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.

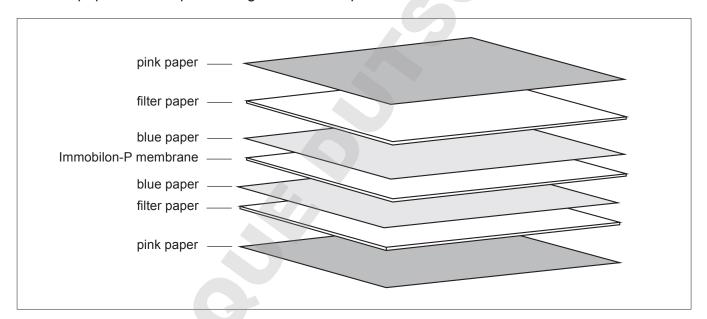


Figure 1. Configuration of Immobilion-P Blotting Sandwich

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

- Immobilion-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.

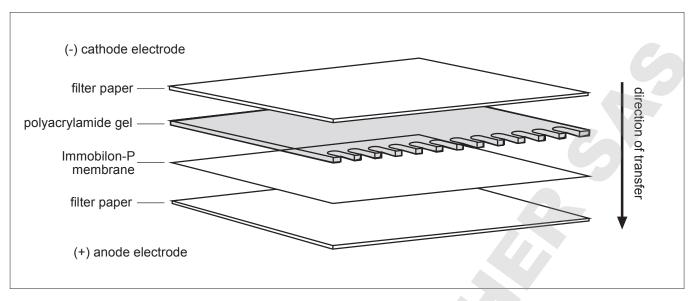


Figure 2. Transfer stack assembly for wet transfer

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

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

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

Туре	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

Туре	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

Туре	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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