THE SEMI-DRY BLOTTING SYSTEMS OPERATIONS AND MAINTENANCE MANUAL

MODELS

IB44000

IB45000

MANUFACTURER'S UNCONDITIONAL WARRANTY

If for any reason SEMI-DRY BLOTTING DEVICE SYSTEM fails to perform in a satisfactory manner for the purchaser, it can be returned for credit within 30 days of purchase.

MANUFACTURER'S LIMITED WARRANTY

This semi-dry blotting device is warranted against defective material or workmanship for a period of one (1) year from date of purchase. Extended warranties can be purchased by contacting the manufacture. In the event of a failure of the product to conform to this written warranty you should return the product, along with proof of purchase date, and a written statement about the nature of the problem to your point of purchase representative

If the manufacture's inspection indicates that a problem has been caused by defective material or workmanship within the limitation of the warranty, The Manufacturer will repair or replace at it's option the product free of charge and return the product. Repairs made necessary by normal wear, or repairs made for product outside of the warranty period, if they can be made, will be charged at regular factory rates.

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This warranty gives you specific legal rights and you may also have other rights which vary from state to state, country to country. The obligation of the warrantor is solely to repair or replace the product; the warrantor is not liable for any accidental or consequential damages due to such defects. Some states do not allow the exclusion or limitations of incidental or consequential damages, so the above limits may not in these areas. For prices and warranty fulfillment , contact your distributor.

TECHNICAL MANUAL OF SEMI-DRY BLOTTING APPARATUS
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PRINCIPLE OF OPERATION

Introduction:

Mixtures of biomedical products can be separated on the basis of their molecular sizes and ionic characteristics by polyacrylamide or agarose gel electrophoresis. Different proteins will appear as bands on the gels after staining. In order to further identify the characteristics of these proteins, components can be transferred and immobilized on a solid support in order that further detection can be achieved using radioisotopic or immunological methods. The transfer of these components from the gels to a solid support requires either an electrical current, vacuum suction or diffusion to move the components to the solid support. The SEMI-DRY BLOTTING APPARATUS (See Figure 1) is a device that can migrate these components from the gel to a solid support, by an electrical current.

The fastest method for the transfer of proteins, RNA and DNA from a gel to a solid support is electrophoretic transfer. To do this, the gel is layered in contact with the membrane sandwiched between several layers of filter paper soaked with buffered solutions forming a stack (Trans-unit in Figure 2). A constant current is applied perpendicular to the gel, and filter papers causing the sample to migrate from the gel onto the membrane. The membrane is then ready for staining or other developmental methods utilizing a PTS instrument [1], autoradiography, or enzymatic methods.

The SEMI-DRY BLOTTING APPARATUS uses a semi-dry method which is safer and considerably more efficient and economical than previously used wet tank methods. It can also be used for transfer of proteins from isoelectrofocused gels or slab gels.

THE SEMI-DRY BLOTTING APPARATUS CONSTRUCTION & CONCEPT OF PRODUCT

This apparatus consists of a specially created and tested graphite Cathode Plate Cover and a graphite Anode Plate (Figure 1). A TRANS-UNIT stack is formed by the gel and the membrane being in contact and sandwiched between filter papers soaked in buffer solution

An electric current is applied perpendicular to the gel and filter papers. The Cathode Paper is saturated with the Cathode buffer and the Anode Paper with Anode buffer. In electrotransblotting, a pH gradient between the Cathode and the Anode side of the TRANS-UNIT is established so that negatively charged molecules can migrate towards the positively charged side of the TRANS-UNIT. The bands from the gels migrate out of the gels and bind to the nitrocellulose membrane (or other membrane supports depending on which type of blotting is being accomplished). Once the bands reach the membrane they are immobilized until the portion of the membrane is saturated with the migrated material. Excess unbound material would migrate into the Anode Filter Paper until it reaches the Dialysis Membrane which then blocks further migration of the material into the next TRANS-UNIT assembly. Several stacks of TRANS-UNIT's can be run at the same time. The relief slot on the cover of the model semi-dry blotting apparatus allows for up to (6) six TRANS-UNITS to be run simultaneously. The design for multiple gel transfers, 2-D transfers and larger gels such as those found in DNA are also an intended use. As a safety feature, the design of this device requires the operator to plug into the power supply after the Cathode Plate Cover is put in place.

The graphite plates serve as the electrodes which also dissipate the potential build up of heat during the electrophoretic transfer. The time needed to transfer bands from various types of gels to the membranes using various current densities are listed in table 1. After the transfer is completed, the filter papers and the gels are removed and the membrane is ready for post-transfer processing.

Figure 1 - Basic Blotting Device

WARNING

TURN OFF YOUR DC POWER SUPPLY BEFORE CONNECTING THE SYSTEM ANODE AND CATHODE WIRE LEAD SET TO THE POWER SUPPLY DC OUTLETS.

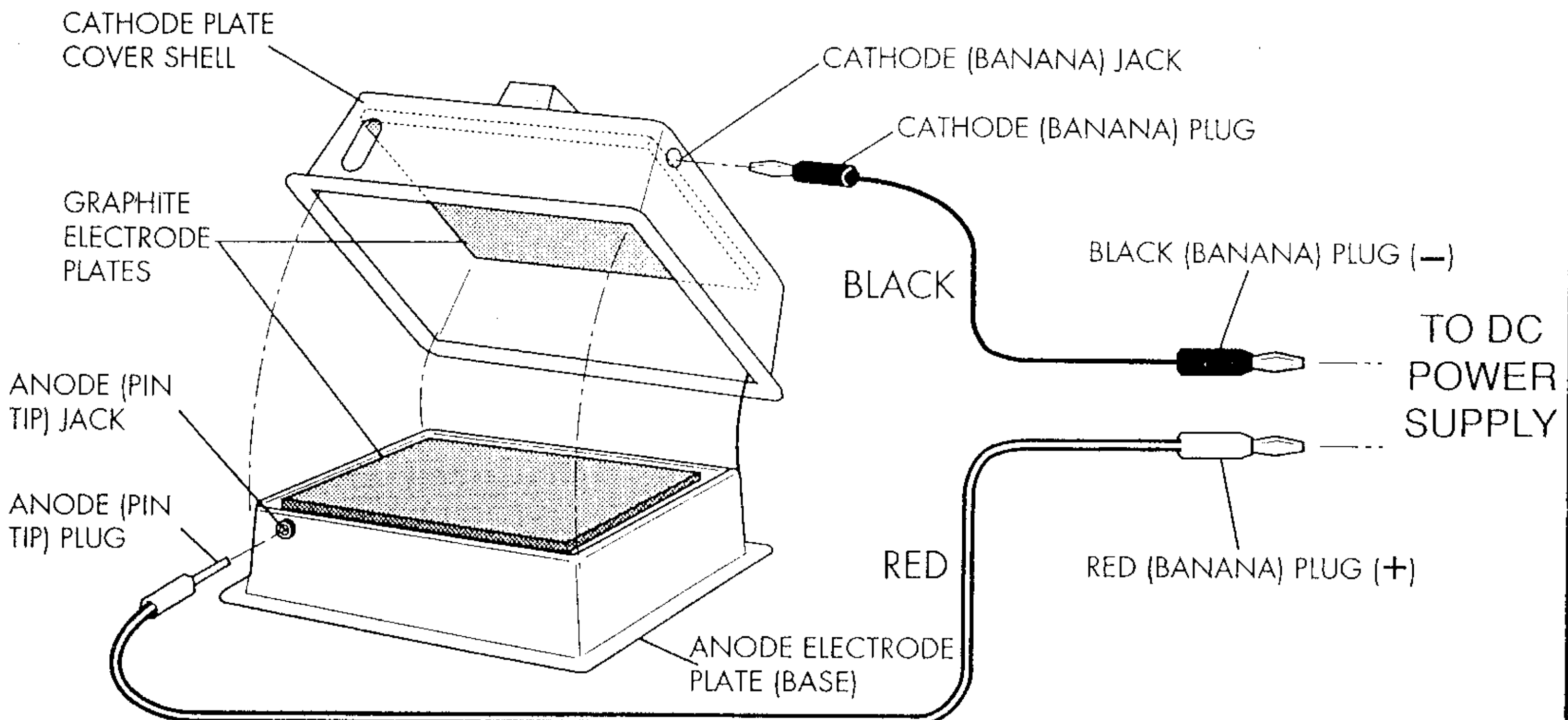

Figure 2 - Building A Trans-Unit Stack

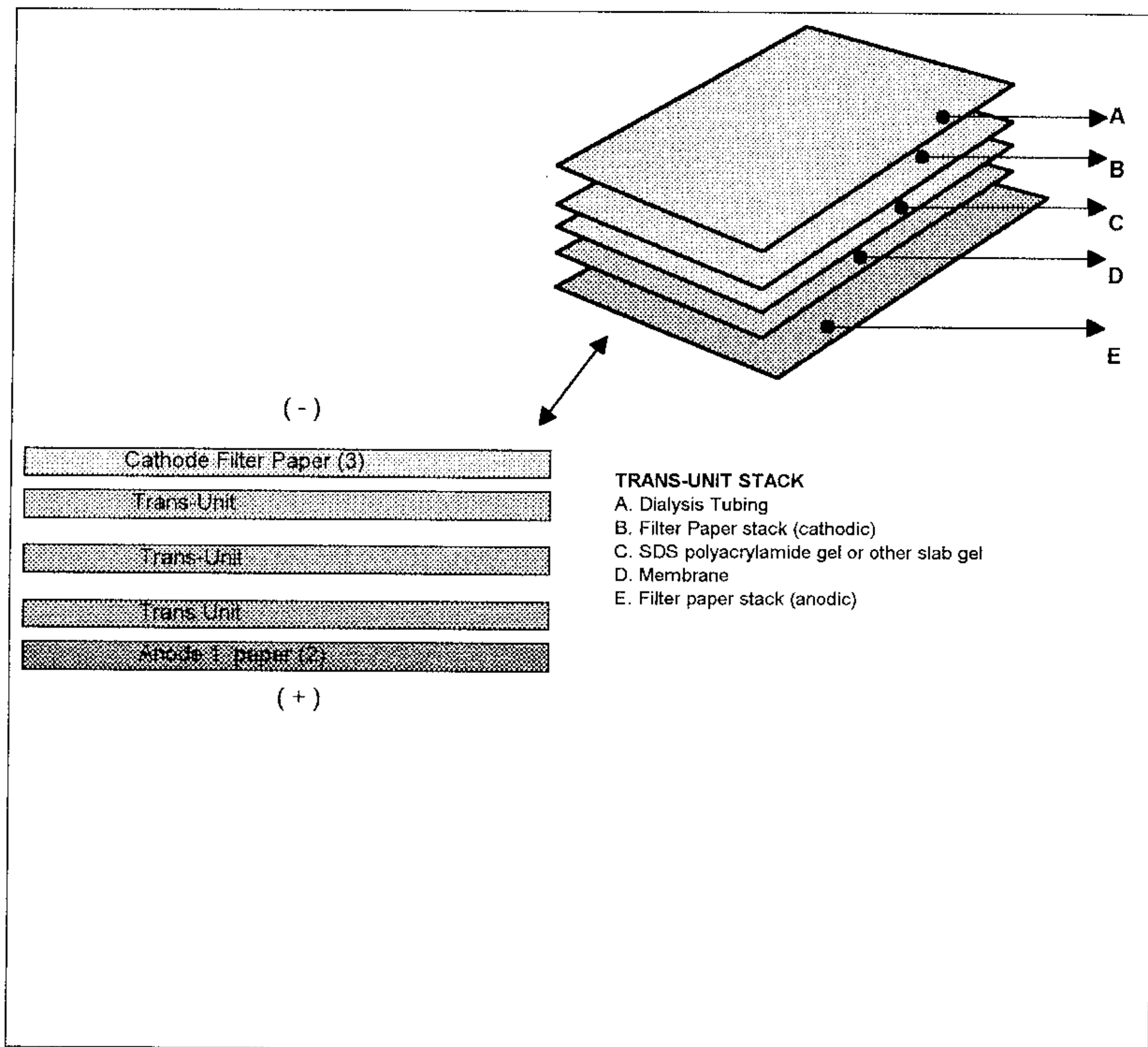

Table 1. Recommended current requirements for electr-transblotting proteins.

Current Density	Trans-Units	Time Limit
0.8 mA/cm2	1-6	1-2 Hours
2.5 mA/cm2	1-6	30-45 Minutes
4.0 mA/cm2	1-6	10-30 Minutes

TECHNICAL DATA SEMI-DRY BLOTTING APPARATUS

Surface Dimensions	9" x 9"	24 x 30 cm
Weight	3.5 lbs	9.5 lbs
Graphite Plate Dimensions	16 x 16 cm	24 x 30 cm
Cathode Electrode plug (wire)	32 inches	42 inches
Anode Electrode plug (wire)	32 inches	42 inches
Recommended Maximum Capacities	6 TRANS-UNITS	2-3 TRANS-UNITS

Necessary Extra Materials And Equipment Provided By User **

TRANS-UNIT

Ready Now™ Pre-Soaked Buffer Pad Kit, Gel (polyacrylamide or Agrose)

Solid support membrane (e.g. Nitrocellulose, PVDF, Nylon)

Filter papers (Schleicher & Schuell # 470 or Dialysis Membrane GB004)

Power Supply

Standard power supply unit capable of delivering 1 ampere or more, with amperage adjustable control

Buffer Solutions

Anode Buffer 1:0.3 M Tris, 20% Methanol, pH 10.4

Anode Buffer 0.025 M Tris, 20% Methanol, pH 10.4

Cathode Buffer = 0.025M Tris/0.04M amino caproic acid.
20% Methanol, pH 9.4

INSTALLATION INSTRUCTIONS

Contents List:

Part Description	Part Number	Number Enclosed
Cathode Plate Cover (black)	Top	1ea
with Cathode graphite electrode	T Electrode	1ea
Anode Plate Base (red) with	Bottom	1ea
Anode graphite electrode	B-Electrode	1ea
Cathode Plate Cover (black)	Top	1ea
with Cathode graphite electrode	T Electrode	
Anode Plate Base (red) with	Bottom	1ea
Anode graphite electrode	B-Electrode	
Anode & Cathode wire lead	(Model 1)	1 set
Set (Black and red)	(Model 2)	1 set
Warranty Registration Card		1ea
Instruction Book		1ea

UNPACKING UNIT

Carefully remove the packing material from the device and check for any damage on the device or cracks on the graphite electrode plates. Report any damage immediately upon receipt of the device.

DEVICE ASSEMBLY

Carefully wipe the electrode with a dampened cloth with distilled water to remove residual dust from the manufacturing process. Unpack the electrode plug wires. Insert the TRANS-UNITS (refer to operations section) and place the Cover Plate on the Base Plate aligning with the a node jack relief slot. Connect the Anode and Cathode plug wires from the device to the proper outlets of the power supply and the device is ready foroperation (see Figure 1).

PROCEDURE OF OPERATION AND TECHNIQUES WESTERN BLOTS

TRANS-UNIT ASSEMBLY

1. Pre-soak the gel in Anode buffer 2 for 10 minutes at room temperature.
2. Pre-cut the filter paper and the solid support membrane to the same size of the gel.
3. Pre-soak the membrane in distilled water at room temperature. If membrane is PVDF soak in methanol.
4. Place 2 sheets of filter paper saturated with Anode Buffer 1 onto the Anode Electrode plate of the Semi-dry Blotting device, followed by a sheet of filter paper (Ready-now) saturated with Anode Buffer 2 (see Application Notes & Recipes for the Anode Buffer 1 and 2).
5. Lay the wet nitrocelluose from step 3 on the filter paper stock.
6. Lay the pre-soaked gel on the membrane stack carefully (between the gel and the membrane) without allowing any air bubbles.
7. Lay one sheet of filter paper soaked with Cathode buffer on the gel.
8. If stacking another TRANS-UNIT on top of the first one, lay a dialysis membrane (pre-soaked in distilled water and cut to the same size as the filter paper) on to the Cathode filter paper and proceed to build additional stack of the TRANS-UNIT. (see Figure 2)
9. Complete the TRANS-UNIT stack with 2 sheets of filter paper soaked with Cathode buffer solution. (see Application Notes)

OPERATION PROCEDURES

1. Place the Cathode Plate Cover (black) on the assembled TRANS-UNIT stack, aligning the Anode jack relief slot.
2. Connect the red Anode jack to the red Anode plug and the black Cathode jack to the black Cathode plug (see Figure 1).
3. Connect the Anode and Cathode Electrode wire to the corresponding outputs of a power supply.
4. Turn on the power supply with constant current at the recommended amperage for the recommended time (see Figure 3).
5. Removal of the transblotted membrane should be as follows:
   a. Remove the cover plate.
   b. Carefully peel off the filter paper, and discard.
   c. Carefully peel off the gel which can stained or discarded.
   d. Peel off the membrane with forceps and ready for fixation and post-transblotted development process.

TECHNIQUES OF OPERATION AND SAFETY NOTES

1. Wear gloves when handling gels, membranes of filter papers, and processing chemicals.
2. Avoid air-bubbles between the gel and the membrane by laying a well soaked gel onto the membrane gently at an oblique angle.
3. The membrane and the gel should have the same dimensions. If the membrane is smaller than the gel, part may not be blotted. If the membrane is larger than the gel, the membrane may come into direct contact with the electrode plate(s) or filter papers which may cause uneven current distribution.
4. Pre-stained molecular weights standards may be used to visualize the transfer as soon as it is completed.
5. Be careful not to tear the dialysis membrane used to separate the TRANS-UNITS
6. Avoid salt crystals accumulating on the electrode plates. These can generate localized heat and cause damage on the unit rinse plates thoroughly with water after use.
7. Use caution with extreme low pH buffers because strong acids can damage the electrode plates if exposed for a prolonged period of time. Rinse plates thoroughly with water after use.

MAINTENANCE AND PRECAUTIONS

Maintenance

1. After each use, the device should be cleaned by thoroughly rinseing plates with water with distilled water. Store in a dry area.
2. Similarly, salt deposits or crystals should be rinsed off of the surfaces of the electrodes before use.

Precautions

1. **DO NOT USE** organic solvents such as acetone, toluene or petroleum ever to clean the device.
2. **DO NOT** immerse the device in ANY solution.
3. **DO NOT** place strong acids or strong bases in contact with the electrode plates. Be sure to clean the device immediately after exposure to any strong corrosive chemicals.
4. **BE CERTAIN** to close the Plate Cover before connecting the device to a power source.
5. **TURN OFF** power source prior to disconnecting electrode wires from the device or taking the device apart.

electrode with distilled water.

TROUBLESHOOTING CONTINUED

PROBLEM	CAUSE	SOLUTION
Poor electrophoretic transfer of RNA or DNA	Buffer may be too concentrated causing gel to carry too much current	Reduce buffer concentration (0.5x - 1x TBE) to maintain proper current
	Power conditions during transfer may have changed. If the voltage is not set high enough, the current will drop below the optimum range thereby reducing RNA/DNA transmigration.	Set voltage limits higher. Optimum transfer of plasmid, vector and PCR DNA appears to occur when the current is set between 3 - 3.55 MA/cm^2 for 10 - 15 minutes. Optimum transfer of RNA appears to be best achieved when current is set between 2.5 - 3 MA/cm^2 for 30 - 40 minutes. More difficult transfers like gnomic DNA may require longer transfer conditions at lower currents.

When searching for answers to problems involving the process of semi-dry electrophoresis, it is important to note that there are a number of factors that each individual scientist mu sttake into consideration regarding their specific experimentation. The semi-dry process works very effectively when all human and non human components are working together.

APPLICATION NOTES AND FORMULATIONS

Buffer Recipes

SDS-PAGE (Protein)

Anode Buffer #1 = 0.3M Tris, 20% Methanol, pH 10.4

Anode Buffer #2 = 0.025M Tris, 20% Methanol, pH 10.4

Cathode Buffer = 0.025M Tris, 0.04M amino caproic acid, 20% Methanol pH, 9.4

DNA Solutions-See Separate Insert

Application Notes

Western Blot (Protein)

The preceding operation procedure is formulated for application with SDS-PAGE. If higher molecular weight protein > 100,000 daltons are being electr-transblotted, the addition of 0.1% SDS to the Cathode buffer may facilitate the transfer.

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Figure-3 Basic Schematic

110 V AC

PS 1

SW

J1 1 — P1 1

J2 2 — P2 2

110 V

AC

Power supply

\-

\+

W-12-000-105

W-13-000-4911

10

9

\- DC

\+ AC

IMM-1A

IMM-1A-2

IMM-1A-3

IMM-1A-4

IMM-1B-1

IMM-1B-2

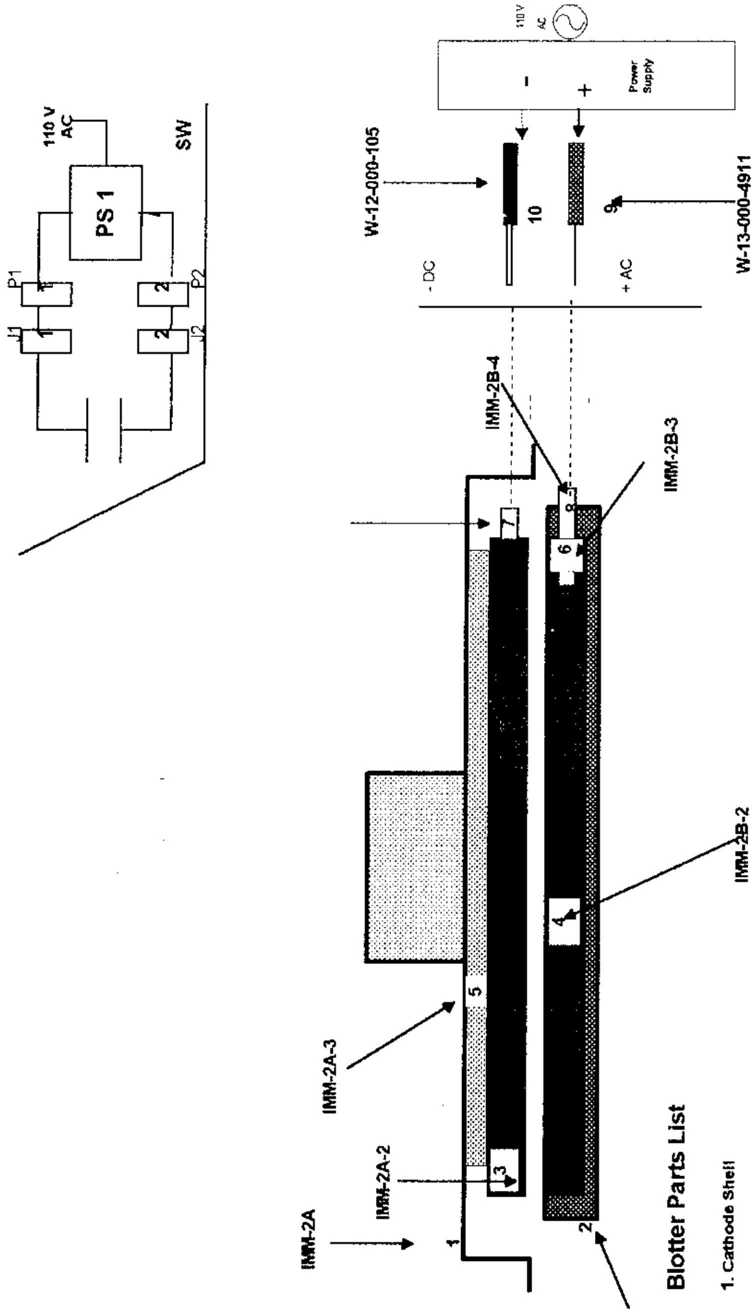
IMM-1B-3

IMM-1B-4

Blotter Parts List

1. Cathode Shell
2. Anode Shell
3. Cathode Graphite Electrode
4. Anode Graphite Electrode
5. Plastic Insulator
6. PVC Jack Cover (J2)
7. Bannana Jack (J1)
8. Gold Pin Tip Jack (J3)
9. Pin Tip Plug (P2)
10. Bannana Plug (P1)

Figure-4 Schematic



TRANSFERRING OF DNA AND RNA

DNA OR RNA BLOT TRANSFERS
USING THE SEMI-DRY BLOTTING DEVICE

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SUGGESTED PROCEDURES OF OPERATION AND TECHNIQUES FOR SEMI-DRY DNA BLOTTING

If the user is not using an automated developing device and is developing blots and gels by a manual method the following is an overview of suggested solutions and methods.

1. DNA is separated on HGT agarose gel (0.7 - 1%) poured with 1x TBE buffer. For genomic DNA use 0.7% and for plasmid digest use (0.8 - 1%).

2. After electrophoresis, trim away non essential portions of the gel. Prepare two buffer pads, two spacer pads, and one charge modified nylon membrane cut to exactly size of the trimmed gel.

3. Soak gel twice in two gel volumes of distilled H_2O for 10 minutes.

4. Soak gel twice in two gel volumes of denaturing *solution 1* for 30 minutes at room temperature with constant shaking.

5. Soak gel twice in two gel volumes of neutralizing *solution 2* for 30 minutes at room temperature with constant shaking.

6. Soak gel twice in two gel volumes of *solution 3* ,1x TBE for 30 minutes.

7. Wet one blotter pad, and one spacer, in *solution 3*, 1x TBE and place on the semi-dry blotter Anode (red/bottom) electrode plate. Using a clean pipette, roll out any air bubbles that may be trapped under the blotter pad.

8. Equilibrate the charge modified nylon transfer membrane in *solution 3*, 1x TBE for at least 10 minutes and place it on top of the spacer pad and eliminate any trapped air as described above. (Step 7)

9. Carefully place equilibrated gel on transfer membrane, making certain that **no** air bubbles are trapped beneath it. Do not move the gel at any time once it is in place.

10. Wet a second spacer pad, and buffer pad in *solution 3*, 1x TBE buffer and place spacer on top of gel. Finally place the buffer pad on top of the space making certain that there are no air bubbles trapped in the sandwich.

11. Carefully lower the semi-dry blotter top (black/top) Cathode electrode in place on top of the sandwich.

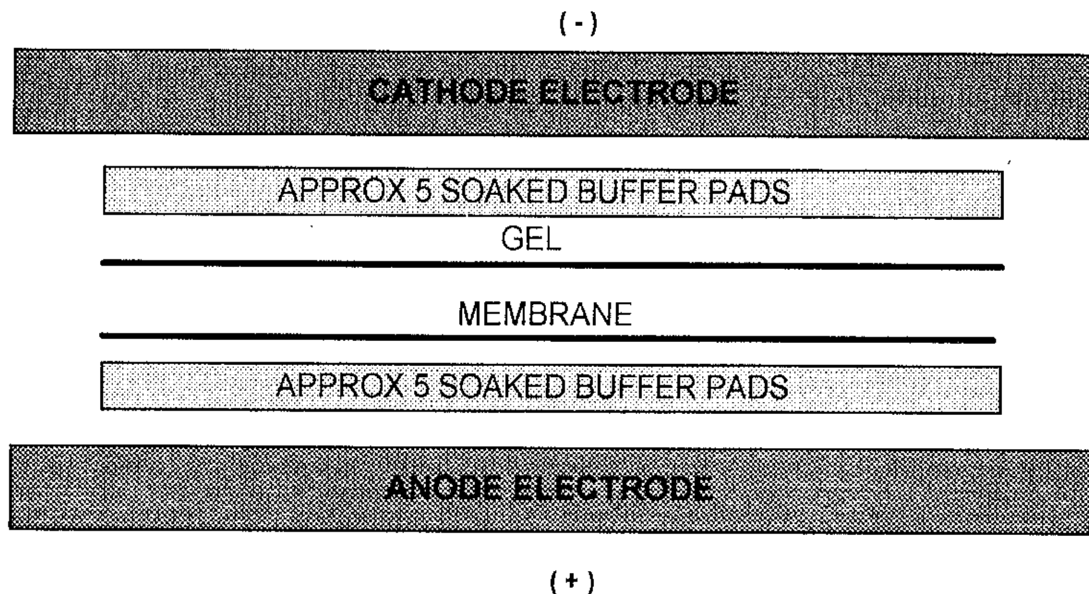
SUGGESTED PROCEDURES OF OPERATION AND TECHNIQUES FOR SEMI-DRY DNA BLOTTING- continued

12. Connect semi-dry blotter to suitable power supply and set transfer conditions between 1.5 - 3.5 mA/cm^2 of gel area. (i.e. 7 x 7/cm gel with an area of 49 cm^2 will require between 17 and 73/mA constant current.)

Power conditions and transfer times will vary with DNA/RNA type and size, thickness of gel and size of the electroblot sandwich. Typically 20-60 minutes will be required at current densities of 1.5 - 3.5 mA/cm^2.

During the electro transfer the voltage should be monitored for large fluctuation. During the transfer the voltage will slowly increase to maintain constant current. If the voltage is lower than 15V increase the length of transfer time. If the voltage increases significantly (i.e., greater than 25V) the buffer capacity may have been exhausted and the run should be terminated. If the run is not stopped the gel will overheat and eventually melt. If the voltage requirement is significantly lower than normal (i.e., <15V), the buffer solution may be more concentrated than 1x, and, therefore, less volume may be required to maintain the specified current. If this is the case, the recommended transfer can be completed as long as the current is adjusted to specified current setting. CARE MUST BE TAKEN NOT TO OVERHEAT THE GEL OR THE BLOTTING DEVICE.

Figure -5 DNA Trans-Unit Assembly



SUGGESTED PROCEDURES OF OPERATION AND TECHNIQUES FOR SEMI-DRY RNA BLOTTING

(Denaturing Formaldehyde Agarose Gels)

1. RNA is separated on to a 1.2% HGT Agarose gel with 1x MOPS and 1.8% formaldehyde. (See solutions).
2. The sample of total RNA are heated to 65^{0}C for 10-15 minutes and added to loading buffer. The loading buffer consists of 50% formamide, 6.5% formaldehyde and 1x MOPS. Typically 5-10ug of RNA are loaded per lane. The samples are then heated to 55^{0}C for 10-15 minutes and mixed 1/10 with bromophenol blue dye.
3. The gels are electrophoresed in a submarine apparatus in 1x MOPS buffer for 3-4 hours at 4-5v/cm.
4. Following electrophoresis, the gel is soaked in 5 gel volumes of 1x TBE buffer containing 0.1ug/ml EtBr for 30 minutes, after which soaked in 1x TBE without EtBr for an additional 30 minutes.
5. The gel should remain in the 1x TBE prior to semi-dry transferring.
6. Trim away nonessential portions of the gel. Prepare two buffer pads, two spacer pads and one charge modified nylon membrane cut to the exact size of the trimmed gel.
7. Saturate the trimmed buffer pads, two spacer pads, and the nylon membrane in 1x TBE buffer.
8. Place one blotter pad on the semi-dry blotter anode (red) electrode plate. Using a clean pipette or test tube, roll out any air bubbles that may be trapped under the buffer pad. Place a spacer on top of the buffer pad making certain that no air bubbles are trapped beneath it.
9. After equilibration (at least 10 minutes) on the nylon transfer membrane in 1x TBE, place membrane on top of the spacer making certain that no air is trapped beneath it.
10. Carefully place the equilibrated gel on top of the nylon transfer membrane, making certain that no air bubbles are trapped beneath it. Do not move the gel once it is in place.
11. Place a second spacer on top of the gel. Finally place buffer pad on top of the spacer making certain that no air is trapped beneath it.

12. Carefully lower the semi-dry blotter cathode (black top) electrode in place on top of the electroblotting sandwich.
13. Connect semi-dry blotting device to a suitable power supply and set transfer conditions to 2-3.5mA/cm^2. Typical transfer time should be 30-35 minutes.

TECHNIQUES FOR SEMI-DRY RNA BLOTTING

(Non-Denaturing Formaldehyde Agarose Gels)

1. Prior to electrophoresis, heat samples in RNA sample/denaturing buffer (4.5ul RNA in ultra pure water, 2ul 5x TBE buffer, 3.5ul formaldehyde, and 10ul formamide).
2. RNA is separated on a 1-1.5 agarose gel, omitting formaldehyde in gel.
3. The gels are electrophoresised in a submarine apparatus in 1x TBE buffer.
4. Following electrophoresis the gel is soaked in 5 gel volumes of 1x TBE buffer containing 0.1ug EtBr for 30 minutes and after which soaked in 1x TBE buffer without EtBr for an additional 30 minutes.
5. The gel should remain in 1x TBE buffer prior to semi-dry transfer.
6. Follow steps 6-13 as outlined above.

Note: 1x MOPS BUFFER MAY BE SUBSTITUTED FOR 1X TBE.

Table 2. Recommended current requirements for electr-transblotting DNA and RNA from either Polyacrylamide Gels, or Agarose Gels. Results may vary according to experiments being conducted, and materials being used.

Method	Recommended Currents	Gel Size	Recommended Time Limit
DNA/RNA from Polyacrylamide DNA from Agarose	1.5-3.5 mA/cm2	9 x 9 16 x 16	30-60 Minutes 1 Hour
RNA from Agarose	2.0-3.5 mA/cm2	9 x 9 16 x 16	30-40 Minutes 30-40 Minutes

Gel thickness and buffer solutions will affect final outcome.

SUGGESTED SOLUTIONS

DNA Solutions

Solution 1	Denaturing Solution	0.5 M NaOF, 1.5 M NaCl
Solution 2	Neutralizing Solution	1.0 M Tris-Hcl/pH 8, 1.5 M NaCl
Solution 3	Tris-Borate-EDTA	0.089 M Tris 0.089 M Boric Acid 0.002 M EDTA, pH 8.3

10x TBE (per liter*), pH 8.3

Quantity	Final 1x Concentration
108 g Tris Base	89/mM Tris Base
55 g Boric Acid	89/mM Boric Acid
40 ml 0.5 EDTA, pH 8.0	2/mM EDTA

1x MOPS, 1 L

Quantity	Final 1x Concentration
4. 186 g MOPS (3-N-Morpholino) propanesulfonic acid)	20 mM
41 g Sodium Acetate	5 mM Sodium Acetate
2 ul of 0.5 M EDTA, pH 8.0	1 mM EDTA

Dissolve 4.186 g MOPS, 0.41 g sodium acetate, and 2 ul EDTA in 800 ml of double distilled H_2O.

37% Formaldehyde Stock Solution

1.8% Formaldehyde - 5 ml of 37% stock/100 ml

6.5% formaldehyde - 1.75 ul of 37% stock/10 ul