional mixing until no granules of agarose are visible.
- 4. Weigh the flask and adjust to the original weight with deionized water to compensate for evaporation.
- 5. Put the capped flask in a water bath at 50°C to 60°C, and allow the agarose to equilibrate at that temperature before pouring gels.

5.1.3 Preparing Samples and Loading the Gel

The amount of DNA that can be loaded per well is variable and depends upon the number and size of the DNA fragments and the cross-sectional area of the well (well width \times gel thickness). As a general rule, the minimum amount of DNA detectable by ethidium bromide staining is 1 ng in a 5-mm wide band on a 3-mm thick gel. For preparative purposes on a 3-mm thick gel, the amount of DNA loaded should not exceed 50 ng per 5-mm wide band. Overloading the gel causes trailing and distortion of bands.

Table 4 contains a formula for a sample loading buffer, which should be added to DNA samples prior to loading. For alternative formulas for sample loading buffers, see chapter 6, references 1 and 2.

Component	Amount	Concentration
Glycerol	5 ml	50% (v/v)
Na ₂ EDTA•2H ₂ O	0.37 g	100 mM
Sodium dodecyl sulfate	0.1 g	1% (w/v)
Bromophenol blue	0.01 g	0.1% (w/v)
Deionized water	to 10 ml	

Table 4. 10X Sample Loading Buffer.

Note: This is a 10X concentration solution. Add 0.1 volume of buffer to samples and apply directly to gel. If the samples contain λ -cohesive ends, as with λ DNA restriction fragments, the samples in buffer should be heated at 65°C for 5 to 10 min prior to loading.

The sample volumes that can be loaded per well for each standard HORIZON Apparatus comb are listed in tables 5 and 6. For analytical purposes, keep sample volumes to a minimum. Generally, 1-mm thick combs provide sharper band definition than 2-mm thick combs.

 Table 5. Sample Volumes for HORIZON 11•14 Apparatus Combs as a Function of Gel Thickness.

Comb Type	Tooth Width (mm)	Comb Thickness (mm)	Gel Thickness (mm)	Capacity/Well (µl)
Prep*	92	2	3 4	410 600
			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
	el Pipet Comb			
12-tooth	7.2	1.0	3	15
			4	23
			5	30
24-tooth	2.7	2.0	3	11
			4	17
			5	22

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

*Tooth width and capacity values are for the central, preparative well.

5

Table 6. Sample Volumes for HORIZON 20•25 Apparatus Combs as a Function of Gel Thickness.

Comb	Tooth Width	Comb Thickness	Gel Thickness	Capacity/Well
Type(mm)	(mm)	(mm)	(μl)	
	. ,	. ,	,	
Prep [*]	165	2	3	1,100
	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
10 10001	0.0	I	4	31
			4 5	40
			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
Multichannel Pipe	at Combs:			
21-tooth	7.2	1.0	3	15
			4	23
			5	30
42-tooth	2.7	2.0	3	11
			4	17

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

*Tooth width and capacity values are for the central, preparative well.

5.1.4 Using Multiple Combs

The multiple comb alignment slots in the HORIZON 11•14 and 20•25 Apparatus lend themselves to a variety of applications. For example, using two rows of wells on the same gel doubles the number of samples of "mini-prep" plasmid DNA that can be screened. A row of wells at the bottom of the gel provides a convenient way to include quantitative standards on a gel for Southern blot hybridization.

Note: To use this feature, add the standards to the bottom row and let them migrate into the gel for just a few minutes before electrophoresis is complete.

5.2 Considerations for Electrophoresis Buffers

5.2.1 Resolution Effects

For electrophoresis of agarose gels of the same concentration and at a fixed voltage, TAE buffer provides better resolution of fragments >4 kb in length, while TBE buffer offers better resolution of 0.1- to 3-kb fragments. TBE has a higher buffering capacity and lower conductivity than TAE and is therefore better suited for high voltage (>150-V) electrophoresis. TBE buffer also generates less heat at an equivalent voltage and does not allow a significant pH drift.

Note: Because of its lower buffering capacity, TAE requires circulation or mixing periodically for full-length electrophoresis, particularly at higher voltages.

Band compression of fragments of high molecular weight (>5 kb) occurs as voltage increases. This effect is observed with both TBE and TAE buffers. Band definition remains sharp, even above 200 V, provided that the gel is not over-loaded. Linear DNA fragments from 0.15 to 10 kb (25 ng total) are easily resolved on a 0.8% agarose gel in 0.5X TBE buffer electrophoresed for 30 min at 200 V.

TAE buffer provides better results for analysis of supercoiled DNA. Anomalous migration of supercoiled DNA, particularly with high molecular weight (>7-kb) fragments, occurs when TBE buffer is used at >75 V. Use of TBE buffer also reduces the ability to resolve supercoiled DNA from nicked circular and linear DNA in the absence of ethidium bromide. For accurate size determination with supercoiled DNA, supercoiled DNA of known sizes (such as the Supercoiled DNA Ladder) must be electrophoresed in an adjacent lane of the gel (3).

5.2.2 Heat Effects

Electrophoresis at high voltages generates heat, and high conductivity buffers such as TAE generate more heat than low conductivity buffers. Caution should be exercised in agarose gel electrophoresis at >175 V. Heat buildup can cause gel artifacts such as S-shaped migration fronts, and in prolonged electrophoresis, can melt the agarose gel. Low-melting-point agarose gels should never be electrophoresed at high voltages. Nominal electrophoresis times for agarose gels in TBE and TAE buffers at various voltages are listed in table 7.

Note: Electrophoretic procedures originally developed with Models H4 and H5 and discontinued Models H1 and H3 often generate additional heat when performed with the HORIZON Apparatus. At higher voltages, these heat effects can melt the agarose gel. Additional buffer circulation and cooling steps may be necessary to adapt such procedures for use with the HORIZON Apparatus.

To prevent drying of the gel and ensure an even voltage gradient across the gel bed, submerge the gel with electrophoresis buffer to a depth of only 1 to 2 mm. Submerging the gel at a depth >2 mm is unnecessary and increases electrical current and heat.

Caution: Electrophoresis at >175 V generates sufficient heat to melt agarose gels. Do not exceed the high voltage electrophoresis times listed above without cooling the gel during electrophoresis. Do not perform electrophoresis using low-melting-point agarose gels at high voltages.

Table 7. Nominal Electrophoresis Times for 1% Agarose Gels at Various Voltages.

Electrophoresis		Tim	ie ^b (h)
Voltage (V)	Buffer ^a	11•14	20•25
25	TBE	18	60
	TAE	30	76
	0.5X TBE	17	50
50	TBE	7.4	26
	TAE	7.0	29
100	TBE	3.4	11
	TAE	2.7	12
	0.5X TBE	3.6	11
150	TBE	2.0	7.2
	TAE	1.4	4.4
250	TBE	0.9	3.0
	TAE	nd	nd
	0.5X TBE	1.1	2.8

Note: Values were determined with the gel submerged 1.5 mm; the operating current ranged from 4 to 360 mA. Current and electrophoresis time vary with buffer volume, gel thickness, and applied voltage.

^aFormulations for TAE and TBE electrophoresis buffers are listed in tables 1 and 2.

^bValues represent the time required for BPB dye to migrate 13 cm from the origin in the HORIZON 11•14 and 22 cm in the HORIZON 20•25. In a 1% agarose gel, BPB comigrates with DNA fragments of approximately 200 bp in 1X TBE buffer and 400 bp in 1X TAE buffer.

5.3 Ethidium Bromide Staining of Double-Stranded DNA

To visualize double-stranded DNA after electrophoresis, the gel should be transferred from the UVT tray to a 0.5 μ g/ml solution of ethidium bromide in deionized water. Approximate staining time is 10 to 15 min for a 3-mm thick gel and longer for thicker gels. As an optional subsequent step to reduce background fluorescence, the gel can be destained in deionized water for 15 to 30 min.

Alternatively, ethidium bromide may be added directly to the agarose prior to casting, so that the gel is electrophoresed in the presence of ethidium bromide. However, this procedure reduces the migration rate and may alter the relative electrophoretic mobility of nucleic acids (3).

5.4 Gel Photography

A darkroom or light-tight enclosure, camera, and UV light source are required for photography of gels stained with ethidium bromide. For best results, place the stained gel directly on top of a 300-nm or 254-nm transilluminator. If the camera contains Polaroid Type 57 or equivalent file (ASA 3000), the required exposure at maximum aperture (f/4.5) should be between 1/4 and 2 s. The intensity of the light source, distance between the gel and the camera lens, film speed, lens aperture, and choice of photographic filters will all affect the exposure time. Use of a 300-nm transilluminator allows gels to be photographed while in place in the UVT tray, although this will increase the required exposure time.

Transmitted UV light yields the highest sensitivities (1 ng of DNA in a 5-mm wide band) in photographing gels. Photography under incident UV light is approximately 10 times less sensitive. A UV-blocking filter (Kodak 2B Wratten filter) used in conjunction with a red gelatin filter (Kodak 23A Wratten filter) provides the highest contrast. Due to the fluorescence of the 2B filter, the two filters must be oriented so that the red 23A filter is adjacent to the camera lens. The ethidium bromide-DNA complex fluoresces at 590 nm upon excitation at 302 nm (2). Short-wave (254 nm) sources provide an equivalent level of sensitivity; however, high-energy UV causes photodimerization and nicking of the DNA. Long-wave transilluminators (366 nm) are much less efficient.

References

- 1. Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- 2. Rickwood, D. and Hames, B.D. (eds.) (1982) *Gel Electrophoresis of Nucleic Acids: A Practical Approach,* IRL Press, Oxford, England.
- 3. Longo, M.C. and Hartley, J.L. (1986) *Focus® 8:3,* 3.

Related Products

Product	Size	Cat. No.
Concert [™] Rapid Gel Extraction System	50 reactions	11456-019
	250 reactions	11456-027
CONCERT Matrix Gel Extraction System	150 reactions	11457-017

The following replacement parts and accessories are available separately from Life Technologies for use with the HORIZON 11•14 and 20•25 Apparatus.

Product	Cat. No.
HORIZON 11•14 Apparatus Accessories:	
HORIZON 11-14 Gel Casting System	11068-046
Blot Transfer Apparatus 11•14	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 2.0-mm thick	11951-068 11951-084
14-tooth: 1.0-mm thick 2.0-mm thick	31081-011 31081-029
20-tooth: 1.0-mm thick 2.0-mm thick	11951-076 11951-092
Custom DELBIN Combs	*
UVT Tray $(11.0 \times 14.0 \times 2.0\text{-cm})$	11084-019
Gel Casting Dams (pair)	11068-053
Gel Casting Clamps (pair)	10245-371
Bull's Eye Level	11957-016
TygoN [®] Tubing (50 ft.) 0.25-in. I.D.	31048-010
HORIZON 20•25 Apparatus Accessories:	
HORIZON 20•25 Gel Casting System	21069-067
Blot Transfer Apparatus 20•25	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 2.0-mm thick	11953-072 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

7

Related	Product	Cat. No.
	Custom Delrin Combs	*
Products	UVT Tray (20.0 × 25.0 × 3.5-cm)	31006-026
	Gel Casting Dams (pair)	21069-059
	Gel Casting Clamps (pair)	10245-397
	Bull's Eye Level	11957-016
	TYGON Tubing (50 ft.) 0.25-in. I.D.	31048-010
	*Please call the TECH-LINE SM for information about custom combs.	
	HORIZON 11-14 Apparatus Replacement Parts (see Figure 5):	
	Power Cords (48-in., pair)	11099-041
	Electrode Replacement	11068-061
	Electrode Hardware Repair Kit	11980-059
	(includes: 2 rubber washers, 2 hex nuts, 2 electrode boots, 1 red nylon cap nut, 1 black nylon cap nut, 2 banana plugs and 2 lock washers)	
	Leveling Foot Replacement	11964-129
	Quick Connect Ports	11962-024
	Replacement Quick Connect Fitting (pkg. of 2)	11940-012
	HORIZON 20•25 Apparatus Replacement Parts (see Figure 5):	
	Power Cords (48-in., pair)	11099-041
	Electrode Replacement	21069-042
	Electrode Hardware Repair Kit	11980-059
	(includes: 2 rubber washers, 2 hex nuts, 2 electrode boots, 1 red nylon cap nut, 1 black nylon cap nut, 2 banana plugs and 2 lock washers)	
	Leveling Foot Replacement	11964-129
	Quick Connect Ports	11962-024
	Replacement Quick Connect Fitting (pkg. of 2)	11940-012

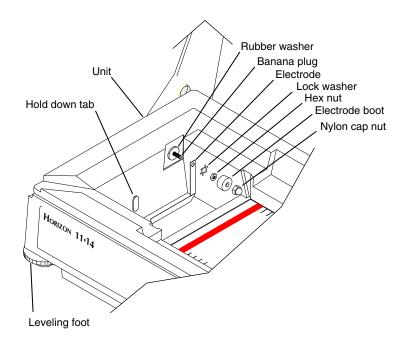


Figure 5. HORIZON 11-14 and 20-25 Replacement Parts.

8

Additional Information

8.1 Care and Handling

The components of the HORIZON 11•14 and 20•25 Apparatus are fabricated from ABS, cast acrylic, and aluminum. 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.

8.2 Specifications

"Installation Category I"

HORIZON 11•14 Apparatus:	
Weight	1.7 kg (3.7 lb.)
Dimensions $(W \times L \times H)$	$\ldots \ldots \ldots \ldots .21.5 \times 31.5 \times 10.5 \text{ cm}$
	$(8.5 \times 12.4 \times 4.1 \text{ in.})$
Construction	Flame retardant ABS, acrylic, aluminum
Gel dimensions (W \times L)	
	(4.3 × 5.5 in.)
Maximum gel thickness	
Working buffer volume	
Electrode material	platinum/niobium laminate
Comb (included)	14-tooth, 1.0-mm thick
Voltage Range	
Current Range	4-360 mA, 0.5 A Max
Operating Temperature Range	4-30°C (non-condensing atmosphere)

"Installation Category I"

HORIZON 20•25 Apparatus:	
Weight	
Dimensions $(W \times L \times H)$	$\ldots \ldots \ldots .32.0 \times 42.5 \times 12.0 \text{ cm}$
	(12.6 × 16.7 × 4.7 in)
Construction	Flame retardant ABS, acrylic, aluminum
Gel dimensions (W \times L) \ldots	
	(7.9 × 9.8 in.)
Maximum gel thickness	
Working buffer volume	
Electrode material	
Comb (included)	
Voltage Range	
Current Range	4-360 mA, 0.5 A Max
Operating Temperature Range	4-30°C (non-condensing atmosphere)

Additional Information

8.3 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.

8.4 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. 14211 Lot No. 1086764