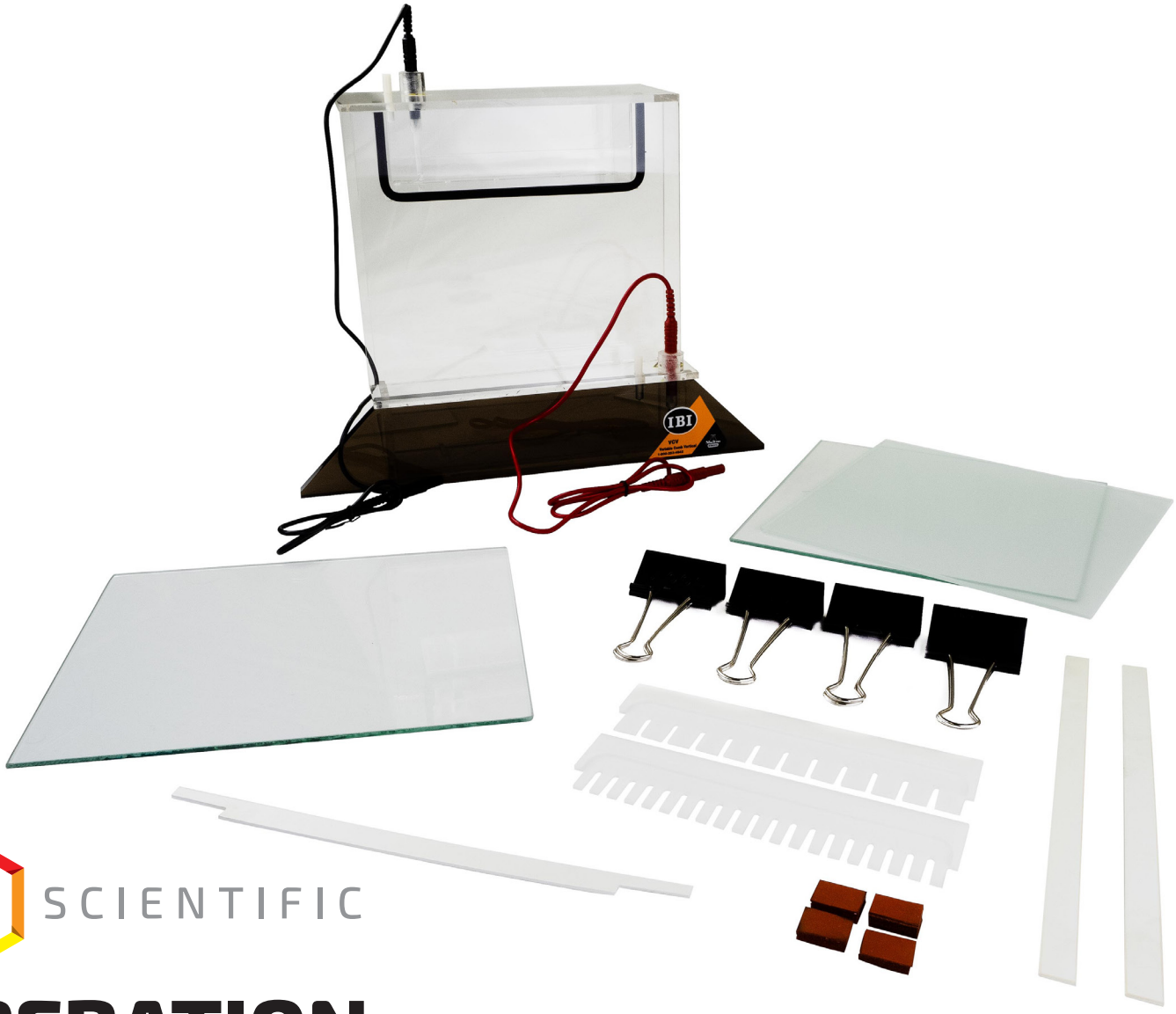


IBI SCIENTIFIC

OPERATION MANUAL

VCV - Variable Comb Vertical System

22cm x 18cm Vertical Gel Electrophoresis System

IB62000

Important Safety Information!

- Please read this manual carefully before operating your new IBI VCV Electrophoresis System.
- This manual contains important operating and safety information.
- To best use this product, please read the entire manual carefully prior to use.
- To avoid possible injury, this product should only be used for its intended purpose.

Package Contents | Unpacking

Upon receiving this product, please verify all of the noted parts and accessories are contained in this package. Awareness of the stated cautions and warnings contained within this manual, compliance with recommended operating parameters, and maintenance requirements are important for safe and satisfactory operation.

- Model IB62000 Vertical Electrophoresis System
- Removable Clear Acrylic Lid w/Red and Black Power Cords
- Inner Glass Plate
- Outer Glass Plate
- Frosted Inner Glass Plate
- 1.5mm Spacer Set*
- 1.5mm x 12 tooth comb*
- 1.5mm x 20 tooth comb*
- (4) Gel Sandwich Clips
- Operation Manual

*Included as a matched set, to ensure thickness does not vary.

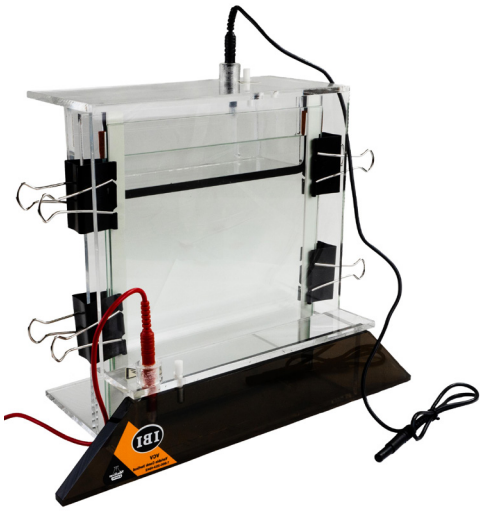
Save all packing material, and these instructions, if the VCV Electrophoresis System is received damaged. This system was carefully packaged and thoroughly inspected prior to leaving the factory. Responsibility for its safe delivery was assumed by the carrier upon acceptance of the shipment; therefore, claims for loss or damage sustained in transit must be made with the carrier.

Note: Carefully inspect all items in the package to insure no items are broken or missing. If there are items broken, please inspect the package carefully for signs of shipping damage. If there is ANY sign of shipping damage, please contact the carrier and file a claim with them immediately. Contact the distributor from which you purchased the item or IBI Scientific directly for assistance at (800) 253-4942 or (563) 690-0484.

Product Specifications

Unit Dimensions: 29cm(h) x 36cm(w) x 12.5 cm(l)
Gel Dimensions: 22cm(w) x 18cm(l)

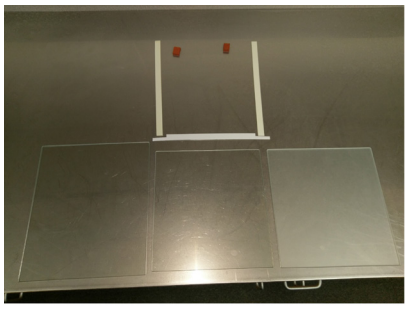
Maximum Sample Capacity: 20 Samples (Using (2) 15 tooth combs)
Buffer Capacity: 550ml Upper Res. / 550ml Lower Res.
Voltage Limit: 600VDC



Operation

Your new VCV Vertical Electrophoresis Unit allows rapid analysis of protein and nucleic acid samples in a 22cm x 18cm format, utilizing either agarose or polyacrylamide gels. This system also allows anaylsis to be completed in less than 90 minutes, while still maintaining comparable gel resolution.

Note: The current to the unit, provided from the external power supply, enters through the lid assembly providing a safety interlock to the user. When the lid is removed, the current to the unit is broken. Do ***NOT*** attempt to use the unit without the safety lid in place, and always turn the power supply OFF before opening the lid.



Assembing the Gel Sandwich

Note: Assembly of the glass plates and spacers are the same for either agarose or polyacrylamide gels. The main difference is for an agarose gel we recommend using the ***FROSTED*** glass plate in the top plate position. The frosted glass plate is included with your VCV Electrophoresis System. The frosted glass plate ensures adhesion of the gel from "slipping" out from between the glass plates after being poured, hardened, and stood vertically.

1.) Before assembly, wash each glass plate with warm soapy water and rinse well. Allow plates to dry completely.

2.) Apply a liberal amount of 70% alcohol to each plate and wipe dry with a lint-free cloth. This will remove any dust, lint, or grease that may have been left behind after washing.

3.) Carefully wipe the polystyrene reversible side spacers with 70% alcohol to remove any dirt or dust that may be residing on them.

4.) Place the longer glass plate on the countertop. Place the side spacers on top of the long plate, with the spacers being flush with the outer edge of the glass plate.

5.) Insert the bottom spacer so that the side spacers fit snuggly into the notched portion of the bottom spacer. See photo to the right.

6.) Place either the frosted (agarose gel) or the clear (polyacrylamide gel) glass plate on top of the spacers, aligning the plate with the outside and bottom edges of the long glass plate and installed spacers.

7.) Clamp the gel sandwich in place using (4) metal spring clamps included with the VCV Electrophoresis System.

8.) Next, remove the backing from the rubber spacer tabs included in the comb/spacer packaging. Stick the spacer tabs to the side spacers at the top of the shorter glass plate (clear or frosted). Placing securely against the top edge of the shorter glass plate, press down on the spacer tab to secure in place

9.) These (2) spacer tabs prevent buffer from running over the top of the shorter glass plate during the gel run. commonly used buffer. elatively low buffer capacity will become exhausted during extended electrophoresis, making buffer recirculation necessary in runs exceeding 140mA-hours. Potential advantages of using TAE buffer over TBE buffer include superior resolution of supercoiled DNA and approximately10% faster migration of double-stranded linear DNA fragments.

Casting the Gel

1.) Rest the plate assembly on one of the bottom corners and angle to approximately 45 degrees.

2.) Using a large pipette, slowly inject your gel solution between the glass plates, starting along the side spacer so the solution runs down along the spacer and begins to fill the bottom of the gel.

3.) Continue this process until the solution begins to near the top of the plate assembly that is lowest. Slowly move the plate assembly from 45 degrees to a straight up vertical position and continue to fill until desired level is reached.

4.) This process will help prevent the formation of air bubbles in the gel sandwich while being poured. If bubbles do form, stop pouring the gel and lightly tap the glass plates to try to remove the bubbles.

5.) Once the desired level is reached, carefully insert comb and ow the gel to set.

Note: Always wear protective gloves when working with acryliamide. Acrylamide is considered to be a neurotoxin.

Removing the Comb and Bottom Spacer

1.) Once the gel is firmly set, carefully remove the comb so as not to tear the gel. Adding a small amount of running buffer around the comb teeth may help allow for easier removal.

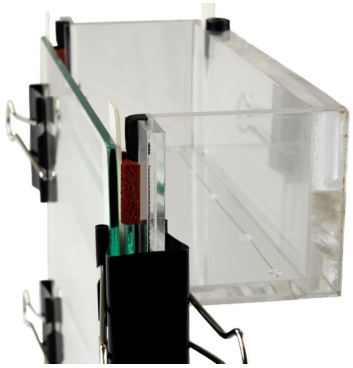
2.) Once the comb is removed, you can now also remove the bottom spacer to expose the lower cross section of the gel for running.

3.) It may be necessary to pour an "agarose plug" into the empty area that is now exposed, after removal of the bottom spacer. The agarose plug can be used for either an agarose gel or polyacrylamide gel.

Note: Another option for the agarose plug would be to use Gel Casting Tape in place of the bottom spacer. This will allow for the running gel to be cast all the way to the bottom of the glass plates. IBI has casting tape available, see model number IB81630.

Assembling the Buffer Chamber

Note: To ensure a leakproof seal, make sure the U-shaped gaskets are clean. Inspect the gasket for small cuts that could result in an upper buffer tank leak. Make sure the side exposed for contact with the gel sandwich is not damaged.



Place the lower chamber on the lab bench, making sure the surface is level. Move the bottom clamps to the center of the gel sandwich and insert the gel sandwich into the lower reservoir, taking care to place the bottom edges against the support blocks. To secure the gel sandwich to the upper reservoir, remove the two upper clamps and firmly place the shorter glass plate face against the gasket and clamp the gel sandwich to the upper reservoir using the same clamps. Make sure gel sandwich is centered in unit.

Note: Lubricating the raised portions of the U-shaped gasket with a drop of running buffer or water helps the gel sandwich slide into place properly.

Buffer Preparation

1.) Prepare 1100ml of electrode buffer by combining 220ml of 5X electrode buffer with 880ml of deionzed water.

2.) Add approximately 550ml of buffer to the upper buffer reservoir. Fill until the buffer reaches a level approxiatemly halfway between the two glass plates. Do NOT overfill the upper buffer reservoir.

3.) Add the remainder of the buffer to the lower reservoir.

Note: The sample buffer must contain either 10% sucrose or 10% glycerol in order to underlay the sample in the well without mixing.

Loading Sample Wells

Sample loading can be done in two ways. The most common method is to load samples into well formed in the gel by a well forming comb. The second method uses the entire gel surface as a single well for liquid samples depending on the gel.

1.) Remove the comb by gently rocking it back and forth

2.) Load the samples into the wells inder the electrode buffer with a pipette. Insert the pipette into the gel about 1 - 2mm from the well bottom before delivery. Disposable pieces of plastic tubing may be attached to the syringe to eliminate the need for rinsing the syringe between sample.

Note: The sample buffer must contain either 10% sucrose or 10% glycerol in order to underlay the sample in the well without mixing.

Running the Gel

1.) Place the lid on top of the upper buffer chamber to fully enclose the cell. The correct orientation is made by matching the black banana jack wit hthe black power cord on the lid.

Note: The lid is indexed so it will only connect one way properly.

2.) Attach the electrical leads to a suitable power supply (200VDC minimum) along with the proper polarity.

3.) Apply the power to the VCV unit and begin electrophoresis. The recommended power condition for optimal resolution with minimal thermal band distortion is 150 volts, constant voltage setting. No adjustment of the setting is necessary for thickness or number of gels. The usual run time is approximately 90 to 120 minutes. Current should be approximately 1000mA per gel at the beginning of the run. During the 90 minute run, the current will slowly drop to 200mA per gel. The drop in amperage is caused by the change in buffer ions in the gel., causing a slow rise in the resistance in the gel. As one would expect from the Ohms law (V = I x R), at constant voltage (V), a rise in resistance (R) results in a drop in the current (I).

Removing the Gel

1.) After electrophoresis is complete, turn off the power supply and disconnect the electrical leads from the power source.

2.) Remove the lid and clamps, earing gloves, carefully pull the Gel Sandwich outo f the lower buffer chamber.

3.) Place the gel sandwich horizontally on the benchtop. Take care to remove any casting tape that may remain on the glass plates. Gently separate the glass plates by inserting a flat edge spatula in-between them and lifting in an upward motion. The gel should come free of the plate.

Choice of Buffer

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

- **TAE -** Tris-Acetate buffer has traditionally been the more commonly used buffer. However, its relativity low buffer capacity will become exhausted during extended electrophoresis, making buffer recirculation necessary in runs exceeding 140mA/hours. Potential advantages of using TAE buffer over TBE buffer include; superior resolution of supercoiled DNA and approximately 10% faster migration of the double-stranded linear DNA fragments.
- **TBE -** Tris-Borate buffer has a significantly greater buffering capacity and its relatively low current draw eliminates the need for recirculation in all but the most extended runs (>300mA/hrs). TBE buffer systems are not recommended when fragments are to be recovered from the gel after electrophoresis.

TRIS-ACETATE-EDTA BUFFER (TAE) - e.g. IB70160:

1X Working Concentration	10X Stock Solution
40mM Tris Base	48.4gm Tris Base
20mM Glacial Acetic Acid (NaOAc)	16.4gm or 11.42ml NaOAc
2.0mM EDTA	7.4gm EDTA or 20ml 0.5M EDTA (pH 8.0)
pH 8.3	H_2O to 1L

TRIS-BORATE-EDTA BUFFER (TBE) - e.g. IB70150, IB70153, IB70154:

1X Working Concentration	10X Stock Solution
89mM Tris Base	108gm Tris Base
89mM Boric Acid	55gm Boric Acid
2.0mM EDTA	6.72gm EDTA or 40ml 0.5M EDTA (pH 8.0)
pH 8.0	H_2O to 1L

Polyacrylamide Gels

Acrylamide is a primary component of polyacrylamide gels for protein electrophoresis.

SDS-PAGE Gels

Product 7.5% T 10% T 12% T
Water

Premixed Acrylamide & Bisacrylamide

InstaPAGE	IB70014	40%	19:1 38% Acrylamide	2% Bisacrylamide
Instabis-2	IB70012	2%	w/v Bisacrylamide	
Acyliquid-40	IB70010	40%	w/v Acrylamide solution	

Use the supplied reference table to determine the correct volumes of Acryliquid-40 and the InstaBis-2 to make 100ml of (X)% Gel. Acryliquid-40 is a 40% (w/v) solution of ultra-pure acrylamide in deionized water. To determine the volumes of InstaBis-2 and Acryliquid-40 to add for gel percentages not listed, use the following formula:

(ml) InstaBis-2:	(ml) Acryliquid-40:	Cross-Linker Content	Monomer Content
=2.500 (gel %) 19:1	=2.375 (gel %) 19:1	0.05	0.95
=1.667 (gel %) 29:1	=2.417 (gel %) 29:1	0.033	0.966
=1.299 (gel %) 37.5:1	=2.435 (gel %) 37.5:1	0.026	0.974

Volume of Acrylliquid-40% to use (ml) = $\frac{\text{MC X Gel Concentration needed X Gel Volume needed}}{40\%}$

Volume of InstaBis-2 to use (ml) = $\frac{\text{cC X Gel Concentration needed X Gel Volume needed}}{2\%}$

Volume of InstaPAGE to use (ml) = $\frac{\text{Gel Concentration needed X Gel Volume needed}}{40\%}$

Resolving Gel Preparation

	12%	8%	6%
1.5M TRIS-HCL pH 8.8	10ml	10ml	10ml
10% SDS solution	400µl	400µl	400µl
Acrylamdie/Bis 40% sol	11.4ml	7.6ml	6ml
10% Ammonium persulfate	200µl	200µl	200µl
TEMED	20µl	20µl	20µl
Deionized water	13.4ml	19.4ml	23.38ml
Total volume	40ml	40ml	40ml

Buffers

TRIS- GlycineRunning Buffer 10X Native gels

TRIS	29gm
Glycine	144gm
ddi Water	1.0L

Dilute 10x with ddi water pH of 1X Solution should be 8.3.

TRIS- Glycine SDS Running Buffer 10X Denatured gels

TRIS	29gm
Glycine	144gm
SDS	10gm
ddi Water	1.0L

Dilute 10X with ddi water pH of 1X Solution should be 8.3

TRIS-Tricine SDS Running Buffer 10x

TRIS	12.1gm
Tricine	179.0gm
SDS	10.0gm
ddi Water	Adjust to 1L

Sample Loading Buffers

Sample Loading Buffer SDS 2X*

Bromophenol blue with Glycerol

2X Stock Solution:

10% SDS 4.0ml

50 % Glycerol 2.0ml

0.1% Bromophenol blue 1.0ml

0.5M TRIS-HCL pH 6.8 2.5ml

2-mercaptoethanol 0.2-0.5ml

ddi Water to 10ml

Sample Loading Buffer, 2X

2X Stock Solution:

50% Glycerol 2.0ml

0.1% Bromophenol blue 1.0ml

0.5M TRIS-HCL pH 6.8 2.5ml

ddi Water to 10ml 10ml

*Sample Preparation

Add an equal amount of Sample loading buffer to your sample. Heat the sample for 3-5 minutes at 100°C. If the sample becomes cloudy you can centrifuge for 3 minutes at 6,000RPM.

Maintenance Of Unit

- Care must be observed in the handling of this unit.
- Do **NOT** expose the unit to temperatures above 60°C
- Do **NOT** expose the unit to organic solvents
- Do **NOT** clean the unit with abrasive cleaners or cleaning aids.
- Use mild cleaning solution (dish soap recommended) for routine cleaning. For heavier dirt, hand wash with soft cloth. In most cases, a rinse in deionized water is sufficient to clean the unit. To remove residual Ethidium Bromide from the gel unit, soak occasionally in a 1% solution of commercial bleach and water for 16 hours, rinse well when finished.

Note: The degradation of acrylic by solvents may result in substantial discoloration, cracking, warpage or etching of the electrophoresis unit. Do **NOT** apply any of the following solvents to this unit; benzene, xylene, toluene, chloroform, carbon tetrachloride, alcohol, phenol, ketones, or esters. Do **NOT** use the Delrin™ combs supplied with this system in formaldehyde for long periods of time. The formaldehyde damages these combs with long exposures.

Note: If an electrode should happen to break during cleaning or any other time, contact IBI Scientific Technical Support at (800) 253-4942 or info@ibisci.com for assistance locating replacement parts.

Elimination of RNase Contamination

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

Warning: DEPC is a suspected carcinogen, handle with care.

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

Accessories | Replacement Parts

VCV Accessories and Replacement Parts

Catalog #	Description
IB62200	Replacement Lid w/Power Cords
IB62600	Replacement Gasket
IB62545	VCV Inner Glass Plate
IB62555	VCV Frosted Inner Glass Plate
IB62585	VCV Outer Glass Plate
SPC-M4	Replacement Power Cords

Accessories | Replacement Parts (Con't)

VCV Comb and Spacer Sets

Catalog #	Description	Well Width	Sample Volume (per mm Gel)
IB62010	0.8mm Comb and Spacer Set		
	- 0.8mm x 12 tooth comb	10mm	40µl
	- 0.8mm x 20 tooth comb	5mm	25µl
	- 0.8mm Spacer Set		
IB62020	1.5mm Comb and Spacer Set		
	- 1.5mm x 12 tooth comb	10mm	70µl
	- 1.5mm x 20 tooth comb	5mm	75µl
	- 1.5mm Spacer Set		
IB62030	3.0mm Comb and Spacer Set		
	- 3.0mm x 12 tooth comb	10mm	120µl
	- 3.0mm x 20 tooth comb	5mm	150µl
	- 3.0mm Spacer Set		
IB62345	Prep. Comb, 0.8mm, 2 Markers x 2 Samples	65mm	250µl
IB62355	Prep. Comb, 1.5mm, 2 Markers x 2 Samples	2mm	500µl
IB62365	Prep. Comb, 3.0mm, 2 Markers x 1 Sample	2mm	500µl
IB62375	Prep. Comb, 3.0mm, 2 Markers x 2 Samples	3/2mm	1000µl

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. Volume 1. Cold Spring Harbor Press, N.Y.

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. 4.9.4. Green Publishing Associates and Wiley-Interscience.

Limited Warranty

Our limited warranty for all electrophoresis gel boxes is one (4) years to the original buyer only (nontransferable) from the original date of purchase. This warranty does not apply to electrodes or platinum wires.

Our limited warranty as noted above extends to the direct end user of IBI Scientific products only. This warranty is in lieu of all other warranties whether expressed or implied, including warranties of merchantability or fitness for a particular purpose. In no situation shall IBI Scientific be liable for any incidental or consequential damages of any kind, even though IBI Scientific has been advised of the possibility of such damages arising out of, or resulting from, the products or the use or modification thereof or due to the breach of this warranty or any other obligation of IBI Scientific to the customer, whether based on contract, tort, or any other legal theory. In no such event shall IBI Scientific be liable for damages which exceed the purchase price of any products.

For further assistance contact IBI Scientific Technical Service at **(800) 253-4942**, **(563) 690-0484** or visit us on the web at **ibisci.com**

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