Application-Specific PVDF Membranes: Immun-Blot® PVDF Membrane for Western Blotting and Sequi-Blot™ PVDF Membrane for Protein Sequencing

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Introduction

PVDF membrane was originally introduced to protein use as an ideal medium for the harsh chemical environment of N-terminal, or Edman degradation, sequencing. Even though PVDF is very hydrophobic and requires a prewetting step in alcohol, its high protein binding capacity, target retention, and resistance to cracking have made it an appealing membrane for general laboratory techniques.

The two main applications for PVDF are N-terminal sequencing and immunoblotting, both of which benefit from the qualities of PVDF but rely on different features 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. To provide the best membrane for each technique, Bio-Rad offers two grades of PVDF: Immun-Blot PVDF membrane for western blotting and Sequi-Blot PVDF membrane for protein sequencing.

Immun-Blot PVDF Membrane

The focus with a blotting membrane is on how well it delivers signal while resisting background and nonspecific binding. Immun-Blot PVDF membrane is ideal for chemiluminescent (Figure 1) and colorimetric (Figure 2) western blots because it very strongly retains target protein (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, up to 0.1% SDS can be added to the transfer buffer without affecting the binding of the proteins 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 during common handling, and will hold up under repeated stripping and reprobing applications.

Fig. 1. High signal-to-noise detection using Immun-Blot PVDF membrane. Serially diluted protein transferred to Immun-Blot PVDF membrane was detected using the Immun-Star™ HRP chemiluminescent detection kit and imaged using the VersaDoc™ 3000 system.

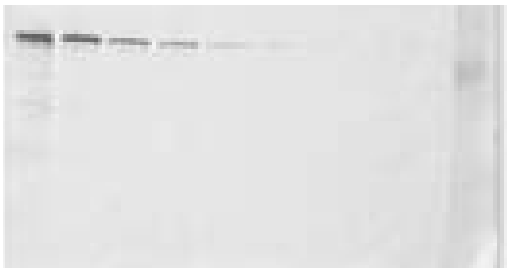


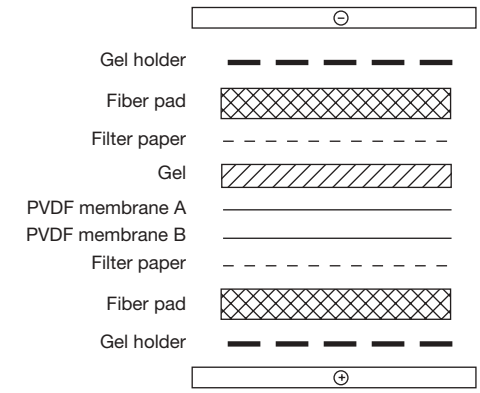
Fig. 2. Low-background results using Immun-Blot PVDF membrane. Protein was transferred to Immun-Blot PVDF membrane and detected with 4CN for low-background results.

Sequi-Blot PVDF Membrane

For protein sequencing applications, Sequi-Blot PVDF membrane is the best choice due to its extremely high protein binding capacity of 170–200 µg/cm^2. This is the original Bio-Rad PVDF membrane, designed to withstand the conditions of N-terminal sequencing while providing the binding capacity to sequence even low-abundance samples. The table shows the increased protein recovery possible with Sequi-Blot PVDF membrane. Recovery of blotted proteins is typically in the range of 80–100% of the initial sample load, resulting in higher initial coupling yields.

The binding efficiency of Sequi-Blot PVDF membrane is illustrated in Figure 3. This experiment showed that Sequi-Blot PVDF membrane is able to retain proteins that transfer through a competitor's product.

A



B

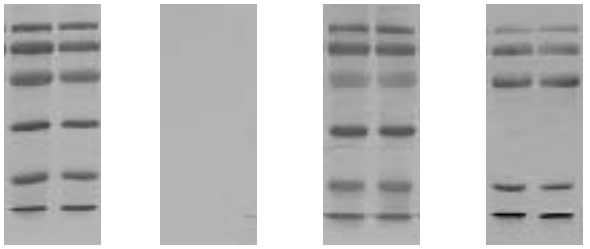


Fig. 3. Superior retention of proteins by Sequi-Blot PVDF membrane. Protein samples were blotted to Sequi-Blot PVDF membrane and a competitor's PVDF membranes using Towbin buffer containing 0.1% SDS. Panel A, illustration of assembled blotting membranes. Membrane A is the one next to the gel. Membrane B is the second membrane, which was layered behind the first. Panel B, all transferred protein was retained by the first Sequi-Blot membrane, while a large percentage of protein transferred through the first membrane of the competitor's product.

	pmol Sequenced	
AA Residue	Sequi-Blot	Competitor's
1. G	45.7	25.0
2. L	38.2	20.0
3. S	8.6	2.8
4. D	39.0	17.0
5. G	29.0	14.0
6. E	32.0	13.0
7. W	12.0	3.3
8. Q	19.8	8.1
9. Q	21.6	8.0
10. V	16.0	6.6
11. L	21.0	9.0
12. N	20.5	7.5
13. V	15.5	5.6
14. W	3.4	0.9
15. G	17.6	7.1
Initial coupling	45.5	22.6
Repetitive yield	93.6	91.7

Table. Analysis of horse apomyoglobin.

Protein (2 mg or 117 pmol) was loaded into wells of an SDS-PAGE gel. After electrophoresis, part of the gel was covered with Sequi-Blot PVDF membrane while the other part was overlaid with a competitor's PVDF membrane. Following blotting, protein bands were identified by Coomassie Brilliant Blue R-250 staining, cut from the blot, and sequenced.

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Bibliography

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Ordering Information

Catalog #	Description
Immun-Blot PVDF Membranes for Western Blotting	
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
162-0255	25 x 28 cm, 10 sheets
162-0177	26 cm x 3.3 m roll, 1
Immun-Blot PVDF/Filter Paper Sandwiches	
162-0238	13.5 x 8.5 cm, 20 pack
162-0239	13.5 x 8.5 cm, 50 pack
162-0218	7 x 8.5 cm, 20 pack
162-0219	7 x 8.5 cm, 50 pack
Sequi-Blot PVDF Membranes for Protein Sequencing	
162-0186	7 x 8.4 cm, 10 sheets
162-0180	10 x 15 cm, 10 sheets
162-0181	15 x 15 cm, 10 sheets
162-0182	20 x 20 cm, 10 sheets
162-0256	25 x 28 cm, 10 sheets
162-0184	26 cm x 3.3 m roll, 1
Sequi-Blot PVDF/Filter Paper Sandwiches	
162-0236	13.5 x 8.5 cm, 20 pack
162-0237	13.5 x 8.5 cm, 50 pack
162-0216	7 x 8.5 cm, 20 pack
162-0217	7 x 8.5 cm, 50 pack

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