Immun-Blot® PVDF Membrane for Protein Blotting

Instruction Manual

BIO-RAD

Bio-Rad Laboratories, 2000 Alfred Nobel Dr., Hercules, CA 94547

4006127 Rev A

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Section 1
Introduction

PVDF (polyvinylidene difluoride) membrane was originally introduced to protein use as an ideal medium for the harsh chemical environment of N-terminal, or Edman degradation, sequencing. The hydrophobicity of PVDF makes it an ideal support for binding proteins in electrophoretic and dot blotting applications. Because of the hydrophobic nature of PVDF, it does require a prewetting step in alcohol. The high protein binding capacity, target retention, and resistance to cracking has made PVDF an appealing membrane for general lab techniques.

The two main applications for PVDF are N-Terminal Sequencing and Immunoblotting, both of which benefit from the qualities of PVDF but in different aspects of the membrane. While sequencing work is concerned with retaining as

much protein as possible, a western blot requires good signal retention with very low background. In order to provide the best possible membrane for each technique, Bio-Rad offers two grades of PVDF, Immun-Blot PVDF for western blotting, and Sequi-Blot PVDF for protein sequencing.

Immun-Blot PVDF

The purpose of a blotting membrane is to deliver good signal results while resisting background and non-specific binding. Immun-Blot PVDF Membrane is ideal for chemiluminescent and colorimetric western blots because it retains target protein very strongly, 140–150 µg protein/cm^2 membrane, but resists background that can obscure high sensitivity detection. Immun-Blot PVDF membrane retains proteins in any transfer format; tank blotting, semi-dry blotting, and dot blotting all deliver excellent results. For proteins that are difficult to transfer, 0.1% SDS

can be added to the transfer buffer without affecting the binding of the protein to PVDF. The results are consistently clean, easy to read blots. The physical strength of Immun-Blot PVDF membrane means that it will not crack or tear in common handling, and holds up under repeated strip and reprobing applications.

Section 2
Membrane Wetting

Immun-Blot PVDF membrane can be used as a direct replacement for the membrane currently being used in your blotting protocol. No changes are required in the procedure, but the special steps given below are required to prepare the membrane for blotting. The hydrophobicity of PVDF makes it impossible to wet the membrane with aqueous solutions. Methanol or an alternative organic solvent is required to prewet the membrane prior to equilibration in

transfer buffer. After equilibration, the membrane can be used in a semi-dry, tank, or capillary blotting system with any acidic or basic blotting buffer.

Note: Always handle membranes using gloves or forceps to prevent contamination.

1. Immerse the membrane in 100% methanol for a few seconds, until the entire membrane is translucent. In methanol, it wets immediately. (Solutions containing 50% methanol concentration can be used to prewet the membrane.)
2. Transfer the wetted membrane to a vessel containing transfer buffer or water. Incubate in buffer until it is equilibrated (² 2–3 minutes). The membrane will float on the surface of the buffer until completely equilibrated. After it is equilibrated it can be easily submerged into the aqueous solution. At this point, the membrane is ready to bind proteins in any blotting application.
3. After the membrane has been wetted with buffer, do not allow it to dry (white spots will form where the membrane is dry). Protein will not bind to the dried membrane, and dry spots will not rewet in aqueous solutions. If the membrane becomes dry prior to blotting, repeat steps 1 and 2 to rewet it.

Section 3
Dot Blotting

Dot blotting requires special precautions to insure that protein is bound to the PVDF membrane before it dries. Directly spot protein to the wetted membrane and allow it to dry. Vacuum assisted dot or slot blotting is not recommended, due to the potential for the PVDF to dry out before the proteins are bound.

Section 4
Electrophoretic Blotting

Immun-Blot PVDF membrane can be used with a variety of transfer equipment, including Bio-Rad's Trans-Blot cell, Mini Trans-Blot cell, and Trans-Blot SD Semi Dry cell. In general, tank blotting is more quantitative with higher binding yields than semi dry transfers. However, very good results can be obtained when a semi dry apparatus is used with a well optimized gel and transfer system. The semi dry transfer is much faster, with transfer times as low as 15 minutes versus tank blotting which requires 1 to 3 hours for transfers.

1. For most proteins, use a Towbin buffer[1] with methanol (MeOH).
2. For proteins that resist transfer out of the gel, up to 0.1% SDS can be added to the transfer buffer. This stabilizes the effect of methanol in stripping SDS off the proteins.

SDS imparts a net negative charge to the proteins and helps facilitate movement out of the gel. Binding of proteins to Immun-Blot PVDF membrane is not affected by the addition of this amount of SDS.

3. PVDF should be clean and free of wrinkles.
4. Wet the membrane following the protocol in the membrane wetting section.
5. Make sure there are no bubbles between the membrane and the gel.
6. After transfer, rinse the membrane three times (5 minutes each) with distilled water.

Solutions

Towbin buffer:

25 mM Tris	3.03 g
192 M glycine	14.4 g
20% methanol	200 ml

Adjust volume to 1 liter with dd H_2O. Prechill the buffer before use.

Towbin/SDS buffer:

Towbin buffer as above with addition of up to 0.1% SDS

Note: Do not add acid or base to adjust pH. The buffer will range from pH 8.1 to 8.5, depending on the quality of Tris, glycine, dd H_2O, and methanol. Methanol should be analytical reagent grade, as metallic contaminants in low grade methanol will plate on the electrodes.

Section 5
Optimization of Blotting Conditions

Immun-Blot PVDF membrane is a very versatile blotting membrane. It can be used with any of the blotting procedures that are used for immunospecific antigen detection, or total protein staining. Use the protocol developed for your lab. If you do not have a standard protocol, most blotting reagent kits will have a good protocol. Bio-Rad's Immun-Blot colorimetric blotting kits, or Immun-Star™ chemiluminescent kits have excellent blotting protocols.

To minimize non-specific background, the blocking step and antibody titers are very important optimization steps. For blocking, we recommend casein or non-fat dry milk. Typical concentrations are from 0.2% to 5%. Antibody dilution factors vary by the manufacture of the antibody, the specificity, and the purity. To optimize blot conditions, cut the PVDF into strips, wet the membrane, and spot the test protein directly to the membrane. Use different blocking concentrations and antibody titers to test the outcomes of these different conditions. Once the blocking and antibody conditions are set, you should have good signal to noise results on the real electrophoretic blots.

Note: The membrane can be stored after proteins have been transferred, but prior to immunoblotting. Store it dry and rewet it when needed. To dry the membrane, place it on filter paper and let it dry at room temperature for a few hours. When you are ready to analyze the blotted samples, rewet the membrane by placing it in 100% methanol or into a staining solution that contains at least 50% MeOH.

In the U.S., technical service is available by calling 1-800-4BIORAD (1-800-424-6723). Our Technical Service representatives are available to answer your questions from 8 AM to 5 PM (PST).

Section 6
References

1. Towbin, H., Staehelin, T. and Gordon, J., *Proc. Natl. Acad. Sci. USA*, **76,** 4350-4354 (1979).

Section 7
Product Information

Catalog Number	Product Description
	Immun-Blot PVDF Membrane
162-0174	**7 x 8.4 cm,** 10 sheets
162-0175	**10 x 15 cm,** 10 sheets
162-0176	**20 x 20 cm,** 10 sheets
162-0177	**26 cm x 3.3 m,** 1 roll
	Blotting Equipment
170-3910	**Trans-Blot Electrophoretic Transfer Cell**
170-3946	**Trans-Blot Cell with Plate Electrodes**
170-3930	**Mini Trans-Blot® Cell**
170-3940	**Trans-Blot SD Semi-Dry Transfer Cell**

Catalog Number	Product Description

Blotting Reagents

161-0305	**Prestained SDS-PAGE Standards,** low range, 500 µl
161-0309	**Prestained SDS-PAGE Standards,** high range, 500 µl
161-0318	**Prestained SDS-PAGE Standards,** broad range, 500 µl
161-0324	**Kaleidoscope Prestained Standards,** 500 µl
170-6527	**Colloidal Gold Total Protein Stain,** 500 ml

Related Blotting Detection Products

Immun-Blot colorimetric detection kits
Premixed Substrate Reagents for Colorimetric Detection
Immun-Star chemiluminescent detection kits

In the US, call Bio-Rad at 1-800-4BIO-RAD (1-800-424-6723), or contact your local office for more information on Bio-Rad blotting equipment and reagents.