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**INSTRUCTION MANUAL
OF
BLOTTING APPARATUS**

NANOBLOT

**(CAT NO. EBNAR-SC0605)
(OLD CAT. NO. E1100060)**

For Western Blotting

TechResource
Scientific Pvt Ltd

101, Marudhar Ind Estate, Navghar, Vasai (E) 401210
Tel: 9819095429 Email: sales@techresource.in

Essential components of the Apparatus:

- a) One tank.
- b) One cathode and one anode plate.
- c) One cathode-facing blotting sandwich frame bearing two pins with stoppers.
- d) One anode-facing narrower frame.
- e) Two fibre sponge pads.
- f) Two locking nuts.
- g) One pair of connecting cords.
- h) One lid.

CAUTION

Use the apparatus with care. It should be washed and rinsed with soft water, air dried at room temperature and stored covered.

It should **NOT BE**:

subjected to impact,

exposed continuously to temperatures outside 40°C,

handled with abrasive or sharp objects, exposed to organic solvents such as aromatic or halogenated hydrocarbons, ketones, esters, alcohols over 30% or concentrated acids over 25%,

exposed to other solutions which will attack plastics such as acrylic, acetal, Celcon, Noryl, Dacron and epoxy.

To avoid electrolytic corrosion, always dry the connecting pins on central electrode unit before attaching the electrical cords to them.

Items needed to perform a Western blotting experiment:

- a) Two white filter paper sheets; $10.5 \times 6.5\text{cm}^2$ (thickness equivalent to Whatman # 3).
- b) Hydrophilic nitrocellulose membrane; $8.5 \times 6.5\text{cm}^2$ (pore size $0.22\text{ }\mu\text{m}$) for the transfer of proteins.
- c) Methanol-containing transfer buffer.
- d) Aluminium foil, gel slicer.
- e) A constant voltage (CV) or constant current (CC) power supply.
- f) Gel containing the separated proteins.
- g) A 500 mL beaker, 10 mL pipettes.
- h) Blunt-edged membrane-forceps.
- j) Pair of gloves

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DETAILED PROCEDURE FOR ASSEMBLING THE NANOBLOT:-

I Preparatory tasks:

- 1) Prepare 500 mL of transfer buffer. Add 100 mL methanol to 400mL of tank buffer and cover it with foil. Cool it to 4 °C. (The dissolution of methanol in water liberates heat. Hence, it is best to add it first and then cool the mixture.)
- 2) Cut two pieces of filter paper (thickness equivalent to Whatman # 3) to the size of the sponge pad ($10.5 \times 6.5\text{cm}^2$). Also cut the 0.22 μm nitrocellulose membrane to the size of the gel (size should be $\leq 8.5 \times 6.5\text{cm}^2$) that is to be blotted. Mark the corners with a pencil.
- 3) Clean all the parts of the Nanoblot (except sponges) and the heavy- walled tray with water. If necessary, use detergent. Ensure that all the parts are finally rinsed with tap (soft) water. Drain off the excess water.
- 4) The assembly has been fitted with threaded stoppers on the guide pins to ensure that the gel does not suffer distortion due to excessive pressure within the sandwich. To adjust the position of the stoppers, place the cathode facing frame with its pins upwards. Place rectangles of filter paper and one of the two sponges over the array of holes. Place the narrower frame over the sponges such that two guide-pins slide into their corresponding holes. Move the stoppers up or down to allow the frame to rest lightly on the sponge. The stoppers may then be further raised or lowered to the desired height depending on the thickness of the gel, number of filter paper sheets employed and the pressure that can be tolerated by the gel (a function of its composition such as %T and %C of polyacrylamide).

II Assembling the sandwich

- 1) After the electrophoresis has been terminated, pour chilled buffer into the tray and immerse the fiber-sponges in it. The presence of methanol facilitates wetting of the sponges and most of the entrapped air can be easily squeezed out. If the buffer does not contain methanol, squeeze the sponges repeatedly till all the air is removed. Transfer the wet sponges to the beaker containing the buffer.
- 2) Remove the gel-sandwich from the MICROKIN and pry the plates apart. Sprinkle some buffer on the gel to prevent it from drying. Detach the gel from the bottom plate and trim away the unnecessary gel portions. Mark the orientation of the gel.
- 3) For the transfer of anionic proteins, assemble the blotting sandwich under buffer in the sequence listed below. Ensure that no bubbles are trapped between the layers. Perform the following steps wearing gloves.
 - a) Place the broader cathode-facing frame on the floor of the tray with its pins upwards. Note that the engraving 'GEL' should be at the top.
 - b) Place a wet sponge pad over the array of holes of the frame. The border strips of the frame will prevent the sponge from moving.

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- c) Wet a filter paper with buffer, and beginning from one edge, gently lower it onto the sponge. Push the fine bubbles to the side.
 - d) Gently lift the gel and place it on the filter paper. Position the gel appropriately. It is difficult to see the gel against the white background. The ink spots help delineate the boundaries.
 - e) Wet the nitrocellulose filter paper in methanol-containing transfer buffer to remove the bubbles. With membrane forceps, carefully place the membrane on the gel. Begin at one edge and proceed to the other whilst lowering the membrane onto the gel.
 - f) Place a buffer-soaked filter paper over the membrane.
 - g) Then place a wet sponge pad over it.
 - h) Finally, place the narrower frame (surface with the larger diameter of the holes downward) over the sandwich such that the guide pins, protruding from the winged frame, slide into the smaller holes in the narrower frame.
 - i) Place one's palm over the narrower frame and push it down such that it comes to rest on the stoppers.
 - j) Secure the assembled blotting sandwich with the locking nuts.
 - k) Orient the tray such that the engraving 'GEL' is on the top.
- 4) Pour chilled buffer into the tank to fill half of it.
 - 5) Slide the electrode plates into the tank. Ensure that the connectors go into their respective slots in the face of the tank.
 - 6) To put the blotting sandwich into the tank, lift it out of the tray and slide the cathode facing plate into the central channels provided. Ensure that the broader frame with 'GEL' engraving faces the cathode (black connector). The sandwich will not fit properly in any other orientation.
 - 7) Adjust the buffer level so as to ensure that the gel is completely immersed in it.
 - 8) Cover the unit with its lid.
 - 9) Connect the black electrical cord to the cathode plate and the red cord to the other plate.
 - 10) Connect the male end of the cords to the power supply. Perform the transfer at constant voltage or constant current*. (See note below.)
 - 11) The time for transfer depends on gel thickness, molecular mass of biomolecule(s), ionic strength of transfer buffer, presence of ionic detergent and amperage used during transfer.
 - 12) The resistance of the system with the commonly used chilled Tris-glycine-methanol buffer (pH 8.3) is around 1000 ohms. The transfer can be done using a voltage gradient of 3-6 V/cm (14-27 V or 14-28 mA in a refrigerator) overnight or at 10 V/cm (45 V or 50 mA in an ice-water bath) in 1.5 hr

***Note:** If your power supply has a CC feature, then it is safest (especially for rapid transfers) to perform the blotting using this mode. When blotting is done at CV, and the joule heat produced during blotting is greater than that removed from the system, the temperature of the buffer will keep on rising. In such cases, the resistance of the system decreases, as the buffer gets warmer. If the voltage is maintained at a constant value, more current flows through the system, which further heats up the buffer, setting up a dangerous positive- feedback loop. In the CC mode, the voltage will drop as the temperature of the buffer rises. Consequently, less heat will be

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generated and the system will stabilize at a steady-state value, where the heat extracted from the system will be equal to that produced due to the passage of the current.

- 13) After the selected time-interval has elapsed, switch off the power supply; remove the unit from the ice-water bath/ refrigerator. Disconnect the cords.
- 14) Remove the lid.
- 15) Pull out the blotting-sandwich and unbolt the frames by turning the locking nuts counter-clockwise.
- 16) Remove the narrower frame, sponge pad and filter papers.
- 17) With the help of a blunt-edged membrane-forceps, carefully peel off the nitrocellulose membrane from the gel.
- 18) Transfer the membrane to a dish and process it for protein staining or immunoanalysis.
- 19) Transfer the gel to a staining tray and stain for proteins to ascertain the effectiveness of the transfer.
- 20) Wash the sponge pads with copious amounts of water, rinse them finally with soft water and allow to drip dry.
- 21) Clean all the parts of the apparatus with water. Use mild liquid detergent if necessary. Ensure that all the parts are finally rinsed with tap (soft) water. Drain off the excess water.
- 22) Dry the parts, fit them into the tank and store at room temperature in the box provided.

TIPS AND NOTES

- 1) Proteins can be fixed on nitrocellulose paper by either drying the paper at 37°C, or exposing the moist paper to fumes of paraformaldehyde, or by soaking the paper for 5 min (20°C) in 1% KOH, followed by rinsing, 2 × 10 min with TBS.
- 2) Binding capacity of nitrocellulose (NC) membranes is 250 µg/cm² and that of PVDF membranes is 172 µg/cm².
- 3) Methanol retards transfer of high (>10⁵) M_r proteins.
- 4) NC membranes are not compatible with reagents used in micro-sequencing procedures. Hence, use PVDF membranes when the blotted proteins have to be sequenced. Also, amino buffers such as Tris and glycine should be avoided as they interfere with the sequencing.
- 5) The recommended amperage for blotting is 0.8 mA/cm² of electroblotting buffer cross-sectional area, in the direction of transfer.
- 6) Binding of nucleic acids to nitrocellulose requires high salt concentrations. Such buffers will have a high conductivity and hence, will not result in efficient electro transfers. Therefore, nitrocellulose membranes are incompatible with northern/Southern electroblotting.

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- 7) Nylon membranes, which can bind nucleic acids even in low ionic strength buffers, are recommended for the electroblotting of nucleic acids.

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