

# PVDF Membrane (0.45 $\mu\text{m}$ )

E801

Version 24.1



## Product Description

PVDF Membrane (0.45  $\mu\text{m}$ ) is a type of pore size 0.45  $\mu\text{m}$  polyvinylidene fluoride (PVDF) blotting membrane. This membrane is hydrophobic and offers a uniformly controlled pore structure with a high binding capacity for biomolecules. PVDF membrane is mainly used for immunoblotting (e.g. western blot), which can transfer proteins from various gel matrices to membranes, with higher adsorption rates for proteins with molecular weight >20 kDa.

## Components

Components	E801-01	E801-02
PVDF Membrane (0.45 $\mu\text{m}$ )	27.5 cm $\times$ 3.75 m	90 mm $\times$ 60 mm, 50 sheets

## Storage

Store at 15 ~ 25°C and ship at room temperature.

## Applications

It is not only applicable for western blot protein transfer, but also applicable for amino acid analysis, glycoprotein color development, lipid polysaccharides analysis, N-terminal protein sequencing, dot hybridization, and slot blot detection, etc.

## Notes

1. This product requires pre-treatment with anhydrous methanol for 30 sec - 1 min before use, activating the positively charged groups on the membrane, making it easier to bind with negatively charged proteins and avoid leaving the dry spots.
2. After activating the membrane with methanol, equilibrate it in the transfer buffer for 30 sec - 1 min to avoid residual methanol affecting the transfer effect.
3. Wear a lab coat and disposable gloves for protection.

## Experiment Process

### Step 1: Protein transfer procedure

▲ Please determine the buffer concentration according to the specific experiment, and it is recommended to conduct a preliminary experiments to find the optimal concentration.

Transfer buffer: 25 mM Tris base, 192 mM glycine, 20% methanol.

Wash buffer: TBS or PBS containing 0.1% Tween-20.

TBS: 18.5 mM Tris base, pH 7.5, 0.9% NaCl.

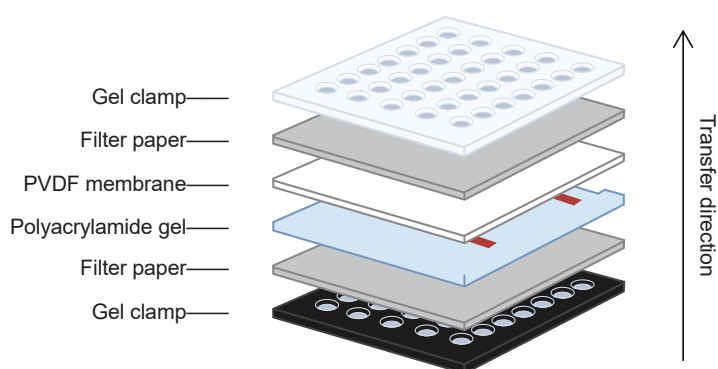
PBS: 10 mM phosphate salt, pH 7.4, 0.9% NaCl.

Blocking buffer: 0.5% - 5% (W/V) blocking buffer (BSA, casein, non - fat powdered milk) in wash buffer.

Table 1. Common usage methods for blocking buffer

Name	Application	Usage ratio
Bovine Serum Albumin (BSA)	Commonly used in the blocking step of western blot	
Casein	Used for the blocking step of routine proteins in western blot, not applicable for blocking phosphorylated proteins	0.5% - 5% (W/V)
Non - fat powdered milk		

1. After the electrophoresis, immerse the gel in the transfer buffer and allow it to equilibrate for 10 - 15 min.
2. Activate the PVDF membrane in methanol for 30 sec - 1 min. The membrane appearance will uniformly change color from white to semitransparent gray.
3. Equilibrate the membrane in the transfer buffer for 30 sec - 1 min to avoid residual methanol affecting the transfer effect.
  - ▲ Carefully handle the membrane with tweezers to avoid damage and contamination. Do not leave dry spots that can inhibit the transfer.
4. According to the following image or based on the transfer device instructions for transfer assembly.



### Step 2: Immune detection procedure

▲ The experimental system can be optimized according to the actual situation.

1. After the transfer, remove the PVDF membrane from the transfer system, briefly wash the membrane with wash buffer, then place it in blocking buffer and incubate on a shaker for 1 h at room temperature. Dilute the primary antibody with wash buffer or blocking buffer to prepare the working solution of the primary antibody.
2. Place the blotting membrane in diluted primary antibody solution, incubate on a shaker for 2 h at room temperature or overnight at 4°C.
3. Wash the membrane with wash buffer 3 - 5 times for 5 min each wash, dilute the secondary antibody with wash buffer or blocking buffer to prepare the working solution of secondary antibody.
4. Place the blotting membrane in diluted secondary antibody solution, incubate on a shaker for 1 h at room temperature.
5. Wash the membrane with wash buffer 3 - 5 times for 10 min each wash.
6. Remove the blotting membrane with flat tweezers, and drain the washing solution on the filter paper. Evenly drop the ECL working solution onto the blotting membrane to ensure uniform coverage, and incubate at room temperature for 30 sec - 1 min. Perform an X-ray film exposure or directly follow the instructions for the CCD imager.
  - ▲ The appropriate ECL luminescence detection kit can be selected according to the abundance of the target protein. It is recommended to choose SuperFemto ECL Chemiluminescence Kit (Vazyme #E423) for detecting low abundance protein targets, SuperPico ECL Chemiluminescence Kit (Vazyme #E422) for detecting conventional protein targets.

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