user manual

Hoefer HE-FLEX

Horizontal Gel Electrophoresis System

Hoefer®

Contents

Important Information

- If this equipment is used in a manner not specified by Hoefer, Inc. the protection provided by the equipment may be impaired.
- This instrument is designed for indoor laboratory use only.
- Only accessories and parts approved or supplied by Hoefer, Inc. may be used for operating, maintaining, and servicing this product.
- Warning! Because this instrument can develop sufficient voltage and current to produce a lethal shock, care must be exercised in its operation.
- This instrument is designed in accordance with the EN61010-1:2001 electrical safety standard. Nevertheless, it should be used only by properly trained operators. Read this entire manual before using the instrument and use only according to the instructions.
- The instrument must always be used with the earth lead of the power cord correctly grounded to earth at the mains outlet.
- Use only undamaged electrical wire and equipment specific for the voltages you will use. All equipment connected to high voltage should be in accordance with EN61010-1:2001.
- Keep the instrument as dry and clean as possible. Wipe regularly with a soft, damp cloth. Let the instrument dry completely before use.
- Do not operate the instrument in extreme humidity (above 80%). Avoid condensation by letting the unit equilibrate to ambient temperature when taking the instrument from a colder to a warmer environment.
- To permit sufficient cooling, ensure that the vents of the instrument are not covered.

Waste Electrical and Electronic Equipment (WEEE)

This symbol indicates that the waste of electrical and electronic equipment must not be disposed as unsorted municipal waste and must be collected separately. Please contact an authorized representative of the manufacturer for information concerning the decommissioning of your equipment

Packing List Hoefer HE-FLEX Gel System

Units include:

Tank with lid, power supply, power cord, and standard casting set .

A:Front

B:Reverse side

Standard casting kit contains:

A&B: casting stand

C: Large Gel trays 13.0 x 13.0 cm

D: Medium Gel trays 13.0 x 6.5 cm

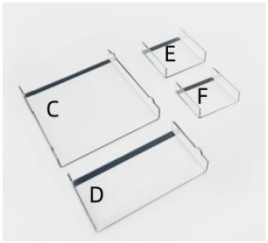
E/F: Mini Gel trays 6.5 x 6.5 cm

G: Medium comb 7+7/14 Wells, 1.5 mm thick

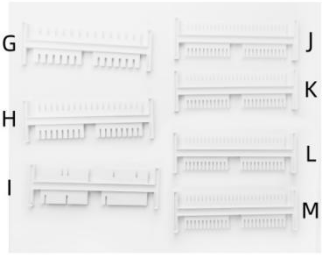
H: Medium comb 9+9/19 Wells, 1.5 mm thick

I: Mini Comb 2+3/3+3 Wells, 2 mm thick

J/K/L/M: Large comb 12+12/27 Wells, 1 mm thick



The packing lists should be referred to as soon as the units are received to ensure that all components have been included. The unit should be checked for damage when received, save packaging for carrier. Please contact your supplier if there are any problems or missing items.



Usage Guidance and Restrictions

- Temperature range between 4 °C and 40 °C.
- Maximum relative humidity 80% for temperatures up to 31 °C decreasing linearly to 50% relative humidity at 40 °C.
- Not for outdoor use.

This apparatus is rated POLLUTION DEGREE 2 in accordance with IEC 664. POLLUTION DEGREE 2, states that: "Normally only non-conductive pollution occurs. Occasionally, however, a temporary conductivity caused by condensation must be expected".

Specifications

Unit dimensions (W × L × H)	19.5 ×26.3 × 6.2 cm
Gel dimensions (W × L)	13.0 × 13.0 cm
Maximum sample capacity	108 samples (4 combs, 27 samples each)
Buffer capacity	500 ml
Distance between electrodes	13.5 cm

Electrophoresis Tank

Overall dimension (W × L × H)	15.0 × 15.0 × 4.0 cm
Material characteristic	UV transmitting (50% at 254 nm, 80% at 312 nm)
Solution volume	500 ml (includes buffer and gels)

Safety Lid

Material characteristic	UV non-transmitting acrylic

Power Supply

Weight	830 g
Input voltage	AC 100 – 240 V, 50/60 Hz
Output voltage	10 to 150 volts in 10 V steps; Constant peak voltage of 150 V
Output amperage	10 to 400 mA in 5 mA steps
Maximum wattage	55 W
Timer	99 hours 59 min, and continuous model
Safety switch	A microsensor in the power supply prevents output without the safety lid in place.
Memory function	Automatic memory (the last used Volt and Time)

Operating Instructions

A. Guidelines for Selecting Electrophoresis Buffers and Gel Concentrations

The two most commonly used buffers for horizontal electrophoresis of double stranded DNA in agarose gels are Tris-Acetate-EDTA (TAE) and Tris-Borate-EDTA (TBE). While the resolving powers of these buffers are very similar, the relative buffer capacities are very different, conferring different run attributes which are summarized below:

TAE

Tris-acetate has traditionally been the more commonly used buffer. However, its relatively low buffer capacity will become exhausted during extended electrophoresis, making buffer recirculation necessary in runs exceeding 140 mA-hours. Potential advantages of using TAE buffer over TBE buffer include superior resolution of supercoiled DNA and approximately 10% faster migration of double-stranded linear DNA fragments.

TBE

Tris-borate's significantly greater buffering capacity and its relatively low current draw eliminates the need for recirculation in all but the most extended runs (> 300 mA-hours). TBE buffer systems are not recommended when fragments are to be recovered from the gel after electrophoresis.

1

Make 600 ml of either 1X TAE or 1X TBE electrophoresis buffer.

2

Weigh an appropriate quantity of agarose (see Table 1) and place it into a 250 ml flask. Add a sufficient quantity of either 1X TAE or 1X TBE buffer (prepared in step 1) to achieve a final volume of 100 ml agarose solution.

Table 1: Gel Concentrations and Resolving Ranges

Concentration of Agarose in Gel (%w/V)	Agarose (g) per 100 ml Buffer	Efficient Range of Separation of Linear DNA (Kb)
0.3	0.3	5 – 60
0.6	0.6	1 – 20
0.7	0.7	0.8 – 10
0.9	0.9	0.5 – 7
1.2	1.2	0.4 – 6
1.5	1.5	0.2 – 3
2.0	2.0	0.1 – 2

Table taken from Sambrook, J., Fritsch, E.F., & Maniatis, T. (1989) Molecular Cloning, A Laboratory Manual, 1, 6.8 613.

3

Make note of the total solution volume so that degree of evaporation can be determined and corrected for.

4

Heat the agarose slurry in a microwave oven for 90 seconds. Swirl the flask to make sure any grains sticking to the walls enter into the solution. Undissolved agarose appears as small "lenses" floating in the solution. Heat for an additional 30 – 60 seconds. Re-examine the solution and repeat the heating process until the agarose completely dissolves.

5

Add deionized water to replace any volume lost through evaporation during the heating process.

6

Add your detection reagent (i.e. SYBR Safe) to the manufacturers' recommended concentration. Mix by gently swirling the flask.

Note: The addition of SYBR Safe to both the gel and the running buffer provides high sample fluorescence with a uniformly low background, resulting in improved detection sensitivity.

B. Casting the Gel

1

Place the gel casting stand on a lab bench.

2

Insert the gel casting tray into the casting stand. If you need to cast a 130x130 mm gel, please choose a large gel tray 130x130 mm.

3

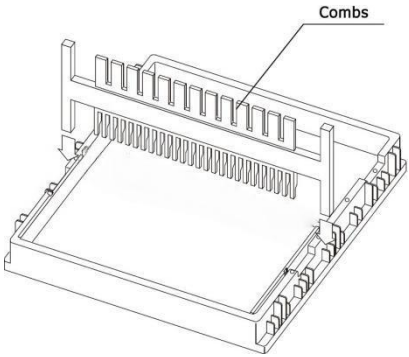
When the gel solution has cooled to approximately 55 °C, slowly pour it into the gel tray. If hotter gel solutions are routinely poured, the tray may warp over time.

4

If bubbles form on the surface of the gel upon pouring, use the comb to either pop them or lightly brush them to the sides of the gel. If large bubbles are allowed to harden within the gel, they may cause artifacts to occur during electrophoresis.

5

Insert one or more combs by placing them into the slots in the casting stand. For best results, place the comb in the slot nearest the end of the casting fixture. If two combs are desired, place the second in the center comb slot.



6

Allow the gel to harden undisturbed for at least 30 minutes.

C. Removing the Comb

1

When the gel is solidified and fully opaque, carefully remove the comb with a gentle wiggling, upward motion. If the comb is difficult to remove or if a low percentage gel is being used, overlay the comb area with a small volume of 1X electrophoresis buffer to preserve the integrity of the wells. Check the wells to ensure their bases are intact.

D. Loading the Samples onto the Gel

1

Remove the casting tray containing the hardened agarose gel from the casting fixture by lifting the ends. Place the tray and gel into the main unit assembly such that the sample wells are on the same end as the negative (black) electrode.

2

Fill the unit with the remaining 1X electrophoresis buffer containing SYBR Safe made previously, covering the gel to a depth of 1– 5 mm. Approximately 350 ml of buffer will be required.

3

Load the samples into the wells with a micropipette or similar device taking care not to puncture the bottom of the wells or load the sample onto the top of the gel.

Note: Use of the same batch of electrophoresis buffer for both the gel and the running buffer is very important. Slight variations in buffer composition between gel and running buffer may result in ionic or pH gradients that can significantly impact the mobility of the samples.

Note (Lid Alarm Setting)

- **Default Setting**

The Lid Alarm is enabled each time the unit is powered on.

- **To Disable the Lid Alarm**

1. With the unit powered off, press and hold the SET button.
2. While holding SET, turn on the unit.
3. When "LID" appears on the screen, release the SET button.
4. After "LID" flashes three times, the Setting Menu will appear.
5. Use the ▲/▼ buttons to select the desired value:
   0000 – Lid Alarm ON
   0001 – Lid Alarm OFF
6. Select 0001, then press SET to save and exit.
7. The unit will now operate according to the selected setting.

E. Electrical Connections to the Safety Lid

The Hoefer HE-FLEX Gel System can be operated with the safety lid in place (If you want to disable the Lid Alarm Setting, please refer to the note on the left).

Electrical current is supplied through the tank electrodes from the power supply. A micro switch connector in the power supply ensures a complete current path, yet allows the lid to be removed from the unit without disturbing the loaded samples.

1

Make sure the power supply is turned off.

2

Plug the male ends of the black (–) and red (+) electrodes into the jacks on the side of the power supply.

3

After the samples have been loaded into the gel, place the lid over the unit so that the lid covers align with the tank.

Set the lid straight down so that the lid rests squarely on the tank. Ensure the inside of the lid makes contact with the power supply.

4

Plug the power supply into a wall outlet.

F. Setting the Power Supply

1

Ensure an approved power cord that satisfies your regional voltage standard is used. Input voltage is automatically detected by the system.

2

Use the set key > to move between voltage, amperage and time parameters.

3

To increase or decrease voltage use up (^) and down (v) arrow keys.

To increase or decrease amperage use up and down arrow keys.

4

Set the timer. Increase or decrease the value with the up and down arrow keys. Between 99 hours and 59 minutes can be set as the run time. Set "0" for Continuous.

On the left side of the Output key the flashing LED indicates that the timer operation has been paused. When setting up the timer in this state, set up after having pushed the output button for a long time so as to reset the timer.

5

Select the required output voltage up to 150 volt or 400 mA.

6

Press the start/pause key to start the run.

G. To Pause a Run and/or Change Parameters

CAUTION: Do not jar or bump the gel box once the lid is place. The electrical connection is made by gravity once the lid is in position. While this design helps to minimize sample disturbance during lid placement, it also may result in a disruption of power to the unit if the lid or unit are disturbed during the run.

1

To pause the run select Pause. During the pause mode the voltage amperage or time can be changed by highlighting the function and using the arrow keys then pressing enter. Once the changes have been made the start button can be pressed to resume the run.

2

To stop the run press the pause button for 30 seconds. Stop will appear.

H. Sample Electrophoresis

CAUTION: DO NOT EXCEED THE MAXIMUM OPERATING VOLTAGE OF 150 VOLTS.

1

The maximum suggested applied voltage for the electrophoresis of DNA in agarose gels using the Hoefer HE-FLEX Gel System is 150 volts.

2

In a 1% TBE gel, this translates into a run time of approximately 1 hour. Lower voltages may be used, of course, and as a general rule, a 70 V run will take twice as long as a 145 V run. Higher voltages may be used to decrease run time, however, if the unit is being operated at higher voltages than 150 V, the heat generated during electrophoresis may decrease sample resolution. Such artifacts may be avoided by running the unit in a cold room or adding 1X electrophoresis buffer "ice cubes" to keep the unit properly cooled.

3

Follow the sample migration into the gel using the loading dye as an indicator. (See page 12 for the Sample Loading Buffer recipe.) Allow the samples to migrate until the fragments have separated, normally until the bromophenol blue dye front has migrated ¾ of the way down the gel.

I. Detection and Documentation of Separated Fragments

1

At the completion of the run, turn off the power supply and disconnect the power cord. Remove the lid and remove the gel tray. Alternatively the entire tank can be placed on a Transilluminator.

2

SYBR Safe stained samples are visualized by exposing them to medium wavelength (312 nm) UV light. Because the gel casting tray is UV transmittant, the gel does not need to be removed from the tray before viewing. Place the gel casting tray containing the gel on the filter surface of a UV Transilluminator for convenient viewing.

Note: If the gel contains SYBR Safe, the progress of electrophoresis may be monitored during the run by turning off the power supply, removing the lid, and illuminating the gel with a medium-wave UV light. The resolved bands will appear as bright green fluorescence against a dark background.

J. Separate operation of tank and power supply

1

Hold the power supply firmly with your right hand to keep it steady on the bench.

2

With your left hand, lift the left side of the electrophoresis tank lid gently upward using the handle.

3

The tank will disconnect smoothly from the power supply.

Troubleshooting Guide

Problem	Cause	Solution
The LCD screen remains blank and the fan does not run when the power is turned on.	AC power cord is not connected.	Check AC power cord connections at both ends. Use the correct cords.
	The fuse has blown.	Replace the fuse.
	Power controller failure.	Contact the manufacturer or supplier.
Operation stops with alarm: The screen displays "NO LOAD".	Electrophoresis tank is not connected to the power supply or there is a broken circuit in the electrophoresis cell.	Check the connections to the power supply and on your electrophoresis cell to make sure the connection is intact; check condition of wires in electrophoresis unit. Close the circuit by reconnecting the cables. Press RUN/PAUSE to restart the run.
	Buffer concentration incorrect.	Replace buffer.
Operation stops with alarm: Display shows "OVER VOLTAGE".	Circuit is interrupted.	Verify that the running buffer is correct. Verify the all connections are attached correctly. Turn the Power Switch off and on again; restart application. If you cannot restart the instrument, turn off the power, disconnect the power cord from the outlet, and contact Technical Service.
Operation stops with alarm: Display shows "LEAKAGE".	Ground leak detected during run.	Check the electrophoresis system for improper grounding. Restart the power supply by turning the Power switch off and on. Turn power off then check the gel tank for buffer leakage.
LID alarm.	Cover of gel tank not in correct place.	Turn off power supply. Place the lid so the magnet is pressed against the power supply and restart. If you cannot restart the instrument, turn off the power, disconnect the power cord from the outlet, and contact Technical Service.

Solutions

Tris Acetate EDTA Buffer (TAE)

1X Working Concentration:	
40 mM	Tris base
20 mM	Glacial Acetic Acid (NaOAc)
2.0 mM EDTA	
pH 8.3	

10X Stock Solution:	
48.4 g	Tris Base
16.4 g or 11.42 ml NaOAc	
7.4 g EDTA or 20 ml 0.5 M EDTA (pH 8.0)	
H_2O to 1 liter	

Tris Borate EDTA Buffer (TBE)

1X Working Concentration:	
89 mM	Tris Base
89 mM	Boric Acid
2.0 mM EDTA	
pH 8.0	

10X Stock Solution:	
108 g	Tris Base
55 g	Boric Acid
6.72 g EDTA or 40 ml 0.5 M EDTA (pH 8.0)	
H_2O to 1 liter	

Sample Loading Buffer, DNA

6X Stock Solution:	
30%	Glycerol in H_2O
0.25%	Xylene cyanol
0.25%	Bromophenol blue
pH 8	

Ordering Information

Product	Code No.
HE-FLEX Electrophoresis complete system 100-240 V. Includes gel tank, safety lid, power supply and standard casting kit.	HE-FLEX

Accessory Items

HE-FLEX Large Gel tray 13.0 × 13.0 cm, 1 pcs	HE-FLEX11
HE-FLEX Medium Gel tray 13.0 × 6.5 cm, 1 pcs	HE-FLEX12
HE-FLEX Mini Gel tray 6.5 × 6.5 cm, 1 pcs	HE-FLEX13
HE-FLEX Casting Stand for all 3 gel sizes	HE-FLEX-CS
HE-FLEX Large comb 12+12/27 Wells, 1 mm thick, 1 pcs	HE-FLEX1227
HE-FLEX Medium comb 9+9/19 Wells, 1.5 mm thick, 1 pcs	HE-FLEX0919
HE-FLEX Medium comb 7+7/14 Wells,1.5 mm thick, 1 pcs	HE-FLEX0714
HE-FLEX Mini Comb 2+3/3+3 Wells, 2 mm thick, 1 pcs	HE-FLEX0506
HE-FLEX Casting Kit Includes: 1 large gel tray, 1 medium gel tray, 2 mini gel trays, 4 large combs (12+12/27 wells), 2 medium combs (9+9/19 wells and 7+7/14 wells), 1 mini comb (2+3/3+3 wells) and casting stand	HE-FLEX14

Care and Maintenance

Important: The units should never come into contact with the following cleaning agents, these will cause irreversible and cumulative damage:

Acetone, Phenol, Chloroform, Carbon tetrachloride, Methanol, Ethanol, Isopropyl alcohol, Alkalis.

Cleaning

Units are best cleaned using warm water and a mild detergent. Water at temperatures above 60 °C can cause damage to the unit and components. The units should not be left in detergents for more than 30 minutes. The tank should be thoroughly rinsed with warm water and distilled water to prevent build up of salts but care should be taken not to damage the enclosed electrode. Vigorous cleaning is not necessary or advised. Air drying is recommended before use.

RNase Decontamination

This can be performed using the following protocol:

- Clean the units with a mild detergent as described above.
- Wash with 3% hydrogen peroxide (H_2O_2) for 10 minutes.
- Rinse with 0.1% DEPC- (diethyl pyrocarbonate) treated distilled water.
- **Caution!** DEPC is a suspected carcinogen. Always wear gloves and safety glasses.

RNaseZAP™ (Ambion) can also be used. Please consult the instructions for use with acrylic gel tanks.

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