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.

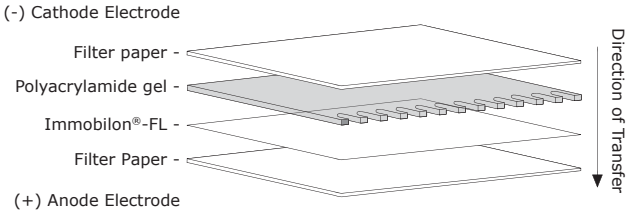
Protein Transfer

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.

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 air dried for storage.
9. 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.

1. Rewet the dry blot in alcohol (>70% methanol, ethanol, or isopropanol) 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

Purchase products online at 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	WBAVDFL01
Immobilon® blotting filter paper, 7 cm × 8.4 cm sheet, 100/pk	IBFP0785C
Immobilon® blotting filter paper, 8.5 cm × 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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