User Guide

Immobilon®-P^{SQ} Transfer Membrane

PVDF membrane for protein sequencing and immunodetection of proteins with molecular weights less than 20 kilodaltons (kDa)

Introduction

The Immobilon®-P^{SQ} membrane has a nominal pore size of 0.2 µm. It is a microporous polyvinylidene fluoride (PVDF) membrane that has been developed to maximize protein binding. This membrane is well-suited for direct protein sequencing and immunodetection following electroblotting of protein from electrophoresis gels (or dot binding of purified protein samples), especially for proteins with molecular weights less than 20 kilodaltons (kDa). Immobilon®-P^{SQ} membrane is compatible with tank and semi-dry electroblotting systems.

NOTE: If proteins in the molecular weight range of 10 to 20 kDa are to be electroblotted, Immobilon®-P^{SQ} and Immobilon®-P membranes should both be evaluated to identify the membrane that will offer optimum detection.

Membrane Wetting

The Immobilon®-P^{SQ} membrane is extremely hydrophobic and will not wet in aqueous solutions until prewetted with >70% methanol, ethanol, or isopropanol.

1. Wet the membrane in alcohol for 15 seconds. When wet, the membrane will change from an opaque white to a uniform, translucent gray.
2. Immerse the membrane in water for 1–2 minutes to displace the methanol. If the membrane floats on top of the water, push it into the water with forceps until it remains submerged.
3. Equilibrate the membrane in transfer buffer by soaking it in buffer for 5 minutes to displace the water. The membrane is now ready for blotting.

   CAUTION: Once the membrane has been wet with water, do not allow it to dry out until the proteins have been transferred to it. If the membrane dries out (turns opaque white) even partially, repeat steps 1 through 3.

Protein Blotting

In tank and semi-dry electroblotting, variables which may affect transfer efficiency include:

- Buffer composition, methanol concentration, and pH of transfer buffer.
- Amount of current used.
- Duration of the transfer blocking solutions.
- Size of the proteins.
- Thickness and density of the gel.

Optimize transfer conditions for each protein before proceeding with your blotting procedure.

1. After electrophoresis of the gel, equilibrate it for 10 minutes in transfer buffer (20 minutes for gels thicker than 0.75 mm).
2. Place the equilibrated gel in direct contact with the wet membrane, removing any air bubbles which may have formed between the gel and the membrane.
3. Place the membrane/gel sandwich in the electroblotting device. Follow the specified blotting procedures for the device used, based on your particular application.

Tips:

- To enhance protein binding of low molecular weight proteins (< 20 kDa), include 20–30% methanol in the anode buffer(s) and reduce the applied current.
- To enhance protein binding of high molecular weight proteins, extend the transfer duration and include low concentrations of sodium dodecyl sulfate (< 0.01% w/v) in the transfer buffer.

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Protein Detection

For Sequencing Procedures:

Stain blotted proteins directly with Coomassie brilliant blue or another dye that will not interfere with subsequent protein sequencing protocols. Destain the membrane using standard protocols that are appropriate for the stain used.

For Immunodetection Procedures:

Adjustments may need to be made to the protocol, such as increasing the concentration of blocking agent to compensate for the elevated binding capacity of Immobilon®-PSQ membrane compared with that of Immobilon®-P membrane.

Membrane Storage

If the membrane is not going to be stained immediately after protein transfer, it can be stored dry without any loss in performance.

1. Place the Immobilon®-PSQ membrane on filter paper and let it dry for one to two hours at room temperature.
2. Cover the membrane with plastic wrap and keep it in a cool, dark area.
3. Before continuing the staining/destaining and sequencing protocol, rewet the membrane using one of the following methods:
   - Place the membrane in 100% methanol, wash it with water, and then equilibrate it in the solvent used for staining.
   - Place the membrane directly into a staining solution that contains a minimum of 50% alcohol.

Product Ordering

Purchase products online at SigmaAldrich.com/products.

Immobilon®-PSQ 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 × 1,000 cm roll	1	ISEQ85R
26.5 cm × 375 cm roll	1	ISEQ00010
26.5 cm × 187.5 cm roll	1	ISEQ00005
10 cm × 10 cm sheet	10	ISEQ10100
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®-P Membrane (0.45 µm pore size) for General Western Blotting Applications

Size	Qty/Pk	Catalogue Number
8.5 cm × 1,000 cm roll	1	IPVH85R
26.5 cm × 375 cm roll	1	IPVH00010
26.5 cm × 187.5 cm roll	1	IPVH00005
8 cm × 10 cm sheet	10	IPVH08100
7 cm × 8.4 cm sheet	50	IPVH07850

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

Size	Qty/Pk	Catalogue Number
8.5 cm × 1,000 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®-E Membrane (0.45 µm pore size) for Western Blotting Applications. No Alcohol Prewet Required.

Size	Qty/Pk	Catalogue Number
8.5 cm × 1,000 cm roll	1	IEVH85R
26.5 cm × 187.5 cm roll	1	IEVH00005
26.5 cm × 187.5 cm roll	50	IEVH07850

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Related products for General Western Blotting Applications

Description	Catalogue Number
Immobilon® NOW Dispenser for 8.5 cm x 1,000 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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