User Guide

Immobilon®-E Transfer Membrane

For High Sensitivity Immunodetection

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Introduction

Immobilon®-E transfer membrane is a polyvinylidene fluoride (PVDF) microporous membrane used for protein blotting applications. Unlike other PVDF transfer membranes, Immobilon®-E membrane does not require an alcohol pre-wet step prior to blotting. It can be wetted with standard transfer buffers. When compared to a nitrocellulose membrane, it has improved handling characteristics and staining capabilities, increased solvent resistance, and a higher signal-to-noise ratio for enhanced sensitivities.

The Immobilon®-E membrane has a nominal pore size of 0.45 micron (µm) and is optimal for blotting proteins with molecular weights greater than 20 kilodaltons (kDa) (Immobilon®-P^{SQ} membrane is optimal for proteins less than 20 kDa). It is an ideal substrate for immunodetection, since it is compatible with standard blocking agents and detection protocols, including chemiluminescence. This user guide provides basic protocols for electroblotting and rapid immunodetection.

For more information, refer to the Protein Blotting Handbook available at SigmaAldrich.com/WesternHelp.

Table 1. Immobilon-E® Membrane Properties and Applications

Composition	**PVDF**
Pore size	0.45 µm
Applications	Western immunoblot assays
Detection methods*	Chemiluminescent (Immobilon® HRP substrates) Chromogenic (TMB, Insoluble) Radioactive
Protein visualization methods	
Transillumination	n/a
Reversible Stains	Ponceau-S Sypro® blot stains
Irreversible Stains	Coomassie brilliant blue dye, Amino black, India black, Colloidal gold

*For fluorescence detection methods, low-autofluorescent Immobilon®-FL membrane is recommended.

Guidelines for Working with Immobilon®-E Membrane

- Always wear gloves when handling the membrane, in order to avoid fingerprints.
- Use blunt forceps to prevent membrane damage.
- Keep the patapar (blue paper) with the membrane during cutting or handling, but discard when wetting the membrane.
- Handle with care to avoid scratches on the membrane surface. Do not fold the membrane.
- Immobilon®-E membrane should be wetted with transfer buffer. It does not require an alcohol pre-wet step prior to blotting.
- If the membrane dries out after initial wetting, it must be wetted in an alcohol solution (> 50% methanol, ethanol, or isopropanol), rinsed in Milli-Q® water, then equilibrated in transfer buffer before use.
- After protein transfer, wash the blot with Milli-Q® water to eliminate any gel residues.
- Blots can be air dried and stored at 4 °C for several months (for later use) or they can be used immediately.

Materials Recommended for Western Blotting

- Immobilon®-E membrane cut to the dimensions of the gel
- Milli-Q® water
- Transfer buffer: 25 mM Tris-base, 192 mM glycine, pH 8.3, 10% alcohol (for tank transfer) or 48 mM Tris, 39 mM glycine, pH 9.2, 10% alcohol (for semi-dry transfer)
- Sheets of filter paper, cut to the dimensions of the gel and soaked in transfer buffer for at least 30 seconds
- Blocking buffer: Immobilon Block®-CH buffer (Cat. No. WBAVDCH01) or 0.5–5% (w/v) blocking agent (bovine serum albumin, casein, nonfat dry milk) in wash buffer

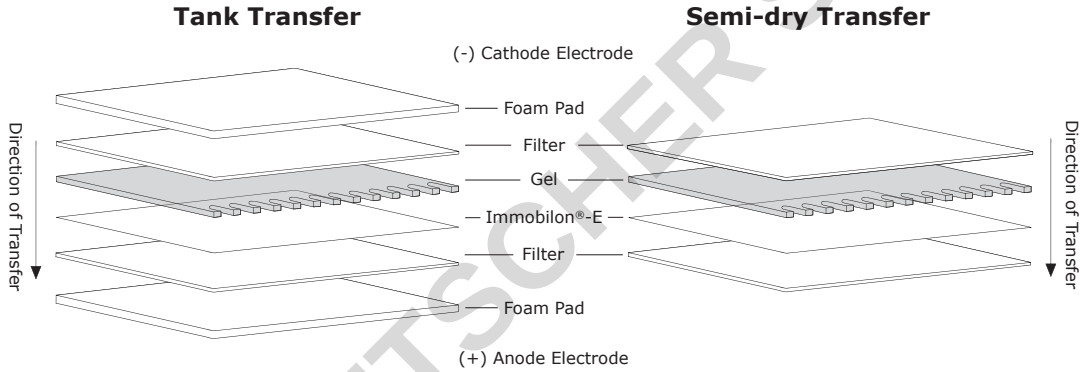
- Wash buffer: Phosphate-buffered saline (PBS) or Tris-buffered saline (TBS) containing 0.5–0.1% Tween® 20 surfactant (PBST or TBST)

  PBS: 10 mM sodium phosphate, pH 7.2, 0.9% NaCl
  TBS: 10 mM Tris, pH 7.4, 0.9% NaCl
- 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 detection enzyme (e.g., horseradish peroxidase [HRP] or alkaline phosphatase [AP]), diluted in blocking buffer or wash buffer

Protein Transfer

Proteins can be transferred to Immobilon®-E membrane by two common electro-transfer methods: tank and semi-dry transfer. Table 2 describes the general conditions and major differences for the two methods.

Table 2. Transfer Methods



Conditions	Membrane gel stack immersed in buffer	Filter paper soaked in buffer
Buffer volume	0.5 L or higher depending on system	~ 0.05 L per mini-gel
Transfer time	Slow (1 or more hours)	Fast (7–45 minutes)
Typical run	Constant voltage	Constant current
Gel equilibration time	Not necessary but recommended	Minimum of 15 minutes equilibration
Required buffer system	Continuous (single buffer)	Continuous (single buffer) or Discontinuous (3 buffers)
Typical continuous buffer name/ composition	Towbin buffer, pH 8.3 25 mM Tris, 192 mM glycine	Bjerrum-Shafer-Nielsen buffer, pH 9.2 48 mM Tris, 39 mM glycine
Typical discontinuous buffer name/ composition	N/A	Anode buffer I: 300 mM Tris, pH 10.4 Anode buffer II: 25 mM Tris, pH 10.4 Cathode buffer: 25 mM Tris, 40 mM amino-caproic acid, pH 9.4
% Alcohol*	10–20% alcohol	10–20% alcohol
% SDS*	0–0.05% SDS	0–0.05% SDS

* Alcohol (methanol, ethanol, or isopropanol) in the transfer buffer has two important functions; it stabilizes the gel dimensions and strips complexed sodium dodecyl sulfate (SDS) from protein molecules, improving protein binding to the membrane. However, for large proteins, or proteins that exhibit solubility problems, it is recommended that the alcohol concentration be decreased and that a small amount of SDS be added to the transfer buffer. This improves protein elution from the gel while maintaining protein solubility during the transfer process.

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Immunodetection

Immunodetection is an antibody-based method that allows the detection, identification, and quantitation of a protein or antigen in the blotting membrane. The typical protocol follows these six general steps:

1. Block unoccupied membrane sites to prevent nonspecific binding of antibodies.
2. Incubate the membrane with a primary antibody that binds to the protein of interest.
3. Wash to remove any unbound primary antibody.
4. Incubate the membrane with a conjugated secondary antibody, which binds to the first antibody.
5. Wash to remove any unbound secondary antibody.
6. Incubate the membrane with a substrate that reacts with the conjugated secondary antibody to reveal the location of the protein.

Standard immunodetection takes at least 4 hours and is widely used, but the SNAP i.d.® 2.0 Protein Detection System can perform the same process with significant time savings (Table 3).

Table 3. Comparison of Standard vs. SNAP i.d.® 2.0 Immunodetection

	Standard Immunodetection	SNAP i.d.® 2.0 Immunodetection
Block membrane	1 hour	10 seconds
Incubate with primary antibody	1 hour	10 minutes
Wash membrane	3 times, 5 minutes each	4 times, ~10 seconds each
Incubate with secondary antibody	1 hour	10 minutes
Wash membrane	3 times, 5 minutes each	4 times, ~10 seconds each
Total time	4 hours	22 minutes

Typical Protocols for Western Blotting and Immunodetection with Immobilon®-E Membrane

Membrane Wetting

Equilibrate the membrane in transfer buffer for 2–3 minutes or until ready to use.

CAUTION: Once the membrane has been wet out, do not allow it to dry out. It can be kept in buffer until protein transfer. If the membrane dries out (turns opaque white) even partially, it must be wetted with alcohol (>50% methanol, ethanol, or isopropanol), rinsed in Milli-Q® water, then equilibrated in transfer buffer.

Semi-dry 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. Assemble the transfer stack according to manufacturer's instructions for the transfer apparatus used.

   CAUTION: To ensure an even transfer, remove air bubbles by carefully rolling a clean pipette or blot roller over the surface of each layer in the stack. Do not apply excessive pressure, as this may damage the gel and membrane.
4. Transfer proteins according to transfer apparatus manufacturer's instructions.
5. 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, or it can be use immediately for the immunodetection step.

   NOTE: Drying the blot before immunodetection may enhance the binding of some proteins and reduce background noise.

Protein Visualization (Optional)

To visualize the protein transfer efficiency, Immobilon®-E membrane may be stained with any reversible blot stain compatible with immunodetection (e.g., Ponceau-S or Sypro® blot stains) or viewed by transillumination using a light box. For a list of reversible and nonreversible compatible stains and protocols, refer to the Protein Blotting Handbook available at SigmaAldrich.com/WesternHelp.

Immunodetection

The following is a general protocol for immunodetection with Immobilon®-E membrane. Some of the critical factors for obtaining a "perfect" Western blot (protein concentration, blocking solution, and antibody concentration) may require optimization.

Standard Immunodetection

1. If blot has been dried, rewet it in alcohol (>50% methanol, ethanol, or isopropanol) for 15 seconds or until it changes from opaque white to translucent gray.
2. Rinse the blot in Milli-Q® water for 1 minute.
3. Place the blot in blocking buffer and incubate for 1 hour with gentle agitation. Dilute the primary antibody in wash or blocking buffer.
4. Place the blot in diluted primary antibody solution and incubate for 1 hour with gentle agitation.

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5. Wash the blot with wash buffer (tris- or phosphate-buffered saline solution, supplemented with Tween® 20 surfactant (TBST or PBST)) 3–5 times for 5 minutes each wash. Prepare secondary antibody in wash or blocking buffer.
6. Place the blot in diluted enzyme-labeled secondary antibody solution and incubate for 1 hour with gentle agitation.
7. Wash the blot with wash buffer 3–5 times for 5 minutes each wash.
8. Place the blot into a clean container and add the appropriate detection reagent (HRP, AP, or chromogenic).
9. Incubate 1–5 minutes, according to the detection reagent manufacturer's instructions.
10. For HRP or AP chemiluminescent reagents, expose blot to x-ray film or acquire the image using a digital imaging system. For chromogenic detection, add the reagent and wait until signal appears.

SNAP i.d.® 2.0 Immunodetection Using Vacuum Filtration

1. If blot has been dried, rewet it in >50% alcohol (methanol, ethanol, or isopropanol) for 15 seconds, or until it changes from opaque white to translucent gray. Prepare all the required solutions and antibodies ahead of time.

   NOTE: Antibodies should be 3 to 5 times more concentrated than in standard immunodetection, but in volumes of 2.5–10 mL depending on the blot size/blot holder.
2. Wet the SNAP i.d.® blot holder in Milli-Q® water and assemble the blot with the protein side down.
3. Using the blot roller, remove all air bubbles and excess water, and insert the blot holder inside the SNAP i.d.® frame.
4. Block by adding 15–30 mL of blocking solution and immediately turn the vacuum on.
5. Depending on the blot holder size used, add 2.5–10 mL of diluted primary antibody and incubate for 10 minutes.
6. Turn vacuum on to flush the antibody, then with the vacuum still on, wash 4 times with 15–30 mL of wash buffer.
7. Turn vacuum off, add 2.5–10 mL (depending on the blot holder size used) of diluted secondary antibody, and incubate for 10 minutes.
8. Turn vacuum on to flush the antibody, then wash 4 times with 15–30 mL of wash buffer.
9. Remove the blot from the blot holder and continue with the detection method of choice (chemiluminescence or chromogenic).

Guidelines for Choosing an Immobilon® PVDF Membrane

The following table provides general guidelines for choosing the appropriate membrane for a specific post-Western blot application. Due to variations in protein properties such as charge density, conformation, and hydrophobicity, not all proteins behave the same way on a given membrane surface. Experiments with a variety of Immobilon® membranes may be necessary to optimize results for your specific application.

Application after Western blotting	Membrane of choice for most proteins
General immunodetection	Immobilon®-E or Immobilon®-P
Amino acid analysis	Immobilon®-P
Immunodetection of low molecular weight or low-abundance proteins	Immobilon®-P^{SQ}
Sequencing of low molecular weight or low-abundance proteins	Immobilon®-P^{SQ}
Fluorescence immunodetection and chemifluorescence methods	Immobilon®-FL

Safety Data Sheet

Safety Data Sheets (SDS) are available on our web site. Go to SigmaAldrich.com and enter your catalogue number in the search box.

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Product Ordering

Purchase products online at SigmaAldrich.com/products.

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

Size	Qty/Pk	Catalogue Number
8.5 cm × 1000 cm roll	1	IEVH85R
26.5 cm × 187.5 cm roll	1	IEVH00005
7 cm × 8.4 cm roll	4	IEVH07804
8 cm × 10 cm sheet	10	IEVH08100
9 cm × 12 cm sheet	10	IEVH09120
10 cm x 10 cm sheet	10	IEVH10100
Blotting Sandwich 7 cm x 8.4 cm	20	IESN07852
Blotting Sandwich 8.5 cm x 13.5 cm	20	IESN08132
7 cm x 8.4 cm sheet	50	IEVH07850

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

Description	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
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 kilodaltons (kDa)

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

Description	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

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Related Products for General Western Blotting Applications

Description	Catalogue Number
Immobilon® NOW Dispenser for 8.5 x 1000 cm rolls	IMDISP
Immobilon® Block Noise Cancelling Reagents for chemiluminescence detection, 500 mL	WBAVDCH01
Immobilon® blotting filter paper, 7 × 8.4 cm sheet, 100/pk	IBFP0785C
Immobilon® blotting filter paper, 8.5 × 13.5 cm sheet, 100/pk	IBFP0813C
Immobilon® ECL Ultra Western HRP substrate, 100 mL	WBULS0100
Immobilon® Signal Enhancer for immunodetection, 500 mL	WBSH0500
Immobilon® Western HRP substrate, 100 mL	WBKLS0100
Immunoblot Blocking Reagent, 20 g	20-200
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 Immunodetection, 10/pk	IMGDV010
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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