INSTRUCTION MANUAL

Blot Transfer Systems

11 x 14 cm Format, #21054010

Suitable for the Horizon® 11.14 and H5

20 x 25 cm Format, #11056017

Suitable for the Horizon® 20.25 and H4

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1.0 BEFORE YOU BEGIN

1.1 IMPORTANT INFORMATION

The **Horizon® 11.14** and **20.25 Blot Transfer Apparatus** are designed for capillary transfer of nucleic acids from agarose gels to membranes in Southern and Northern blots. Used in conjunction with the appropriate blotting materials and membranes, the included trays and supports offer a simple and efficient method for performing high quality blot transfers.

- Blot transfer apparatus 11.14 is compatible with 11 x 14 cm horizontal gel electrophoresis units including the Horizon 11.14 and the Model H5 apparatus.
- Blot transfer apparatus 20.25 is compatible with 20 x 25 cm horizontal gel electrophoresis units including the Horizon 20.25 and the Model H4 apparatus.

This manual provides instructions on the use of both blot transfer apparatus noted above.

For operating procedures for the Horizon 11.14, 20.25, H4, and H5 Apparatus, as well as general guidelines, practical applications, and suggestions for agarose gel electrophoresis, please refer to the instruction manuals. These systems have not been qualified for use in any human or animal diagnostic or therapeutic application. Use for other than the intended use may be a violation of applicable law.

Please carefully follow the manual's instructions. Do not alter equipment or operate with broken components. Failure to adhere to these directions could result in personal and/or laboratory hazards as well as invalidate the equipment warranty.

1.2 COMPONENTS

The 11.14 and 20.25 blot transfer apparatus are designed for durability and ease of use. Each complete apparatus includes the following items:

One molded buffer tray

- One machined and bent acrylic tray insert
- One machined and bent acrylic gel restrainer
- One instruction manual

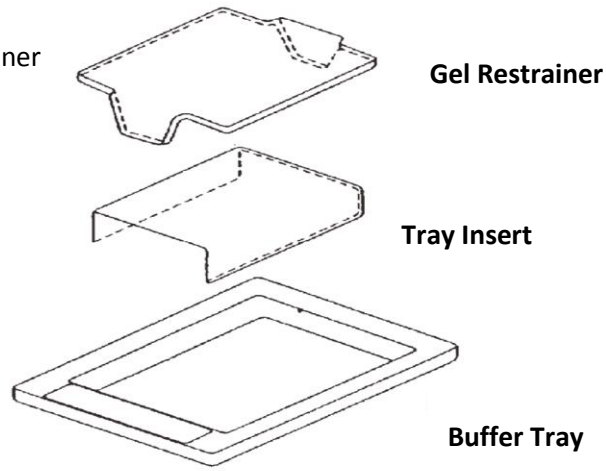


Figure 1: Components

2.0 INSTRUCTIONS FOR USE

2.1 GEL PREPARATION AFTER ELECTROPHORESIS

Refer to Figure 1 to identify the features and components in these instructions.

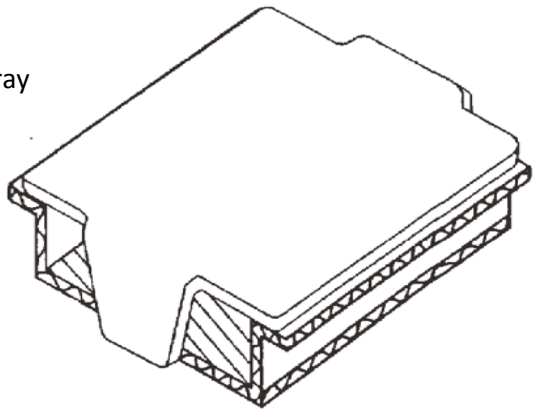
The capillary blot technique originally developed by Southern (1) has been modified and extended in a number of ways since its original publication. Protocols with buffer compositions for DNA and RNA blots can be found in a number of journal articles and laboratory manuals (2 thru 5).

All protocols require one or more soaking steps before actual blotting begins. During these steps, the agarose gels are fragile and easily damaged; whenever possible, handle the gel using the UVT tray on which it was cast.

To help prevent the gel from slipping off the UVT tray during handling, place the gel restrainer over the UVT tray. Orient the gel restrainer so that the bent tabs block the open ends of the UVT tray (Figure 2).

All soaking steps can be carried out in the buffer tray. Transfer the gel, in the UVT tray, into the buffer tray and cover the gel completely with the appropriate soaking solution. Rock the buffer tray and gel gently and regularly during soaking steps. Remove the gel and thoroughly rinse the buffer tray with deionized water before adding each new solution.

Figure 2: Gel restrainer in position over UVT tray



2.2 APPLYING WICKING SHEETS

1. When all soaking steps are complete, remove the gel, in the UVT tray or gel plate, to a clean work surface. Use the gel restrainer as needed while handling the gel in the UVT tray.
2. Wet a wicking sheet in the solution to be used for capillary transfer.
3. Place the wicking sheet on the gel. If using a UVT tray, align the edges of the wicking sheet against the side rails of the tray with the overlap of the wicking sheet equal at the top and bottom of the gel. The wicking sheet should run 90 degrees to the side rails of the UVT tray so that the overlap does not run up the side rails.

Note: Do not trap air pockets between the gel and wicking sheet. Remove trapped air by lifting and replacing the wicking sheet or by gently finger. Small bubbles in the wells will not affect transfer.

4. After the wicking sheet is in position, place the tray insert into a UVT tray at 90 degrees to each other so that the gel is centered between, but not touching, the rounded edges of the insert tray. Again, be careful not to trap air pockets between the tray insert and the wicking sheet (Figure 3). With a gel plate just center the gel on the wicking sheet and insert the tray.
5. Hold the UVT tray (or gel plate) and tray/insert assembly securely but gently together and invert the entire assembly.
6. Carefully remove the UVT tray or gel plate, leaving the gel in place on the wicking sheet on the now inverted insert tray (Figure 4).

Note: The wicking sheet runs over the insert tray legs.

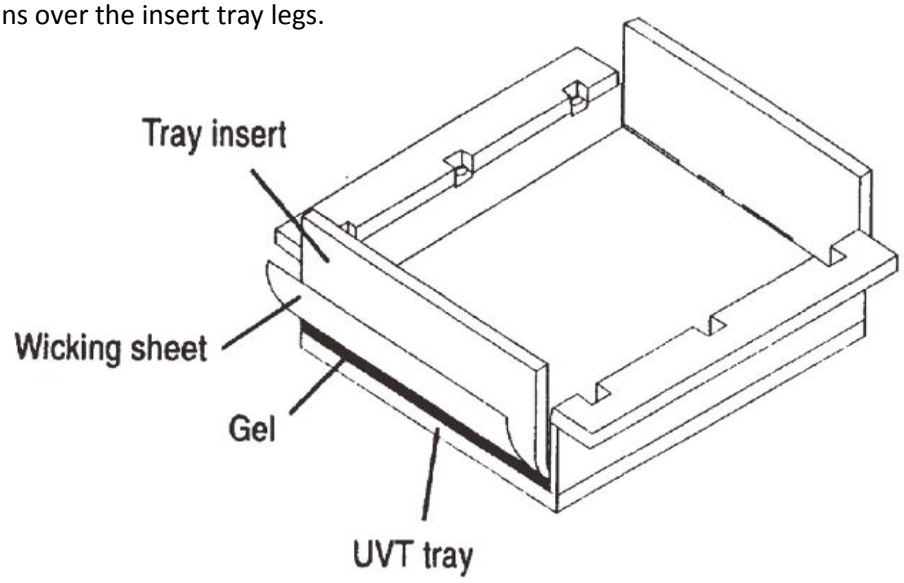


Figure 3: Gel and wicking sheet on tray insert

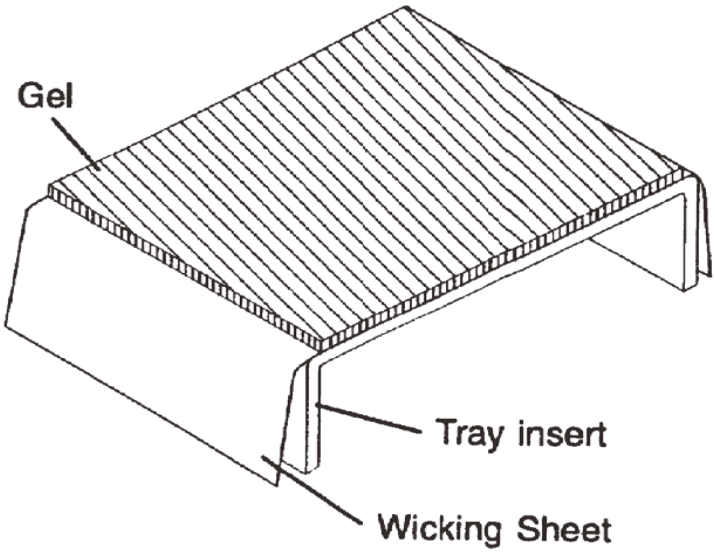


Figure 4: Inverted insert tray

2.3 APPLYING THE MEMBRANE AND BLOTTING PADS

Nitrocellulose and PVDF (positively-charged nylon) membranes are commonly available and both work well.

1. Make any needed identifying marks in soft pencil or preferred marker on a piece of the dry, precut membrane.

Note: Handle the membrane with gloved fingers or smooth-tipped (non-serrated) forceps to prevent oils and proteins on fingers from blocking efficient transfer.

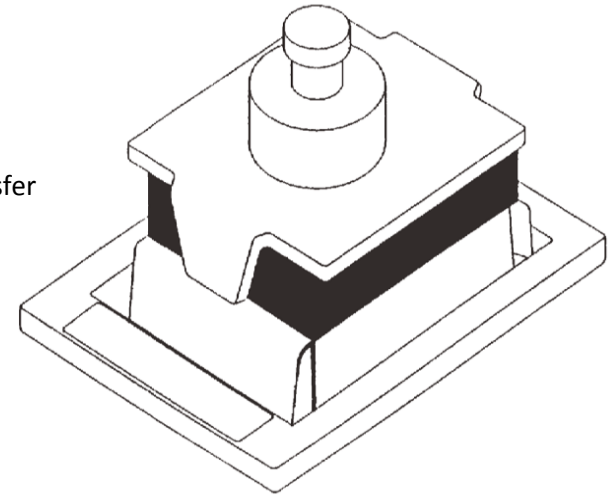
Note: PVDF membranes require pre-treatment by soaking in methanol for 1 to 2 minutes followed by soaking in transfer buffer for 5 minutes.

2. Wet the membrane by floating it on distilled water for a few moments and then immerse it in transfer buffer for 1 to 2 minutes.
3. Align the membrane carefully on the gel taking care not to create any air pockets.

Note: Do not allow the membrane to touch the wicking sheet during transfer. If it does, a 'short circuit' will occur and buffer will move around rather than through the gel.

4. Place the tray insert, with the wicking sheet, gel and membrane on it, into the buffer tray with the ends of the wicking sheets extending into the tray.
5. Place a stack of blotting pads on top of the membrane, and the gel restrainer over the blotting pads (Figure 5). As with the membrane, be careful not to let the blotting pads touch the wicking sheet. To ensure even contact through the blotting stack, place a weight on top of the gel restrainer.

Figure 5: Configuration for capillary transfer



2.4 CAPILLARY TRANSFER

1. Fill the buffer tray approximately half full with transfer buffer. To ensure even capillary flow, make sure the ends of the wicking sheets are equally immersed.
2. Allow from 4 to 24 hours for complete capillary transfer depending upon the size of the DNA or RNA fragments and the percentage and thickness of the gel.

3. After blotting is complete, remove the gel restrainer and blotting pads. Carefully lift the membrane from the gel with forceps and initiate subsequent analysis procedure.

Note: The gel will normally be compressed to a small fraction of its original thickness.

4. Discard all used paper blotting materials properly. Thoroughly rinse and dry all plastic components to remove any residual solution.

3.0 REFERENCES

1. Southern, E.M. (1975*) Journal of Molecular Biology 98, 503.*
2. Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
3. Schatz, D.G. (1989) *in Current Protocols in Molecular Biology*, (Ausubel, F>M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G, Smith, J.A. and Struhl, K., eds.), Vol. 1 p. 2.9.1, Greene Publishing Associates and Wiley Interscience, New York.
4. Thomas, P.S. (1980) *Proceedings National Academy of Acad. Science USA 77, 5201.*
5. Selden, R.F. (1989) in *Current Protocols in Molecular Biology*, (Ausubel, F>M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G, Smith, J.A. and Struhl, K., eds.), Vol. 1 p. 4.9.1, Greene Publishing Associates and Wiley Interscience, New York.
6. Bolt & Mahoney, (1997) High-efficiency blotting of proteins of diverse sizes following sodium dodecyl sulfate–polyacrylamide gel electrophoresis, *Analytical Biochemistry* 247, 185–192.

4.0 RELATED PRODUCTS

4.1 H11.14 AND H5	GEL SIZE	CATALOG #
Horizon 11.14 horizontal gel electrophoresis apparatus Includes ***FastCast*** aluminum wedges and integral safety lid	11 x 14 cm gel	11068012
Model H5 horizontal gel electrophoresis apparatus	11 x 14 cm gel	21087010
H11.14 UVT Tray	11 x 14 cm gel	11084019

4.2 H20.25 AND H4	GEL SIZE	CATALOG #
Horizon 20.25 horizontal gel electrophoresis apparatus Includes ***FastCast*** aluminum wedges and integral safety lid	20 x 25 cm gel	21069018
Model H4 horizontal gel electrophoresis apparatus	20 x 25 cm gel	11025012
H20.25 UVT Tray	20 x 25 cm gel	31006026

5.0 CARE AND HANDLING

5.1 MATERIALS AND CARE

Each apparatus is fabricated from high quality formed PVC/Acrylic and machined acrylic plastic. Both have very good heat, impact, and chemical resistance but will not withstand autoclaving or dry-heat sterilization.

All components may be washed with water and a detergent. To remove grease and oils, use a hexane, kerosene, or aliphatic naphtha. *Never* use abrasive cleaners, window sprays, or any fluid that may contain toluene, methylene chloride, phenol, acetone, benzene, halogenated hydrocarbon solvents, or undiluted laboratory alcohols.

For replacement parts, call your distributor or Apogee Technical Support.

5.2 GENERAL SPECIFICATIONS

Type	**H11.14 Casting System**	**H20.25 Casting System**
Dimensions (W × L x H)	17.6 × 23.2 × 5.6 cm	26.7 x 34.0 x 5.6 cm
Weight	0.37 kg	0.72 kg
Gel Dimensions	11 x 14 cm	20 x 25 cm
Construction	PVC/Acrylic, acrylic	PVC/Acrylic, acrylic

5.3 TECHNICAL SUPPORT AND SERVICE

Should you have any problems with this unit, please contact:

Apogee Designs, Ltd.
Attn: Electrophoresis Support
101 Kane Street
Baltimore, MD 21224 USA

Phone: 443.744.0368 9 to 5PM EST, Monday through Friday
Fax: 410.633.3666
Email: info@apogeephoresis.com

5.4 INSTRUCTIONS FOR RETURN SHIPMENT

IMPORTANT: Before sending the unit back to us, it is absolutely necessary to call our Technical Support department to **get authorization to return products**!

- Return only defective devices. For technical problems which are not definitively recognizable as device faults please contact Apogee Technical Support.

- Use the original box or a similarly sturdy one.
- Please enclose a detailed description of the fault and when, or how, the problem occurred.

Important: Clean all parts from residues and of biologically dangerous, chemical and radioactive contaminants. Please include a written confirmation (use the respective Decontamination Declaration/Certificate following in Section 8 that the device is free of biologically dangerous and radioactive contaminants in each shipment. If the device is contaminated, it is possible that Apogee will be forced to refuse to accept the device. The sender of the repair order will be held liable for possible damages resulting from insufficient decontamination of the device.

Please enclose a note which contains the following:

1. Sender's name and address and,
2. Name of a contact person for further inquiries with telephone number.

5.4.1 CLEANING AND DECONTAMINATION FOR RETURN OF PRODUCTS

Use the original product packaging whenever possible, to avoid damage to the unit being returned. All returned material must be cleaned and decontaminated prior to shipping. The components of apparatus products are fabricated from a variety of materials including: ABS, acrylic, vinyl, glass, silicone, aluminum and stainless steel. Please clean any unit or product to be returned using the following three step procedure.

STEP 1: GENERAL CLEANING PROCEDURE

For materials not contaminated with biological or radiological substances, components may be gently washed with water and a non-abrasive detergent, and rinsed with deionized water. Dry using a soft cloth, paper towel or allow to air dry. A light application of hexane, kerosene, or aliphatic naphtha will remove grease.

To prevent surface damage, never use abrasive cleaners, window sprays or scouring pads to clean these products. Avoid excessive exposure to UV light, phenol, acetone, benzene, halogenated hydrocarbon solvents or undiluted alcohols because they may cause crazing.

STEP 2: BIOLOGICAL CLEANING PROCEDURE

Using a solution of either 5% household bleach in water or 70% ethanol in water, wipe down the apparatus using a clean cloth or sponge. Neutralize the solution by wiping the surface with a mild, nonabrasive detergent and rinse well with water.

STEP 3: RADIOLOGICAL DECONTAMINATION PROCEDURE

To meet various regulatory and safety standards, please follow the decontamination procedure given here if radioactive materials are used with this product or are used in the vicinity of where this apparatus has been used or stored.

WARNING: We cannot and will not accept return of products that are contaminated with any radioactivity.

For beta emitting isotopes such as ^{32}P, use a GM-type radioactivity meter calibrated in counts per minute (CPM) to determine the background readings for your work area. Wearing latex gloves, survey the unit to be returned with the GM meter. If any part of the unit is found to show readings higher than background, wash the area using Radiacwash© (Atomic Products Corp.) and paper towels, or another similar commercially available detergent. If Radiacwash is not available, a mild detergent or a Formula 409© type solution will do. As you clean, discard liquid and solid waste (gloves and paper towels) according to your local and institutional regulations for radioactive material disposal. Continue washing until the GM-meter reading for the contaminated area(s) is equal to or below background.

To decontaminate units where a GM-meter is not as useful for detection, as with 'H, or "S, it will be necessary to perform swipes of the unit and detect using a scintillation counter. The unit should be dry. Wipe surfaces with dry paper circles (these are commercially available or you can make your own). Areas can be charted, so that individual swipes can be done on different surfaces to better isolate areas of contamination.

Swipes should be placed into individual scintillation vials with an appropriate floor and then analyzed on a properly programmed scintillation counter. If contamination above 100 disintegrations per minute dpm/100cm^2 (dpm=CPM/efficiency) is found, wash the area as described above in ^{32}P decontamination. After cleaning the area, swipe it a second time to determine the amount of contamination remaining. If the area still has greater than 100 dpm/cm^2, continue the cycle of swipes and washing until you achieve a reading of less than 100 dpm/cm^2.

Once the unit has been determined to be radiation free (<100dpm/cm^2) remove all the hazardous and radioactive labels from the unit. If the labels cannot be removed, deface them. Failure to do so may result in a significant delay or refusal of repair. If your unit has non removable contamination (detectable with a GM-meter and not with paper swipes, or detectable with paper swipes but after continued washing the dpm/cm^2 remains constant and above 100) of a short half life isotope such as ^{32}P, it may be stored for ten half lives of isotopic decay and the decontamination procedure repeated.

Note: *Units contaminated with non removable, long half life isotopes may not be returned.*

If questions still persist, please contact:

Apogee Designs, Ltd.
Attn: Electrophoresis Support
101 Kane Street
Baltimore, MD 21224 USA

Phone: 443.744.0368 9 to 5PM EST, Monday through Friday
Fax: 410.633.3666
Email: info@apogeephoresis.com

5.4.2 NOTICE REGARDING THE RETURN OF APPARATUS PRODUCTS

US Federal Regulations

In order to comply with US federal regulations and to protect the health and safety of employees, it is imperative that all customers read this notice and adhere to the requirements regarding the return of apparatus products. The US Department of Transportation, the Department of Health and Human Services, and the Nuclear Regulatory Commission have strict regulations on the shipment of hazardous materials (49 CFR Part 173) including etiologic agents (49 CFR Part 173 and 42 CFR Part 72) and radioactive materials (CFR 49 Part 173 and 10 CFR Part 20).

German Law

To comply with German law (i.e. §71 StrlSchV, §17 GefStoffV and §19 ChemG) and to avoid exposure to hazardous materials during handling or repair, completion of this form is required before equipment leaves your laboratory. When equipment is returned for repair, evaluation, credit or exchange, the customer becomes the shipper and must ensure that the item is free of contamination whether chemical, biological or radioactive. Procedures for decontamination are described above.

Materials received that have not been properly decontaminated or units which do not have hazard labels (such as 'caution radioactive materials') may be decontaminated at the customer's expense (approximately $350) and may result in delay or refusal of repair. In addition, in the case of radioactive contamination, Apogee may be required to notify a licensing authority that in turn may be required to notify the customer's licensing authority.

Please carefully follow decontamination instructions and fill out the Decontamination Declaration that follows. Place the Decontamination Declaration inside the top flap of the box where it can be immediately noticed by the receiver. Any change to this procedure may result in service delay.

6.0 WARRANTY

Apogee 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 and does not include breakage of the electrodes or crazing from cleaning with solvents that attack ABS, PVC/Acrylic or acrylic. Apogee's 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 within three years 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. Apogee makes no other warranty, expressed or implied, including warranties of merchantability or fitness for a particular purpose. Under no circumstances shall Apogee 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.

In the interest of bettering performance, Apogee reserves the right to make improvements to the design, construction, and appearance without notice.

7.0 DECONTAMINATION DECLARATION

RGA Number (IMPORTANT): _____

Customer Name: _____

Institute: _____

Address: _____

TEL #: _____ FAX #: _____

E-mail: _____

Unit type: _____ Serial number: _____

DESCRIPTION OF PROCEDURES USED TO DECONTAMINATE UNIT (LOOK AT 5.4.1)

☐ 1. Gently washed with water and a non-abrasive detergent, and rinsed with deionized water.

☐ 2. Using a solution of 5% household bleach in water or 70% ethanol in water, the unit was wiped down using a clean cloth or sponge and neutralized with deionized water.

☐ 3. To meet various regulatory and safety standards, please follow the decontamination procedures given in 5.4.1 if **radioactive materials** were used with this product.

This piece of equipment **<u>has not</u>** been decontaminated. Reason:

☐ To the best of my knowledge, unit is free of chemical, biological, or radioactive contamination.

I understand that if the equipment is found to be contaminated, regardless of the signature on this document, the equipment may be decontaminated at my expense. Also, if the equipment is found to be contaminated, the response time for repairs will be delayed.

Signature: _____

Title: _____

Date: _____

*Please place **completed and signed** form inside the box with the equipment where it can immediately be noticed by the receiver. We appreciate you taking the time to perform the necessary precautions to ensure that equipment being returned can be safely handled by our employees.*