

05317 Immobilon™-FL Transfer Membrane

Product Ordering Information, continued

Immobilon-P⁵⁰ 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.

Standard Warranty

Millipore Corporation ("Millipore") warrants its products will meet their applicable published specifications when used in accordance with their applicable instructions for a period of one year from shipment of the products. **MILLIPORE MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE.** The warranty provided herein and the data, specifications and descriptions of Millipore products appearing in Millipore's published catalogues and product literature may not be altered except by express written agreement signed by an officer of Millipore. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized and if given, should not be relied upon.

In the event of a breach of the foregoing warranty, Millipore's sole obligation shall be to repair or replace, at its option, the applicable product or part thereof, provided the customer notifies Millipore promptly of any such breach. If after exercising reasonable efforts, Millipore is unable to repair or replace the product or part, then Millipore shall refund to the customer all monies paid for such applicable product or part. **MILLIPORE SHALL NOT BE LIABLE FOR CONSEQUENTIAL, INCIDENTAL, SPECIAL OR ANY OTHER INDIRECT DAMAGES RESULTING FROM ECONOMIC LOSS OR PROPERTY DAMAGE SUSTAINED BY ANY CUSTOMER FROM THE USE OF ITS PRODUCTS.**

MILLIPORE

P36572, Rev. B, 05/06

© 2006 Millipore Corporation. Printed in U.S. All rights reserved.
Millipore and Milli-Q are registered trademarks of Millipore Corporation. Immobilon is a trademark of Millipore Corporation.
Sypro is a registered trademark of Molecular Probes, Inc.
Tween is a registered trademark of ICI Americas, Inc.

Immobilon™-FL Transfer Membrane

For Optimal Fluorescent Immunodetection

Introduction

The Millipore Immobilon-FL transfer membrane is a polyvinylidene fluoride (PVDF) microporous membrane for binding proteins that are 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. The membrane exhibits very low auto-fluorescence across a wide range of excitation/emission wavelengths. This property makes it ideal for blotting applications involving fluorescence-based immunodetection. In addition, Immobilon-FL membrane can be used for standard chemiluminescent or chromogenic detection.

The Immobilon-FL membrane has a nominal pore size of 0.45 µm and offers optimal blotting for proteins ≥20 kDa. Immobilon-FL membrane is compatible with standard blocking agents and buffers.

This insert provides a general protocol for immunodetection using Immobilon-FL membrane. The protocol should be optimized for your specific blotting application. Further information on related protocols, troubleshooting and background information on Western blotting, can be found in the "Protein Blotting Handbook" (publication number TP001). Request a free copy of the handbook at www.millipore.com/eblotting.

Materials Recommended for Western Blotting

- Immobilon-FL membrane cut to the dimensions of the gel
- Sheets of filter paper, cut to the dimension of the gel and soaked in a transfer buffer (such as 25 mM Tris-base, 192 mM glycine, 10% methanol) for at least 30 seconds
- 100% methanol
- Milli-Q® water
- Wash buffer: Phosphate-buffered saline (PBS) or Tris-buffered saline (TBS) containing 0.05-0.1% 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 imager and fluorescent reagent manufacturers also 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), labeled with a fluorescent dye of choice, 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-FL membrane in 100% methanol for 15 seconds. The membrane will uniformly change from opaque to semitransparent.

CAUTION: Use care when handling the membrane to prevent tearing. Do not leave dry spots that can inhibit the transfer.

4. Equilibrate the membrane for at least 5 minutes in the transfer buffer.
5. Assemble the transfer stack as shown in Figure 1.

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.

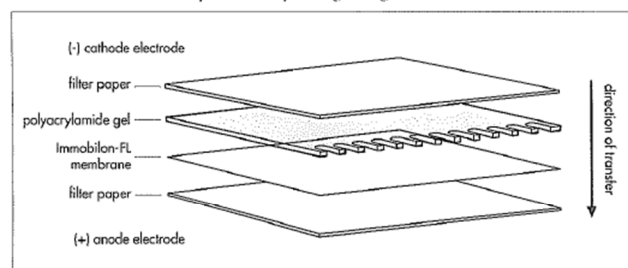


Figure 1. Transfer stack assembly for wet transfer

6. Transfer proteins according to transfer apparatus manufacturer's instructions.
7. 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.
8. To visualize the transferred proteins, Immobilon-FL 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).

Further information on related protocols, troubleshooting and background information on Western blotting, can be found in the "Protein Blotting Handbook" (publication number TP001). Request a free copy of the handbook at www.millipore.com/eblotting.

Immunodetection

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

NOTE: If using chemi-fluorescent reagents, follow the procedure below to step 4. Then follow reagent manufacturer's directions.

1. Re-wet the dry blot in 100% methanol for 15 seconds. The blot 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 3–5 times for 5 minutes each. Prepare secondary antibody solution.
5. Place the blot in diluted fluorescent dye-labeled secondary antibody solution and incubate for 1 hour with gentle agitation.
6. Wash the blot with wash buffer 3–5 times for 5 minutes each.
7. Place the blot onto a piece of clean filter paper to dry.
8. Image the blot using an appropriate fluorescence scanner.

Product Ordering Information

Immobilon-FL, Immobilon-P, and Immobilon-P[®] transfer membranes are offered by Millipore. See the Technical Assistance section for information about contacting Millipore Corporation.

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
	7 × 8.4 cm	10	IPFL 078 10

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

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

The vibrant M, Millipore, and Sigma-Aldrich are trademarks of Merck KGaA, Darmstadt, Germany or its affiliates. Detailed information on trademarks is available via publicly accessible resources.
© 2018 Merck KGaA, Darmstadt, Germany and/or its affiliates. All Rights Reserved.

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the US and Canada.

