INSTRUCTIONS

Low-Fluorescence PVDF Transfer Membrane

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22860

Number	Description
22860	**Low-Fluorescence PVDF Transfer Membrane, 0.2 µm, 7 cm × 8.4 cm,** 10 sheets

Storage: Store membranes flat at ambient temperature and away from chemical vapors. Some solvent vapors may partially dissolve the membranes, which will disrupt the pore structure.

Introduction

Polyvinylidene difluoride (PVDF) membranes are hydrophobic and have high binding affinity for proteins and nucleic acids. The Low-Fluorescence PVDF Membrane has been optimized for use with fluorescent probes. This membrane has lower background levels and increased sensitivity for fluorescent probing than regular PVDF or nitrocellulose membranes.

Important Product Information

- Bromophenol blue can emit a strong fluorescent signal that interferes with protein detection. Either do not use bromophenol blue in the gel loading buffer or make sure that the dye front has migrated from the gel before transfer.
- Do not use a ballpoint pen to write on membranes because ink emits a fluorescent signal and can smear across the membrane causing high background levels. If a membrane needs to be marked, use a pencil.
- Once the membrane has been hydrated, only use forceps to move the membrane. Grip only the edges and avoid scratching or bending the membrane, which increases background levels.
- Stripping and reprobing fluorescent Western blots is not recommended because results are typically inconsistent.

Example Procedure for Transferring Proteins to a PVDF Membrane

Note: Always wear gloves when handling PVDF membranes because oils from fingers may prevent proper wetting. Proteins from hands may also bind to the membranes, causing background.

A. Materials required

- Completed gel separation of proteins: Use any suitable protocol to separate proteins by electrophoresis
- 100% Methanol
- Cold transfer buffer: Use any suitable buffer for transferring proteins from acrylamide gels to membranes (see the Related Products Section)
- Western blotting filter paper (see Related Products Section)
- Transfer unit (including fiber pads) and power supply

Method

1. Remove gel from cassette and equilibrate in cold transfer buffer for 10-15 minutes.

   Note: Incubation time is based on 1.0 mm thick gels. Increase incubation time for thicker gels.
2. For orientation with the gel, cut a notch in a corner of the membrane.
3. Wet the membrane in 100% methanol for 15 seconds and rinse with ultrapure water for 2 minutes with agitation.
4. Equilibrate the membrane in cold transfer buffer for 10-15 minutes.

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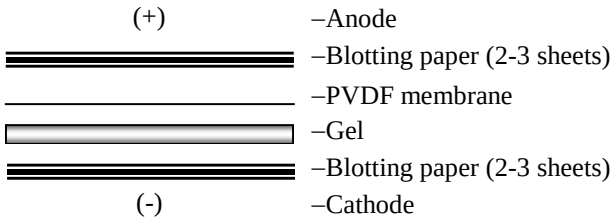
5. Wet the filter paper and fiber pads in cold transfer buffer for 5 minutes.
6. Assemble the components in the following manner for transfer:



7. Attach the leads and perform the transfer at 200 mA for 90 minutes.

   Note: Transfer time and efficiency will vary depending on polyacrylamide concentration, gel thickness, the presence of SDS or methanol, pH and ionic strength of the transfer buffer, and the molecular weight of the protein. Determine the optimal transfer conditions empirically.
8. When the transfer is complete, disconnect the leads from the transfer unit and disassemble the transfer components to remove the membrane.
9. Keep membrane moist until ready to use.

Related Products

Please see the catalog or website for a complete listing of products for Western blotting.

XP04200BOX	**Novex™ Tris-glycine protein gels** (see thermofisher.com/proteingels for a complete listing)
NW04120BOX	**Bolt™ Bis-Tris Plus protein gels** (see thermofisher.com/proteingels for a complete listing)
LC2676	**Novex™ Tris-Glycine SDS Sample Buffer**
LC2675	**Novex Tris-Glycine SDS Running Buffer**
88600	**Western Blotting Filter Paper**, 100 sheets
LC5615	**iBright™ Prestained Protein Ladder**
A32727	**Goat Anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 555**
A32732	**Goat Anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 555**
A32728	**Goat Anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 647**
A32733	**Goat Anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 647**

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