User Guide

Immobilon®-P^{SQ} Transfer Membrane

PVDF membrane for protein sequencing and immunodetection of proteins with molecular weights less than 20,000

Introduction

The Immobilon®-P^{SQ} membrane is a 0.2 µm 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 20,000. Immobilon®-P^{SQ} membrane is compatible with tank and semi-dry electroblotting systems.

NOTE: If proteins in the molecular weight range of 10,000–20,000 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 100% methanol.

1. Wet the membrane in methanol 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
- Size of the proteins
- Thickness and density of the gel

Protein Blotting, continued

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 (< 20,000), 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.

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:

Refer to publication TP001EN, "Protein Blotting Handbook" (go to www.millipore.com and search on TP001EN). In the Protocols section, go to Immunodetection Protocols. 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®-P^{SQ} 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®-P^{SQ} 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% methanol.

Product Ordering Information

This section lists the catalogue numbers for Immobilon®-P^{SQ} and other Immobilon® membranes. See the Technical Assistance section for contact information. You can purchase these products on-line at www.millipore.com/products.

Immobilon®-P^{SQ} Membrane (0.2 µm pore size) for blotting applications of proteins with molecular weights less than 20,000

Size	Qty/Pk	Catalogue Number
26.5 × 375 cm roll	1	ISEQ00010
26.5 × 187.5 cm roll	1	ISEQ00005
26 × 26 cm sheet	10	ISEQ26260
20 × 20 cm sheet	10	ISEQ20200
15 × 15 cm sheet	10	ISEQ15150
10 × 10 cm sheet	10	ISEQ10100
9 × 12 cm sheet	10	ISEQ09120
8.5 × 13.5 cm sheet	10	ISEQ08130
8 × 10 cm sheet	10	ISEQ08100
7 × 8.4 cm sheet	50	ISEQ07850

Immobilon®-P Membrane (0.45 µm pore size) for general Western blotting applications

Size	Qty/Pk	Catalogue Number
26.5 × 375 cm roll	1	IPVH00010
26.5 × 187.5 cm roll	1	IPVH00005
26 × 26 cm sheet	10	IPVH304F0
20 × 20 cm sheet	10	IPVH20200
15 × 15 cm sheet	10	IPVH15150
10 × 10 cm sheet	10	IPVH10100
9 × 12 cm sheet	10	IPVH09120
8.5 × 13.5 cm sheet	10	IPVH08130
8 × 10 cm sheet	10	IPVH08100
7 × 8.4 cm sheet	50	IPVH07850

Immobilon®-FL Membrane (0.45 µm pore size) for fluorescence detection applications

Size	Qty/Pk	Catalogue Number
26.5 × 375 cm roll	1	IPFL00010
26.5 × 187.5 cm roll	1	IPFL00005
20 × 20 cm sheet	10	IPFL20200
10 × 10 cm sheet	10	IPFL10100
7 × 8.4 cm sheet	10	IPFL07810

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Technical Assistance

For more information, contact the office nearest you. In the U.S., call 1-800-645-5476. Outside the U.S., 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

The applicable warranty for the products listed in this publication may be found at www.millipore.com/terms ("Conditions of Sale").

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