Instruction for Use

Semi-dry Electroblotting of IEF Gels with Precast SERVALYT™ PreNets™

Perform the Isoelectric Focusing following the standard protocol given in the instructions manual contained in each package of SERVALYT™ PRECOTES™ and SERVALYT™ PreNets™.
For IEF of standard SERVALYT™ PreNets™ of 125 x 125 mm (gel layer of 0.3 mm) the protocol applies an initial voltage of 200 V, a final voltage of 2500 V and a maximum wattage of 8 W. Temperature is 4 ℃. Duration approx. 3 hrs. or 350 0 to 4000 Vh.

The Blotting Procedure applies a discontinuous buffer system :

Blotting buffer* to be used:
Anode buffer I : 300 mM Tris, 20 % (v/v) methanol
Anode buffer II : 30 mM Tris, 20 % (v/v) methanol
Cathode buffer : 25 mM Tris/HCl (pH 9,4), 40 mM 6-Aminocapronic acid, 20 % (v/v) methanol

The buffers are All contained in the Semi Dry Buffer Kit for Western Blotting (SERVA cat.no. 42559).

* Reference: Kyhse-Andersen J., Biochem Biophys Methods.**10** (1984) 203-209

While the IEF is running:

- Cut to size of gel: 9 pcs. of filter paper (Whatman 3) and 1 sheet of transfer nitrocellulose-membrane (e.g. Nitrocellulose SERVA NC 45, cat.no. 71208)
- Soak 3 pieces each of filter paper in anode buffer I, anode buffer II and cathode buffer. Wet the membrane in anode buffer II.

Upon completion of electrophoresis:

Stop power supply, open flat bed unit, remove electrode wicks and applicator strip from the gel. Remove the gel from flat bed unit. Separate the gel from the film backing by lifting the gel layer slowly starting at one corner using a pair of forceps (the layer contains the inert fabric NetFix™ which reinforces the soft gel preventing from tearing). Transfer the gel layer containing NetFix™ to a stack set up in the order described below.

Set-up and start blotting:

Form the blotting stack starting on top of the anodic plate electrode in the order:

1. 3 layers of filter paper, soaked in anodic buffer I
2. 3 layers of filter paper, soaked in anodic buffer II
3. Transfer membrane
4. Focused SERVALYT™ PreNets™ gel (removed from the film backing); mark the orientation of the gel on the membrane (i.e., cathode/anode and/or marker lane).
5. 3 layers of filter paper, soaked in cathodic buffer

Note: Ensure the filter paper is soaked properly, i.e., moist but not wet, when assembling the stack. Make sure that there are no air bubbles trapped (use a pasteur pipette or glass rod to remove bubbles) Close blotting apparatus and apply 0.8 mA/cm^2 or 125 mA (format 125 x 125 mm). Transfer times depend on the nature of the proteins in the sample, to be determined individually. For example, marker proteins (6000 up to 100,000 Da) are transferred sufficiently within 30 minutes.

While blotting prepare separating buffer :

- Dissolve 9.55 g PBS (phosphate buffered saline) and 3 g Tween 20 (cat.no. 37470) in 1000 ml distilled water.
- Stop blotting, disconnect power supply and open the blotting apparatus. Remove the blotting stack and transfer it in a tray containing 100 ml separating buffer. This step is recommended to avoid the PreNet gel to stick to the membrane; adhering gel fragments may interfere with staining. Place the tray on a shaker for 3 minutes. The various layers will separate from each other, without any problem of adhesion.

Staining:

Unspecific staining procedures are applicable, i.e. Amido Black 10 B or Ponceau S, and also specific detection methods (e.g., using antibodies).

Staining using Amido Black 10 B

- Stock solution: 1 % w/w Amido Black 10B, (cat.no. 12310) in 40 % methanol and 10 % acetic acid)
- Dilute stock solution 1:10 with distilled water, incubate membrane in staining solution until bands appear clearly visible. Destain in distilled water until background is clear. Use a shaker in all steps. Dry blot on paper towel.

Staining using Ponceau S

- Ready-to-use Ponceau S solution (at.no. 33427)
- incubate membrane in Ponceau S solution for 5 minutes (use shaker), destain in distilled water until background is clear and bands are clearly visible.

Note: It is also possible to perform electrotransfer of IEF gels using tank blotting procedures.