ENDURO™ GEL XL

User Manual

HORIZONTAL GEL ELECTROPHORESIS UNIT

E0160
E0160-230V
E0160-230V-UK
E160-CAN

Intertek

Labnet

Lit M00078

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About This Manual

This manual is designed to assist you in the optimal usage of your Enduro GelXL. The manual is available in English, French, German, Italian, Portuguese, and Spanish on our website at **www.labnetinternational.com/document-center**

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I. MAINTENANCE

Please handle the unit with care:

Do not expose the unit or its accessories to temperatures above 60°C.
Do not expose the unit to organic solvents.
Do not clean the unit with abrasive cleaners or cleaning aids.

In most cases, rinsing with deionized water will sufficiently clean the unit. For heavier dirt, use a mild cleansing solution such as dish soap (alkaline cleansers are not recommended). Hand wash and dry with a soft cloth. To remove residual ethidium bromide, occasionally soak the unit in 1% commercial bleach solution for 16 hours. Rinse well.

PLEASE NOTE: The degradation of acrylic due to solvents may result in substantial discoloration, cracking, warpage, or etching of the electrophoresis unit.

Do not apply any of the following solvents: benzene, xylene, toluene, chloroform, carbon tetrachloride, alcohols, phenols, ketones, or esters.

Do not expose the ABS combs supplied with this unit to formaldehyde for extended periods. When casting gels containing formaldehyde, remove the combs promptly upon hardening of the gel and rinse completely with deionized water.

Elimination of RNase Contamination

Should treatment of the unit to eliminate RNase contamination be desired, clean the unit with a mild detergent as described above followed by soaking for 10 minutes in a solution of 3 % hydrogen peroxide, and then for 1 hour in 0.1 % DEPC (diethyl pyrocarbonate). Pour out final rinse, and air dry.

CAUTION: DEPC is a suspected carcinogen; handle with care.

Alternatively, soak the unit and accessories in freshly made 2.2 mM acetic anhydride treated water (200 µl/liter) for at least five minutes. Solutions for RNA work (electrophoresis buffers, etc) may be made from the same acetic anhydride treated water as well.

WARNINGS:

CAUTION! Injury, damage to equipment, or property, may result if used in a manner not specified by the manufacturer.

CAUTION! A pinch hazard exists between the plastic enclosure and the shaking head.

CAUTION! **NOT** for use with flammable liquids.

II. OPTIONS AND SPECIFICATIONS

A. Components and Accessories

Catalog # **Description**

E0160 **ENDURO Gel XL Complete Electrophoresis System**
Comes complete with 1) 12.5 x 12 cm, 2) 12.5 x 6 cm UV Transmittant casting trays, casting stand with divider, and four 1.0 mm thick 28/14 reversible tooth combs, power cord and manual.

Accessories

Catalog #	Description
E0161	(1)12.5 x 12cm UV Transmittant Casting Tray
E0162	(2) 12.5 x 6 cm UV Transmittant Casting Tray
E0163	(4) 6 x 6cm UV Transmittant Casting Tray
E0164	(2) 1 mm x 14/28 tooth Reversible Comb
E0165	**(**2) 1mm 5/8 tooth Reversible comb
E0166	Micro casting set - (4) 6 x 6cm UV Transmittant Casting Tray, 2) 1mm 5/8 tooth Reversible combs, Casting stand with divider
E0167	Casting Stand with divider
E0168	Standard casting set – (1) 12.5 x 12cm tray, (2) 12.5x6cm trays, (4) 14/28 tooth multi-channel compatible combs, casting stand with divider
R1000-100BP	Molecular Weight marker 100 bp
R1000-1KB	Molecular Weight marker 1 Kb

B. Specifications

Unit dimensions	24.5 x 17.0 x 6.2 cm
Gel dimensions	12.5 x 12.0 cm
Maximum sample capacity:	112 samples (4 combs, 26 samples each)
Buffer Capacity:	300 ml
Distance Between Electrodes:	13.5 cm
Electrophoresis Tank	
Overall dimension	18.3 × 16.4 × 5.6 cm
Material characteristic	UV transmitting (50% at 254nm, 80% at 312nm)
Solution volume	300ml (includes buffer and gels)
Safety Lid	
Overall dimension	19.7 × 16.9 × 3.8 cm
Material characteristic	UV non-transmitting Polycarbonate

Power Supply	
Overall dimension	7.5 × 17.0 × 6.2 cm
Weight	410 g
Input Voltage	AC100 - 240V, 50/60Hz
Output Voltage	10 to 150 volts; Constant peak voltage of 150V
Output Amperage	10 to 400 mA
Maximum Wattage	45 W
Timer	99 hours 59 min, and continuous model
Safety Switch	Micro-sensor (hall) in the Power Supply. No output without safety lid,
Memory Function	Automatic memory (the last used V and T)

III. OPERATING INSTRUCTIONS

A. Preparation of the Agarose Gel and Electrophoresis Buffer - DNA

1. Select the percentage gel necessary to effectively resolve your sample, using Table 1 as a guideline.

Table 1: ***Gel Concentrations and Resolving Ranges***

Concentration of Agarose in Gel (% w/V)	Efficient Range of Separation of Linear DNA (Kb)
0.3%	5 - 60
0.6%	1 - 20
0.7%	0.8 - 10
0.9%	0.5 - 7
1.2%	0.4 - 6
1.5%	0.2 - 3
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.

2. Weigh an appropriate quantity of agarose (0.3 % means 0.3 g of agarose per 100 ml of gel volume) and place it into a 250 ml flask. Note a 4mm gel will use 100 mls of agarose solution.

3. Make 500 ml of either 1X TAE or 1X TBE electrophoresis buffer (see below).

Electrophoresis Buffers

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 [(1)] .

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.

4. Add ethidium bromide to the diluted electrophoresis buffer to a final concentration of 0.5 µg/ml.

NOTE: The addition of ethidium bromide to both the gel and the running buffer will result in maximum detection levels by providing high levels of sample fluorescence with an evenly low level of background.

5. Add 6.6 ml of the 1X electrophoresis buffer containing ethidium made in step 4 per millimeter of gel thickness desired, up to a maximum to 100 ml, to the flask containing the agarose. A 100 ml gel solution will make a 7.6 mm thick gel. Thinner gels may be made, however care must be taken that the wells are deep enough to accommodate the desired sample volume.

Catalog #	Comb Description	Well Width	Sample Volume 1mm
E0167	1 mm, 14 tooth	5mm	5ul
E0167	1 mm, 28 tooth	2.5mm	2.5ul
E0168	1 mm, 5 tooth	8mm	8ul
E0168	1mm, 8 tooth	4mm	4ul

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

7. 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.

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

Proceed to Section C, Step 1, "Casting the Gel" on page 12.

B. Preparation of the Agarose Gel and Electrophoresis Buffer - RNA

RNA molecules are separated by electrophoresis through denaturing gels prior to analysis by northern hybridization. Agarose gels containing formaldehyde[1, 2, 3] are commonly used for RNA electrophoresis. Presented below is a general protocol for electrophoresis of RNA using formaldehyde gels.

CAUTION! All equipment and solutions used in the following protocol should treated with DEPC (diethyl pyrocarbonate) or acetic anhydride prior to use to inhibit RNase activity (see Section II, page 4 for protocol). It is recommended that dedicated solutions be made solely for RNA work to minimize the risk of sample degradation due to RNase activity.

NOTE: Staining RNA samples with ethidium bromide has been reported to reduce sample blotting efficiency. Therefore, if samples are to be analyzed by northern hybridization after electrophoresis, run a duplicate lane(s) for staining, or minimize the exposure of RNA samples to ethidium bromide by following the post-electrophoresis staining protocol on page 12.

The following protocol will make 50 ml of a 1.5 % agarose gel containing 1X MOPS [3-(N-morpholino)-propanesulfonic acid]-Acetate-EDTA (MAE) buffer and 2.2 M formaldehyde, resulting in a 7.5 mm thick gel:

1. Weigh out 0.5 g of agarose, and place into a 125 ml flask.
2. Add 43.5 ml of DEPC (or acetic anhydride) treated water.
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 60 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. Allow the solution to cool to 60°C. Place the flask in a hood and add 5 ml of 10X MAE buffer (see Appendix A for recipe), and 1.5 ml of 37 % formaldehyde.

CAUTION: Formaldehyde vapors are toxic. Gel preparation should take place in a hood and solutions and gels containing formaldehyde should be kept covered when possible.

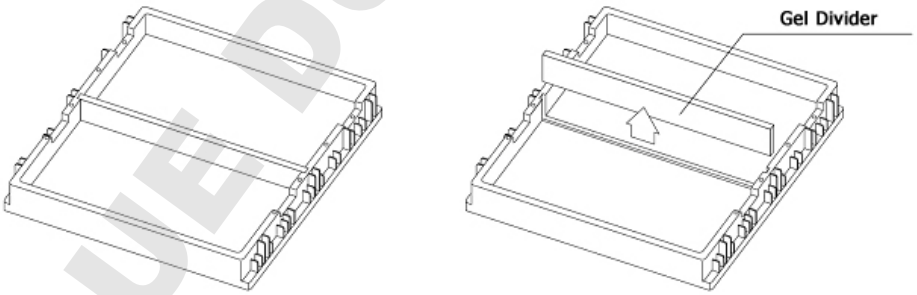
Proceed with Section C, Step 1, "Casting the Gel" on page 12.

C. Casting the Gel

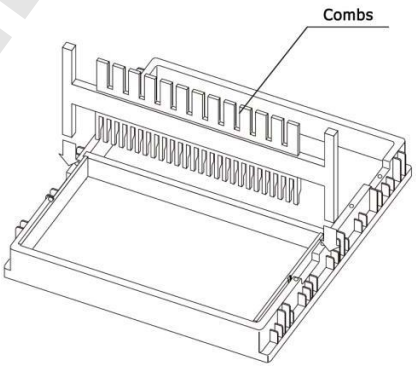
1. Place the gel casting stand on a lab bench.

CAUTION! Cast agarose gels containing formaldehyde in a hood.

2. Insert the gel casting tray into the casting Stand. If you are using the 12 x 6 cm gels place the spacer in the center of the casting Stand, then insert the two 12 x 6 cm landscape gel trays (see direction 2 below).



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.

D. 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.

CAUTION: Prolonged exposure of the combs supplied to gels containing formaldehyde will cause them to degrade. Be sure to remove the comb(s) from formaldehyde gels as soon as gel hardening is complete and rinse them well prior to storage.

If a gel is not to be used immediately after preparation, remove it from the casting fixture and place it in a plastic bag or container and submerge in 1X electrophoresis buffer containing 1 mM NaN_3. Store at +4°C.

E. 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 samples wells are on the same end as the negative (black) electrode.

2. Fill the unit with the remaining 1X electrophoresis buffer containing ethidium bromide made previously (or 1X MAE buffer for RNA gels), covering the gel to a depth of 1-5 mm. Approximately 300 ml of buffer will be required.

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.

3. Pre-run RNA gels at 100 V for five minutes prior to loading the

samples.

4. 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. .

F. Electrical Connections to the Safety Lid and

The ENDURO Gel XL can only be operated with the safety lid in place. Electrical current is supplied through the tank electrodes to the power supply by placing the lid on the tank the circuit is completed. A simple gravity connector in the cover 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.
4. Set the lid straight down so that the lid rests squarely on the tank, connection is inside end of the lid which engages the power supply.
5. Plug the Power Supply into a wall outlet.
   Ensure an approved power cord that satisfies your regional voltage standard is used.
   Input voltage is automatically detected by the system. A transformer is not necessary in Europe and any other region where the standard voltage is higher than 100V.
6. Set the timer. Increase or decrease the value with the Up and Down buttons. Timer can be set between 1min – 99 hours. Set "--:--" for continuous operation.
7. Select the required output voltage up to 150 volt or 400 mA.
8. Press the start/stop button to start the run.

To Pause a run and change parameters.

1. To pause the run press the Run/Pause button once. During the pause mode the voltage amperage or time can be changed by highlighting the function and using the arrow keys then pressing the mode key. Once the changes have been made the start button can be pressed to resume the run.
2. To stop the run press the run/pause button for 3 seconds. Stop will appear on the display.

CAUTION: Do not jar or bump the gel box once the lid is place. The safety switch is operated by a hall-effect sensor which relies on a lid mounted magnet. Moving the gel box can move the lid and cause the unit to pause until the lid is put back into position.

G. Sample Electrophoresis

The maximum suggested applied voltage for the electrophoresis of DNA in agarose gels using the Gel XL is **150** volts. 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 volt run will take twice as long as a 140 V run. Higher voltages may be used to decrease run time, however, if the unit is being operated at higher voltages than 140 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.

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

The suggested run parameters for the electrophoresis of RNA in agarose gels containing formaldehyde is 60 - 80 Volts.

CAUTION: Formaldehyde vapors are toxic. Electrophoresis of RNA in gels containing formaldehyde should take place within a fume hood.

Follow the sample migration into the gel using the loading dye as an indicator. (See Appendix A 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 3/4 of the way down the gel.

NOTE: If the gel contains ethidium bromide, the progress of electrophoresis may be monitored during the run by turning off the power supply, removing the lid, and shining a medium-wave UV light onto the gel. The resolved bands will appear as orange bands against a dark purple background.

H. 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. To stain RNA gels containing formaldehyde post electrophoresis, soak the gel in 1 liter of DEPC-treated water overnight at room temperature. Transfer the gel to a solution of 20X SSC containing 0.5 µg/ml of ethidium bromide, stain for 5 -10 minutes.

3. Ethidium bromide 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.

3. Sample banding patterns may be documented by autoradiography

I. Trouble shooting guide

Problem	**Cause**	**Solution**
The LCD screen is blank	AC power cord is not connected	Check AC power cord connections at both ends. Use the correct cords.
	The power switch is not on	Toggle the power switch
Operation stops with alarm: The screen displays "**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 bufffer
Operation stops with alarm: Display shows "**Lid**"	Lid was removed during a run	• Verify that the lid is properly seated • Verify the all connections are attached correctly • press the RUN/PAUSE button to restart
Other error		• Turn off the power, disconnect the power cord from the outlet, and contact Technical Service

IV. APPENDICES

A. Buffers for Electrophoresis

Tris Acetate EDTA Buffer (TAE):

1X Working Concentration:	10X Stock Solution:
40 mM Tris base	48.4 g Tris Base
20 mM Glacial Acetic Acid (NaOAc)	16.4 g or 11.42 ml NaOAc
2.0 mM EDTA pH 8.3	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:	10X Stock Solution:
89 mM Tris Base	108g Tris Base
89 mM Boric Acid	55g Boric Acid
2.0 mM EDTA pH 8.0	6.72g EDTA or 40ml 0.5M EDTA (pH 8.0)
	H_2O to 1 liter

RNA electrophoresis Running Buffer

MOPS Acetate EDTA (MAE):

Solutions containing MOPS should be wrapped in aluminum foil and stored at room temperature. The buffer tends to yellow with age. Light yellow buffer may be used, however, dark yellow solutions should be discarded.

1X Working Concentration:	10X Stock Solution:
20 mM MOPS (pH 7.0)	41.8 g MOPS
8 mM NaOAc	800 ml DEPC treated H_2O
1 mM EDTA (pH 8.0)	adjust pH to 7 with NAOH and add: 16.6 ml 3M DEPC-treated NaOAc 20.0 ml 0.5 M DEPC-treated EDTA, pH 8 bring to 1.0 liter and filter

Solutions containing MOPS should be wrapped in aluminum foil and stored at room temperature. The buffer tends to yellow with age. Light yellow buffer may be used, however, dark yellow solutions should be discarded.

Sample Loading Buffer, DNA

10X Stock Solution:

50 % Glycerol
100mM Na_3EDTA
1% SDS
0.1% Bromophenol blue
pH 8.0

Sample Loading Buffer, RNA

5X Stock Solution:

1 mM EDTA, pH 8.0
0.25 % Bromophenol Blue
0.25 % Xylene Cyanol
50 % Glycerol

B. Physical Properties of Electrophoretic Plastics

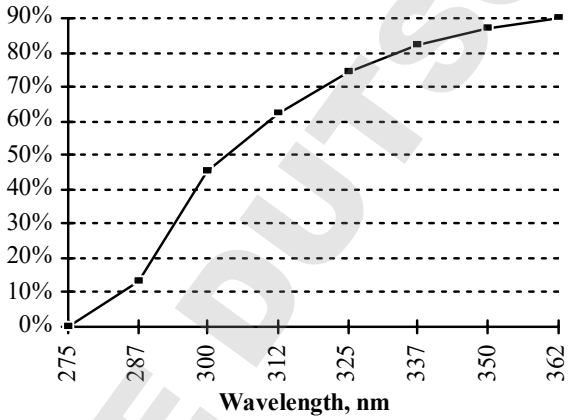


Figure A: UV Transmission Characteristics of UV Gel Tray

The UV transmittant tray is ideal for monitoring the progress of electrophoresis without removing the gel from the tray. Figure A above clearly delineates the absorption specifications of the UV transmittant plastic gel tray. Minimal transmission is seen below

V. REFERENCES

1. Lehrach, H., et al. 1977. *Biochemistry* **16:**4743.

2. Sambrook, J., Fritsch, E.F., and Maniatis, T., (1989). Molecular Cloning, A Laboratory Manual, vol 1. Cold Spring Harbor Press, New York.

3. Selden, R.F. (1988) Analysis of RNA by Northern Hybridization," in *Current Protocols in Molecular Biology*, F.M. Ausubel, et. al, editors, volume 1, p.4.9.1. Green Publishing Associates and Wiley-Interscience.

Technical Support and Information Services*:*

Our staff is available to advise you with any questions regarding our products or their specific application.

For Technical Support and Information in the USA:

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Edison, NJ 08837
Tel: 732-417-0700
www.labnetinternational.com

Symbols and Conventions
The following chart is an illustrated glossary of the symbols that may be used in this manual or on the product.

	The electrical warning indicates the presence of a potential hazard which could result in electrical shock.
	**CAUTION** This symbol refers you to important operating and maintenance (servicing) instructions within the product Instruction Manual. Failure to heed this information may present a risk of damage or injury to persons or equipment.
	This symbol identifies a Protective Earth (PE) terminal, which is provided for connection of the supply system's protective earth (green or green/yellow) conductor.
	This symbol indicates double insulation - no serviceable parts.

EQUIPMENT DISPOSAL-EUROPEAN REGULATIONS

According to Directive 2012/19/EU of the European Parliament and of the Council of 4 July 2012 on waste electrical and electronic equipment (WEEE), Enduro GelXL is marked with the crossed-out wheeled bin and must not be disposed of with domestic waste.

Consequently, the buyer shall follow the instructions for reuse and recycling of waste electronic and electrical equipment (WEEE) provided with the products and available at the following link: www.corning.com/weee

NOTES

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