

User Guide

Immobilon®-FL Transfer Membrane

For Optimal Fluorescent Immunodetection

Introduction

The Immobilon®-FL 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. The membrane exhibits very low autofluorescence 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 with molecular weights greater than 20 kilodaltons (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. For further information on related protocols, troubleshooting, and background information on Western blotting, go to SigmaAldrich.com/WesternBlotting.

Materials Recommended for Western Blotting

- Immobilon®-FL membrane cut to the dimensions of the gel.
- Sheets of filter paper, cut to the dimensions 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.
- Alcohol (>70% methanol, ethanol, or isopropanol) for wetting dry membrane.
- 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 (bovine serum albumin, casein, nonfat dry milk) in wash buffer.

NOTE: Some imager and fluorescent reagent manufacturers also provide blocking solutions. Refer to their protocols, 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

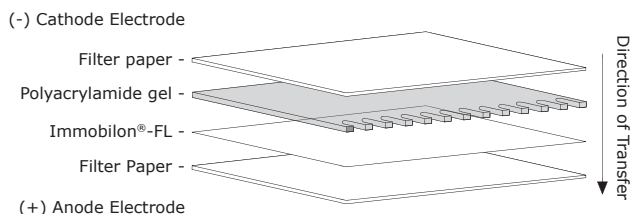
1. Resolve the protein mixture on a 1D or 2D polyacrylamide gel.
2. Immerse the gel in the transfer buffer and allow it to equilibrate for 10-15 minutes.
3. Wet the Immobilon®-FL membrane in alcohol (>70% methanol, ethanol, or isopropanol) for 15 seconds. The membrane will uniformly change from opaque to semi-transparent.
4. Immerse the membrane in Milli-Q® water for 1-2 minutes to displace the alcohol.

CAUTION: To prevent tearing, handle the membrane with care. Do not leave dry spots, as these may inhibit transfer.

5. Equilibrate the membrane for at least 5 minutes in the transfer buffer.
6. Assemble the transfer stack as shown on the next page.

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

- Transfer proteins according to transfer apparatus manufacturer's instructions.



- Remove the blot from the transfer system and rinse the membrane briefly in Milli-Q® water to remove gel debris. The blot may be air dried for storage.
- To visualize the transferred proteins, Immobilon®-FL membrane may be stained with any reversible blot stain compatible with immunodetection (e.g., Ponceau-S, CPTS, Sypro® Ruby or Sypro® Rose blot stains).

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 chemifluorescent reagents, follow the procedure below to step 4, then follow the reagent manufacturer's directions.

- Rewet the dry blot in alcohol (>70% methanol, ethanol, or isopropanol) for 15 seconds. The blot will uniformly change from opaque to semitransparent.
- Place the blot in blocking buffer and incubate for 1 hour with gentle agitation. Prepare primary antibody solution.
- Place the blot in diluted primary antibody solution and incubate for 1 hour with gentle agitation.
- Wash the blot with wash buffer 3–5 times for 5 minutes each. Prepare secondary antibody solution.
- Place the blot in diluted fluorescent dye-labeled secondary antibody solution and incubate for 1 hour with gentle agitation.
- Wash the blot with wash buffer 3–5 times for 5 minutes each.
- Place the blot onto a piece of clean filter paper to dry.
- Image the blot using an appropriate fluorescence scanner.

Product Ordering

Purchase products online at [SigmaAldrich.com/products](https://www.sigmaaldrich.com/products).

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

Size	Qty/Pk	Catalogue Number
8.5 cm × 1000 cm roll	1	IPFL85R
26.5 cm × 375 cm roll	1	IPFL00010
26.5 cm × 187.5 cm roll	1	IPFL00005
10 cm × 10 cm sheet	10	IPFL10100
7 cm × 8.4 cm sheet	10	IPFL07810

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

Size	Qty/Pk	Catalogue Number
8.5 cm × 1000 cm roll	1	IPVH85R
26.5 cm × 375 cm roll	1	IPVH00010
26.5 cm × 187.5 cm roll	1	IPVH00005
10 cm × 10 cm sheet	10	IPVH10100
9 cm × 12 cm sheet	10	IPVH09120
8.5 cm × 13.5 cm sheet	10	IPVH08130
8 cm × 10 cm sheet	10	IPVH08100
7 cm × 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 kDa

Size	Qty/Pk	Catalogue Number
8.5 cm × 1000 cm roll	1	ISEQ85R
26.5 cm × 375 cm roll	1	ISEQ00010
26.5 cm × 187.5 cm roll	1	ISEQ00005
9 cm × 12 cm sheet	10	ISEQ09120
8.5 cm × 13.5 cm sheet	10	ISEQ08130
8 cm × 10 cm sheet	10	ISEQ08100
7 cm × 8.4 cm sheet	50	ISEQ07850

Immobilon®-E Membrane (0.45 µm pore size) for Western Blotting Applications. No Alcohol Prewet Required.

Size	Qty/Pk	Catalogue Number
8.5 cm × 1000 cm roll	1	IEVH85R
26.5 cm × 187.5 cm roll	1	IEVH00005
26.5 cm × 187.5 cm roll	50	IEVH07850

Related products for General Western Blotting Applications

Description	Catalogue Number
Immobilon® NOW Dispenser for 8.5 cm x 1000 cm rolls	IMDISP
Immobilon® Block - CH (Chemiluminescence Blocker), 500 mL	WBAVDL01
Immobilon® blotting filter paper, 7 cm x 8.4 cm sheet, 100/pk	IBFP0785C
Immobilon® blotting filter paper, 8.5 cm x 13.5 cm sheet, 100/pk	IBFP0813C
Immobilon® Signal Enhancer for immunodetection, 500 mL	WBSH0500
Immobilon® Western HRP substrate, 100 mL	WBKLS0100
Immunoblot Blocking Reagent, 20 g	20-200
Immobilon® ECL Ultra Western HRP substrate, 100 mL	WBULS0100
Immobilon® Forte Western HRP substrate, 100 mL	WBLUF0100
Immobilon® Crescendo Western HRP substrate, 100 mL	WBLUR0100
Immobilon® Classico Western HRP substrate, 100 mL	WBLUC0100
Immobilon®-GO for Simple Immunodetection	IMGDV010
SNAP i.d.® 2.0 Protein Detection System-Mini	SNAP2MINI
SNAP i.d.® 2.0 Protein Detection System-Midi	SNAP2MIDI
SNAP i.d.® 2.0 Mini Blot Holders (7.5 cm x 8.4 cm)	SNAP2BHMN0100
SNAP i.d.® 2.0 Midi Blot Holders (8.5 cm x 13.5 cm)	SNAP2BHMD0100
Phosphate-buffered saline with 3% nonfat milk, pH 7.4, dry powder	P2194
Phosphate-buffered saline with Tween® 20 surfactant, pH 7.4, tablet	08057
Ponceau S solution, 0.1% (w/v) in 5% acetic acid, 1 L	P7170
Re-Blot™ Plus Strong Antibody Stripping solution, 10X, 50 mL (Chemicon®)	2504
TMB substrate, insoluble (Calbiochem®), 100 mL	613548
Tris-buffered saline with Tween® 20 surfactant, pH 7.6, tablet	91414
Tris-glycine buffer 10X Concentrate, 1 L	T4904-1L

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