User Guide

Immobilon®-P Transfer Membrane

For High Sensitivity Immunodetection

For more detail, refer to the full length Immobilon®-P user guide at www.millipore.com (search on *Immobilon-P*).

Introduction

Immobilon®-P transfer membrane is a polyvinylidene fluoride (PVDF) microporous membrane used for transfer of proteins from a variety of gel matrices. This membrane is hydrophobic and offers a uniformly controlled pore structure with a high binding capacity for biomolecules. When compared to a nitrocellulose membrane, it has improved handling characteristics and staining capabilities, increased solvent resistance, and a higher signal-to-noise ratio for enhanced sensitivities.

The Immobilon®-P membrane has a nominal pore size of 0.45 micron (µm) and is optimal for blotting proteins with molecular weights greater than 20,000 (Immobilon®-P^{SQ} membrane is optimal for proteins less than 20,000). It is an ideal substrate for immunodetection, since it is compatible with standard blocking agents and detection protocols, including chemiluminescence. Because the membrane is composed of PVDF, it is also compatible with the harsh conditions used in protein sequencing and amino acid analysis. This user guide provides basic protocols for electroblotting and rapid immunodetection.

For more information, refer to publication TP001EN, the "Protein Blotting Handbook" (go to www.millipore.com and search on TP001EN).

Materials Recommended for Western Blotting

- Immobilon®-P membrane cut to the dimensions of the gel
- Alcohol (> 50% methanol, ethanol, or isopropanol) for wetting dry membrane
- Milli-Q® water
- Transfer buffer: 25 mM Tris-base, 192 mM glycine, pH 8.3, 10% alcohol for tank transfer or 48 mM Tris, 39 mM glycine, pH 9.2, 10% alcohol for semi-dry transfer)
- Sheets of filter paper, cut to the dimensions of the gel and soaked in transfer buffer for at least 30 seconds
- Blocking buffer: bløk®-CH buffer (cat. no. WBAVDCH01) or 0.5–5% (w/v) blocking agent (bovine serum albumin, casein, nonfat dry milk) in wash buffer
- Wash buffer: Phosphate-buffered saline (PBS) or Tris-buffered saline (TBS) containing 0.05-0.1% Tween®-20 surfactant (PBST or TBST)

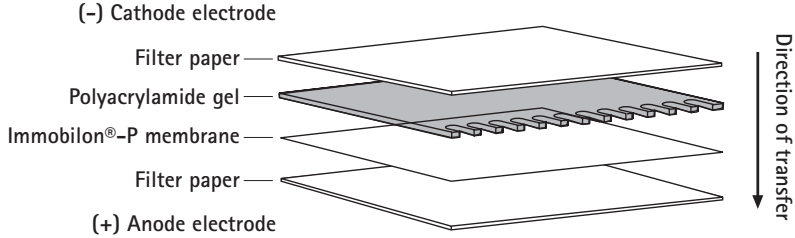
  PBS: 10 mM sodium phosphate, pH 7.2, 0.9% NaCl
  TBS: 10 mM Tris, pH 7.4, 0.9% NaCl
- Primary antibody (specific for the protein of interest), diluted in blocking buffer or wash buffer
- Secondary antibody (specific for the primary antibody), labeled with a detection enzyme (e.g., horseradish peroxidase [HRP] or alkaline phosphatase [AP]), diluted in blocking buffer or wash buffer

Protein Transfer

1. Resolve the protein mixture on a 1D or 2D polyacrylamide gel.
2. Immerse the gel in the transfer buffer and allow it to equilibrate for 10–15 minutes.
3. Wet the Immobilon®-P membrane in alcohol (>50% methanol, ethanol, or isopropanol) for 10-20 seconds, or until the it changes from opaque white to uniform, translucent gray. Do not leave dry spots, as these may inhibit transfer.
4. Immerse the membrane in Milli-Q® water for 1–2 minutes to displace the alcohol.
5. Equilibrate the membrane for 2–3 minutes in the transfer buffer.

   CAUTION: To prevent tearing, handle the membrane with care. Once the membrane has been wet out, do not allow it to dry out until the proteins have been transferred to it. If the membrane dries out (turns opaque white) even partially, it must be wet out again (steps 2–4).

6. Assemble the transfer stack as shown below or according to transfer apparatus manufacturer's instructions.



   CAUTION: To ensure an even transfer, remove air bubbles by carefully rolling a clean pipette or blot roller over the surface of each layer in the stack. Do not apply excessive pressure, as this may damage the gel and membrane.

7. Transfer proteins according to transfer apparatus manufacturer's instructions.
8. Remove the blot from the transfer system and rinse the membrane briefly in Milli-Q® water to remove gel debris. The blot may be used immediately, or air dried for storage.
9. **[Optional]** To visualize all of the transferred proteins, Immobilon®-P membrane may be stained with any reversible blot stain compatible with immunodetection (e.g., Ponceau-S, CPTS, Sypro® Ruby or Sypro® Rose blot stains), or viewed by transillumination using a light box.

Immunodetection

The following is a general protocol for immunodetection with Immobilon®-P membrane. For optimal results, refer to the immunodetection reagent manufacturer's instructions.

1. If the blot was dried, rewet it in alcohol (>50% methanol, ethanol, or isopropanol) for 15 seconds, or until it changes from opaque white to translucent gray.
2. Rinse the blot in Milli-Q® water for 1 minute.
3. Place the blot in blocking buffer and incubate for 1 hour with gentle agitation. Prepare primary antibody solution in wash or blocking buffer.
4. Place the blot in diluted primary antibody solution and incubate for 1 hour with gentle agitation.
5. Wash the blot with wash buffer 3–5 times for 5 minutes each. Prepare secondary antibody solution in wash or blocking buffer.
6. Place the blot in diluted enzyme-labeled secondary antibody solution and incubate for 1 hour with gentle agitation.
7. Wash the blot with wash buffer 3–5 times for 5 minutes each.
8. If developing with a chromogenic reagent, incubate blot in the developing solution until sufficient signal has been generated for the band of interest. To stop development, transfer the blot to Milli-Q® water or follow the instructions provided with the developing reagent. The developed blot may be dried on filter paper and imaged.

Protein Transfer, continued

9. If developing with chemiluminescent detection, incubate in developer 1–5 minutes, according to detection reagent manufacturer's instructions, and then expose the blot to X-ray film or acquire image using a digital imaging system.

Product Ordering Information

This section lists the catalogue numbers for Immobilon®-P and other Immobilon® membranes. See the Technical Assistance section for contact information. You can purchase these products on-line at www.millipore.com/products.

Immobilon®-P Membrane (0.45 µm pore size) for general Western blotting applications

Size	Qty/Pk	Catalogue Number
26.5 × 375 cm roll	1	IPVH00010
26 × 26 cm sheet	10	IPVH304F0
20 × 20 cm sheet	10	IPVH20200
15 × 15 cm sheet	10	IPVH15150
10 × 10 cm sheet	10	IPVH10100
9 × 12 cm sheet	10	IPVH09120
8.5 × 13.5 cm sheet	10	IPVH08130
8 × 10 cm sheet	10	IPVH08100
7 × 8.4 cm sheet	50	IPVH07850

Immobilon®-P^{SQ} Membrane (0.2 µm pore size) for blotting applications of proteins with molecular weights less than 20,000

Size	Qty/Pk	Catalogue Number
26.5 × 375 cm roll	1	ISEQ00010
26 × 26 cm sheet	10	ISEQ26260
20 × 20 cm sheet	10	ISEQ20200
15 × 15 cm sheet	10	ISEQ15150
10 × 10 cm sheet	10	ISEQ10100
9 × 12 cm sheet	10	ISEQ09120
8.5 × 13.5 cm sheet	10	ISEQ08130
8 × 10 cm sheet	10	ISEQ08100
7 × 8.4 cm sheet	50	ISEQ07850

Immobilon®-FL Membrane (0.45 µm pore size) for fluorescence detection applications

Size	Qty/Pk	Catalogue Number
26.5 × 375 cm roll	1	IPFL00010
20 × 20 cm sheet	10	IPFL20200
10 × 10 cm sheet	10	IPFL10100
7 × 8.4 cm sheet	10	IPFL07810

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Technical Assistance

For more information, contact the office nearest you. In the U.S., call 1-800-221-1975. Outside the U.S., go to our web site at www.millipore.com/offices for up-to-date worldwide contact information. You can also visit the tech service page on our web site at www.millipore.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at www.millipore.com/terms ("Conditions of Sale").

PR04178, Rev. 04/15