Immobilon®-P Blotting Sandwiches

PVDF Transfer Membrane and Filter Paper for Western Blotting and Immunodetection of Proteins

Introduction

Immobilon-P Blotting Sandwiches consist of one sheet of Immobilon-P transfer membrane with a sheet of blotting filter paper on either side. The sandwiches are pre-cut and compatible with the most commonly used pre-cast electrophoresis gels.

NOTE: Pink paper separates the sandwiches from one another. Within each sandwich, blue paper protects the Immobilon-P membrane (Figure 1). Remove the pink and blue papers before performing western blot procedures.

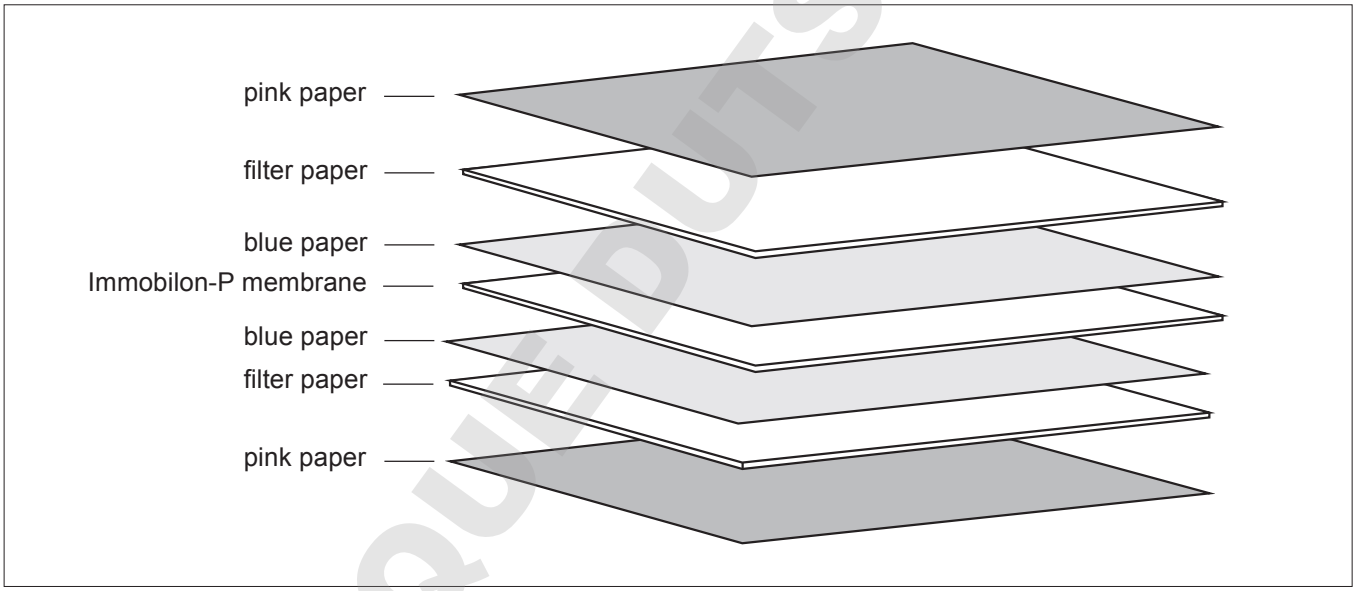

Figure 1. Configuration of Immobilon-P Blotting Sandwich

Immobilon-P transfer membrane is a polyvinylidene fluoride (PVDF) microporous membrane for binding proteins transferred from a variety of gel matrices. This membrane is hydrophobic and offers a uniformly controlled pore structure with a high binding capacity for biomolecules. Immobilon-P membrane has a nominal pore size of 0.45 µm and is useful for blotting proteins >10 kDa. The membrane and blotting paper are suitable for all chemiluminescent or chromogenic detection methods.

This insert provides a general protocol for immunodetection. The protocol should be optimized for your specific application. For detailed western blotting protocols, recommendations and troubleshooting, go to www.millipore.com/immunodetection.

Materials Recommended for Western Blotting

- Immobilon-P Blotting Sandwich
- Transfer buffer (such as 25 mM Tris-base, 192 mM glycine, 10% methanol)
- 100% methanol
- Milli-Q® water
- Wash buffer: Phosphate-buffered saline (PBS) or Tris-buffered saline (TBS) containing 0.05% Tween®-20 surfactant;
  PBS: 10 mM sodium phosphate, pH 7.2, 0.9% NaCl
  TBS: 10 mM Tris, pH 7.4, 0.9% NaCl
- Blocking buffer: 1–5% (w/v) blocking agent (BSA, casein, nonfat dry milk) in wash buffer

  NOTE: Some reagent manufacturers provide blocking solutions. Refer to their protocol, if applicable.
- Primary antibody (specific for the protein of interest), diluted in blocking buffer or wash buffer
- Secondary antibody (specific for the primary antibody) diluted in blocking buffer or wash buffer
- Substrate reagent appropriate for enzyme-conjugated secondary antibody

Protein Transfer Procedure

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. Separate the sheet of Immobilon-P transfer membrane from the two filter paper sheets. Notch or label one corner of the membrane to correspond to a corner of the gel.
4. Wet the Immobilon-P membrane in 100% methanol for 15 seconds. The membrane appearance will uniformly change color from opaque to semitransparent. Then transfer the membrane to a dish containing Milli-Q water for 2 minutes.

   CAUTION: Use care when handling the membrane to prevent tearing. Do not leave dry spots that can inhibit the transfer.
5. Equilibrate the membrane for at least 5 minutes in the transfer buffer.
6. Soak the sheets of filter paper in transfer buffer for at least 30 seconds.
7. Assemble the transfer stack as shown in Figure 2.

   CAUTION: To ensure an even transfer, remove air bubbles by carefully rolling a clean pipette over the surface of each layer in the stack. Applying excessive pressure may damage the gel and membrane.
8. Transfer proteins according to transfer apparatus instructions.
9. Remove the blot from the transfer system and briefly rinse the membrane in Milli-Q water to remove gel debris. The blot may be air dried for storage.
10. To visualize the transferred proteins, Immobilon-P membrane may be stained with any reversible blot stain compatible with immunodetection (for example, Ponceau-S Red, CPTS, Sypro® Ruby or Sypro Rose blot stains). Follow the reagent manufacturer's staining protocol.

    NOTE: Immobilon-P Blotting Sandwiches can be used in wet-tank and semi-dry electroblotting procedures. Semi-dry transfer requires additional sheets of filter paper.

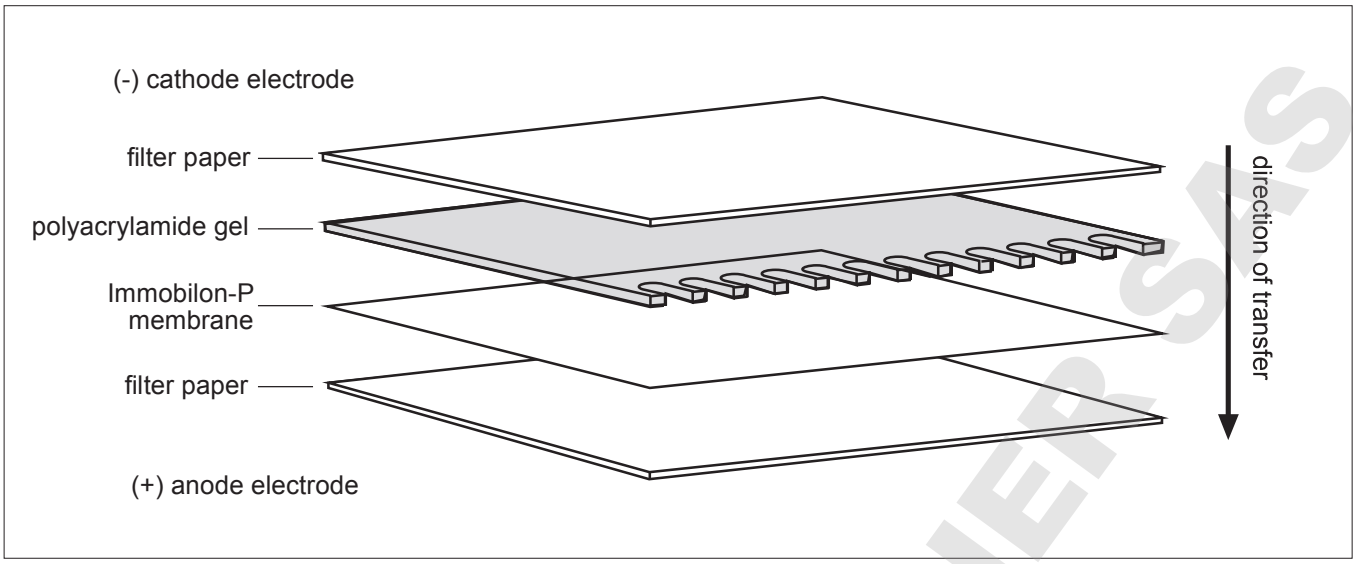

Figure 2. Transfer stack assembly for wet transfer

Immunodetection

The following is a general protocol for immunodetection. For optimal results, refer to the manufacturer's protocol provided with the immunodetection reagents.

1. Re-wet the dry blot in 100% methanol for 15 seconds. The blot appearance will uniformly change from opaque to semitransparent.
2. Place the blot in blocking buffer and incubate for 1 hour with gentle agitation. Prepare primary antibody solution.
3. Place the blot in diluted primary antibody solution and incubate for 1 hour with gentle agitation.
4. Wash the blot with wash buffer 3 times for 5 minutes each wash. Prepare secondary antibody solution.
5. Place the blot in diluted secondary antibody solution and incubate for 1 hour with gentle agitation.
6. Wash the blot with wash buffer 3 times for 5 minutes each wash.
7. Proceed with either chromogenic or chemiluminescent protein detection.

Product Ordering Information

Millipore Corporation offers Immobilon-P Blotting Sandwiches, Immobilon-P, Immobilon-FL, and Immobilon-P^{SQ} transfer membranes. See the Technical Assistance section for information about contacting Millipore Corporation.

Immobilon-P Blotting Sandwiches (0.45 µm pore size) for General Western Blotting Applications

Type	Size	Qty/Pk	Catalogue Number
Pre-cut PVDF Sandwiches	8.5 × 13.5 cm	20	IPSN 081 32
	7 × 8.4 cm	20	IPSN 078 52

Immobilon-P Membrane (0.45 µm pore size) for General Western Blotting Applications

Type	Size	Qty/Pk	Catalogue Number
Roll	26.5 × 375 cm	1	IPVH 000 10
Cut Sheet	26 × 26 cm	10	IPVH 304 F0
	20 × 20 cm	10	IPVH 202 00
	15 × 15 cm	10	IPVH 151 50
	10 × 10 cm	10	IPVH 101 00
	9 × 12 cm	10	IPVH 091 20
	8.5 × 13.5 cm	10	IPVH 081 30
	8 × 10 cm	10	IPVH 081 00
	7 × 8.4 cm	50	IPVH 078 50

Immobilon-FL Membrane (0.45 µm pore size) for Fluorescence Detection Applications

Type	Size	Qty/Pk	Catalogue Number
Roll	26.5 × 375 cm	1	IPFL 000 10
Cut Sheet	20 × 20 cm	10	IPFL 202 00
	10 × 10 cm	10	IPFL 101 00

Immobilon-P^{SQ} Membrane (0.2 µm pore size) for Blotting Applications of Proteins <20 kDa

Type	Size	Qty/Pk	Catalogue Number
Roll	26.5 × 375 cm	1	ISEQ 000 10
Cut Sheet	26 × 26 cm	10	ISEQ 262 60
	20 × 20 cm	10	ISEQ 202 00
	15 × 15 cm	10	ISEQ 151 50
	10 × 10 cm	10	ISEQ 101 00
	9 × 12 cm	10	ISEQ 091 20
	8.5 × 13.5 cm	10	ISEQ 081 30
	8 × 10 cm	10	ISEQ 081 00
	7 × 8.4 cm	50	ISEQ 078 50

Technical Assistance

For more information, contact the Millipore office nearest you. In the U.S., call **1-800-MILLIPORE** (1-800-645-5476). Outside the U.S., see your Millipore catalogue for the phone number of the office nearest you or 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.

Warranty

The applicable Millipore Warranty and limited liability for products listed in this publication may be found at www.millipore.com (search on “Terms and Conditions of Sale”).

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