PVDF Membrane, 0.22 μm

Introduction

Polyvinylidene fluoride (PVDF) membrane is a commonly used solid support in Western blotting. PVDF membrane is highly hydrophobic, mechanically strong, and chemically stable. It is resistant to a variety of organic solvents and tolerates a certain range of acidic and alkaline conditions, making it suitable for protein transfer and subsequent multiple antibody stripping and reprobing experiments. The general protein binding capacity of PVDF membrane is approximately 0.15–0.20 mg/cm^2 (varies slightly among brands and models), which is generally higher than that of nitrocellulose (NC) membranes, providing higher protein retention and detection sensitivity. PVDF membranes are commonly used in protein transfer, amino acid analysis, glycoprotein staining, and slot blot applications.

Due to its hydrophobic nature, the pores of PVDF membrane in the dry state are filled with air, which prevents aqueous transfer buffers from entering. Therefore, it requires activation with low-surface-tension organic solvents such as methanol or ethanol before use. The alcohol solvent displaces the air in the pores, causing the membrane to change from opaque to translucent, indicating complete wetting. The membrane is then rinsed with water to remove residual alcohol and equilibrated in transfer buffer. During electrophoretic transfer, proteins migrate from the gel onto the PVDF membrane surface under the driving force of an electric field. They are tightly adsorbed onto the membrane through hydrophobic interactions between their hydrophobic regions and the PVDF backbone, as well as other non-covalent forces such as van der Waals forces and hydrogen bonds, thereby achieving efficient transfer and immobilization of proteins from the gel to the membrane support.

The membrane is available in two pore sizes, 0.22 μm and 0.45 μm, which are recommended for small proteins (< 20 kDa) and routine proteins (> 20 kDa), respectively. The 0.22 μm membrane has an internal surface area approximately three times that of the 0.45 μm membrane, offering higher retention and stronger binding for small proteins, though the background may be slightly higher.

Components and Storage

Components	Size 1	Size 2	Size 3
PVDF Membrane, 0.22 μm	1 roll (30 cm x 300 cm)	20 sheets (6.6 cm x 8.5 cm)	100 sheets (6.6 cm x 8.5 cm)
Shipping: Room temperature	**Shelf life: 2 years at 10°C to 25°C**		

Protocol

1. Membrane Preparation (Activation and Equilibration)

   a. Cutting: Cut the PVDF membrane to the same size as the gel, and clip a small notch in one corner of the membrane as an orientation marker.

   b. Activation: Immerse the membrane in 100% methanol for 15 seconds, ensuring the entire membrane is completely soaked with no dry areas remaining.

   c. Washing: Transfer the membrane to deionized water and rinse for approximately 1–2 minutes to remove residual alcohol.

   d. Equilibration: Place the membrane into sufficient transfer buffer and equilibrate at room temperature for 15–20 minutes to allow the membrane to fully adapt to the transfer system.

2. Transfer Sandwich Assembly (Wet Transfer)

   Assemble the transfer "sandwich" in the following order from anode to cathode:

(+) Anode

Filter paper (2–3 layers, pre-soaked with transfer buffer)

PVDF membrane (equilibrated)

Gel (equilibratedfor 30 minutes)

Filter paper (2–3 layers, pre-soaked with transfer buffer)

(-) Cathode

*Note: After placing each layer, gently roll over with a glass rod or roller to thoroughly expel any air bubbles between layers; trapped bubbles will cause localized transfer failure.

3. Electrotransfer

   a. Place the assembled transfer sandwich into the electrotransfer tank and add sufficient transfer buffer.

   b. Connect the electrodes and perform the transfer at a constant current of 0.8 mA/cm^2 (based on gel area) for 45–90 minutes.

   c. After transfer is complete, disconnect the electrodes, disassemble the transfer sandwich, and carefully remove the PVDF membrane with forceps.

***Note:** Transfer time and efficiency vary depending on polyacrylamide concentration, gel thickness, presence or absence of SDS and methanol, pH and ionic strength of transfer buffer, and molecular weight of the target protein. Optimal transfer conditions should be determined experimentally. In general, larger proteins (> 100 kDa), higher percentage gels, or thicker gels require extended transfer time; SDS facilitates migration of large proteins from the gel, but high concentrations may reduce protein binding efficiency to the membrane; methanol promotes protein binding to the membrane but simultaneously shrinks gel pores, hindering migration of large proteins; the Tris-Gly buffer system at pH 8.3 is routinely used for transfer. Transfer conditions should be optimized according to the specific experimental system.

4. Post-transfer Processing

a. Immediately after removing the membrane, place it into blocking buffer and block for 1 hour at room temperature or overnight at 4°C to block non-specific binding sites.

b. Proceed with antibody incubation, washing, and signal detection.

***Note:** Keep the membrane wet at all times from completion of transfer until use; avoid drying.

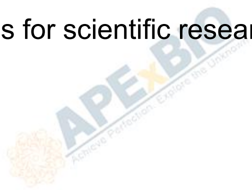
5. Subsequent Processing and Storage of Membrane

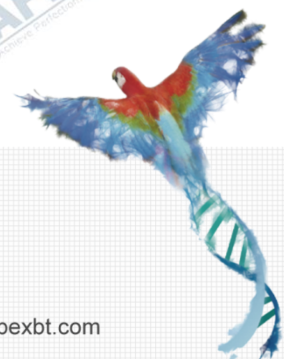
After transfer, the membrane can be immersed in PBS or TBS and stored at 4°C for several days. For stripping and reprobing of PVDF membranes, the use of commercially available stripping buffer is recommended. PVDF membranes can withstand multiple rounds of stripping and reprobing.

Note

1. The recommended storage and transport conditions for this product are temperature 10 to 25°C, humidity 30% RH to 75% RH; transport at room temperature.

2. This product is for scientific research use only.





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