INSTRUCTION MANUAL

Semi Dry Blotting Systems

CAT NO: EPS-B0020 & EPS-B0021

EPS BIOSOLUTIONS

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IMPORTANT USER INFORMATION

This Instruction Manual will explain how to use this product safely and effectively. Please read and carefully follow the instruction manual in its entirety.

The triangle/lighting bolt symbol alerts the user of the product to potentially hazardous electrical exposure.

Always turn off the DC power source prior to disconnecting power cords from the product.

Disconnect power cords from the power source first and then from the product.

For maximum safety, always operate this system in an isolated, low traffic area, not accessible to unauthorized personnel.

Section 1 General Information

1.1 Introduction

Semi-dry blotting is faster than tank blotting. It requires less material and generates less heat. The closely spaced electrodes separated by less conductive saturated filter papers permit rapid and efficient transfer without the very high current.

Both, discountiuous and continuous buffer systems can be applied. Discontinuous buffer systems are particularly recommended because transfer is very homogeneous for a wide range of molecular weights, even larger proteins up to 200KDa can be transferred with an efficiency of > 80%.

The EPS Semi-Dry blotting are equipped with corrosion resistant, long-lasting electrodes made from Graphite and stain less steel without a buffer tank or gel cassettes. Because of this direct contact, only minimum of transfer buffer required (less then 200ml). The electrodes are

resistant to corrosion and built for homogeneous, fast and efficient transfer of protein. This unit is designed with the anode plate serving as the base.

1.2 Standard supply

- Electrode bed (Polished Graphite)
- Cushion pad
- Power cord.

1.3 Specifications

Constructions:

Electrode bed	Acrylic
Electrodes	Polished Graphite
Power cords	7500VDC, 500mA, 65°C

Section 2 Instructions for Use

2.1 For Single Transfers

- Polyacrylamide gel containing the resolved proteins
- Nitrocellulose transfer membrane, cut to the same dimensions as the gel
- Cut two pieces of “extra thick” filter paper the same dimensions as the gel
- Semi-dry transfer system large enough to accommodate gel (10 X 10cm)
- Transfer buffer
- Deionized water

2.2 Set up

- Prepare transfer buffer
- Remove the gel from its glass cassette and trim away any stacking gel
- Equilibrate the gel for 10 min in 50-100 ml of transfer buffer

- Soak the appropriate number of blotting paper, pieces for at least 30 seconds
- Prepare nitro cellulose membrane
- Wet the membrane in methanol for few seconds until the membrane uniformly changes from opaque to semi-transparent.
- Rinse the membrane in leionized water for few seconds
- Equilibrate the membrane in transfer buffer for at least 5 min

2.3 Transfer stack Assembly

Note: Wear gloves throughout the procedure to avoid contamination.

Use 100 ml of each solution for minigels, while for larger gels use enough of the solution to cover completely the gel/membrane/paper sheets in each soaking step.

However, careful attention should be paid to the polarity during the set-up, since reversing the polarity can result in damage to the cathode.

Note: To ensure an even transfer, remove air bubbles between layers by careful rolling a pipette or stirring rod over the surface of each layer in the stack. Do not apply excessive pressure to prevent damaging the membrane and gel.

2.4 Blotting

Remove the stacking gel from the gel. Measure the gel and record its size. Do not pre-soak the gel. Do not cut a corner off the gel, as this may allow a short circuit and inefficient transfer. Blot the gel as soon as possible after electrophoresis to avoid diffusion of the samples through the gel.

Cut pieces of blotter paper to the size of the gel. You will need four pieces, plus one for each gel transferred. It is important that the blotter paper (and the membrane) not be larger than the gel. Larger pieces may make contact around the gel, and allow the current an alternate route, thereby making transfer inefficient.

Wear gloves rinsed of powder, cut a piece of nylon membrane to the size of the gel. Make the lower right-hand corner to allow for orientation. Pre-wet the membrane in transfer buffer for 2 to 5 minutes.

Place a insulation sheet in the bottom of the unit. The insulation sheet should have a central, square opening cut in it that is 2 mm smaller than the gel in both length and height.

In a dish of transfer buffer, saturate two pieces of blotter paper cut in step 2. Place these on top of the insulation sheet, centering them by first placing the center of the paper down first, and rolling the edges out. The filter paper should cover the cut-out in the insulation sheet and slightly overlap it on all sides.

Construct the first transfer sandwich on top of the previously-placed blotter paper by placing the membrane, then the gel, then an additional piece of buffer-soaked blotter paper on the stack.

Stack the components neatly, with edges parallel. As each layer is placed upon the stack, make sure no air bubbles are trapped. Sweep a wetted, gloved finger over the layer, or roll a pipette or test tube over it to remove bubbles. Proteins on the surface of the gel will bind to the membrane as soon as contact is made. The membrane must therefore be positioned correctly the first time. Do not try to adjust.

Place two additional pieces of buffer-soaked blotter paper on top of the stack. Place the cover on the unit (it fits only one way). Hold the cover level and slide it gently down onto the stack.

Do not remove the cover until after the blot is completed. Part of the stack may stick to the cover or otherwise be knocked out of alignment.

2.5 Protein Transfer

1. Insert the black cathode lead (-) into the plate socket.
2. Insert the red anode lead (+) into the anode plate socket.
3. Connect the anode lead and cathode lead to their corresponding power supply outputs.
4. Turn the power supply to zero before turning on. Turn on the power supply and set to approximately 0.8 mA/cm2 of gel. 8 mm x 7 mm mini-gels may be run at 100 mA constant current. Larger gels must be limited to 0.8 mA/cm2 to avoid excessive heat build up.
5. Set the current and let it run for the time indicated.
6. Turn off power supply when the transfer is complete.
7. Disconnect the system leads.
8. Remove the cover.

9. Remove and discard the filter papers. Note: When using graphite plates, graphite particles from the anode electrode plate occasionally appear on the filter paper. These particles do not affect operation.
10. Remove the gel.
11. Remove the blotted membrane with a pair of forceps.
12. Rinse the membrane in deionized water and place it onto a piece of clean Whatman 3MM paper to dry.

Important: Do not allow membrane to dry out if analysis of the bound protein requires the native conformation or enzyme activity.

2.6 Post Tranfer

Once the transfer is complete, the efficiency can be monitored by visually checking the transfer of the pre-stained molecular weight standards.

SECTION 3 Maintenance of Equipment

3.1 Care and Handling

The plastic components of the semi dry blott are fabricated from acrylic. As with any laboratory instrument, adequate care ensures consistent and reliable performance.

After each use, rinse buffer chamber, gel tray and combs with de-ionized water. Wipe dry with a soft cloth or paper towel, or allow to air dry. Whenever necessary, all components may be washed gently with water and a non-abrasive detergent, and rinsed and dried as above.
Never use abrasive cleaners, glass cleaning sprays or scouring pads to clean the components, as these will damage the unit and components.

Additional precautions:

- Do not autoclave or dry-heat sterilize the apparatus or components.
- Do not expose the apparatus or components to phenol, acetone, benzene, halogenated
- hydrocarbon solvents or alcohols.
- Avoid prolonged exposure of the apparatus or components to UV light.

3.2 Maintenance

The following inspection and maintenance procedures will help maintain the safety and reliable performance of the semi dry blotting systems. Replacement parts can be ordered by calling 044-24363199 or by contacting your local distributor.

- Banana plugs and power cords should be inspected regularly. If the banana plugs become loose or do not feel friction tight replace the plugs or power cords.

- Should power cord assemblies (connectors, wire or shrouds) show any signs of wear or damage (e.g. cracks, nicks, abrasions, or melted insulation), replace them immediately.

- The platinum wire is secured to the banana jack by compression between a stainless washer and the jack nut. The nut/washer interface should be tight and free of corrosion.