Print

PVDF Western Blotting Membranes

Cat. No. 03 010 040 001 1 roll, 30 cm × 3.00 m

Version 08
Content version: January 2014

1. Product Overview

Material
Microporous polyvinylidene difluoride (PVDF) membrane.

Pore size
0.2 µm

Phobicity
Hydrophobic

Primary binding mechanism
Electrostatic, hydrophobic

Protein binding capacity

Adsorption of:	
goat IgG	294 µg/cm²
BSA	131 µg/cm²
Insulin	85 µg/cm²

Application
The PVDF membrane is an ideal medium for
- western blotting

and is an excellent solid support for other analytical techniques ranging from
- dot/slot blotting
- blotting from 2 D gels
- protein sequencing
- HIV detection
- hybridoma screening
- cell blotting

The high binding capacity, high mechanical strength, and chemical resistance of the PVDF membrane makes it especially useful for protein blotting.

The combination of strong sample retention with low background binding generates excellent signal-to-noise ratios with chromogenic and radioactive detection techniques. The membrane is also ideal for chemiluminescent substrates.

Tested for the use with Lumi-Light Western Blotting Substrates*.

Storage/Stability
The membrane is stable at +15 to +25°C until the expiration date printed on the label.

2. Procedures and Required Material

2.1 Before You Begin

Handling Instructions
To avoid damage or contamination of the membrane, always wear gloves when handling.
The PVDF membrane is extremely hydrophobic and will not wet in aqueous solution unless pre-wet with methanol.

2.2 Membrane Wetting

Additional solutions required
Methanol

Procedure
Membrane wetting can be done in three steps:
Note: The membrane must not be allowed to dry during any of the steps. If drying occurs, even partially (where it has dried it will become opaque), repeat steps 1 to 3.

❶	Moisten the membrane in methanol for a few seconds (1–3 s). The color will change from an opaque white to a uniform translucent gray.
❷	Incubate the membrane in water for 1 or 2 min to elute the methanol.
❸	Soak the membrane in transfer buffer for a few minutes to displace the water. The membrane is now ready for blotting.

2.3 Electrophoresis and Blotting

Handling Instructions
Carry out electrophoresis. Gels should be incubated for 15 – 20 min in transfer buffer. The transfer buffer may contain up to 20% methanol.
- Place the membrane in contact with the gel. Remove air bubbles carefully from between gel and membrane. Place the membrane/gel into the electroblotting device.
- Ideal transfer conditions should be determined for each protein system. The protein transfer is influenced by, for example, the pH of the transfer buffer, the amount of methanol in the transfer buffer, the amount of current used to transfer the protein, and the amount of time the current is applied.

Transfer of Molecular Weight Markers
The transfer of high molecular weight proteins works best without methanol, while transfer of low molecular weight proteins works best with methanol.

2.4 Protein Detection

Protein Staining

- Proteins can be stained with dyes such as Coomassie blue, Amido black, India ink, or Ponceau S.
- If the membrane will not be stained immediately, it can be stored dry.

Re-wetting of the membranes can be done by one of the following methods:

1) Place the membrane directly into the staining solution that contains a minimum of 50% methanol, or
2) Place the membrane in methanol, wash with double dist. water, and incubate it in the solution used for staining.

- The membrane can be destained using standard protocols appropriate for the stain used. Coomassie blue and Amido black can be destained in destaining solutions with high methanol concentrations.

Immunostaining

To prevent nonspecific binding of antibody, incubate the membrane with a suitable blocking reagent (*e.g.*, bovine serum albumin, non-fat dry milk or Western Blocking reagent*).

If the membrane will not be immuno-stained immediately, it can be air dried before or after blocking.

Before staining, moisten the PVDF membrane in methanol for a few seconds, wash in double dist. Water, and then soak with transfer buffer for 3 min.

The other method for re-wetting the membrane is to incubate it in buffered saline solution containing 0.5% Tween® 20 (v/v) for 15 to 30 min.

For more detailed information, please read the Instructions for Use of the corresponding chemiluminescence or chromogenic (Western) blotting kits.

3. Supplementary Information

3.1 Text Conventions

To make information consistent and memorable, the following text conventions are used in this document.

Text conventions	Use
Numbered Instructions labeled ❶, ❷, etc.	Steps in a procedure that must be performed in the order listed.
Asterisk *	Denotes a product available from Roche Applied Science.

Symbols

In this document, the following symbols are used to highlight important information.

Symbol	Description
	Information Note: Additional information about the current topic or procedure.

3.2 Changes to Previous Version

- Change of the pore size of the membrane

3.3 Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our homepage http://www.roche-applied-science.com.

Product	Pack size	Cat. No.
BM Chemiluminescence Western Blotting Substrate (POD)	1 set (1,000 cm^2 membrane) 1 set (4,000 cm^2 membrane)	11 500 708 001 11 500 694 001
BM Chemiluminescence Western Blotting Kit (Mouse/Rabbit)	1 kit (2,000 cm^2 membrane)	11 520 709 001
BM Blue POD Substrate, precipitating (TMB ready-to-use solution)	100 ml	11 442 066 001
BM Purple AP substrate, precipitating (NBT/BCIP ready-to-use solution)	100 ml	11 442 074 001
Western Blocking Reagent	100 ml (10 blots, 100 cm^2) 6 × 100 ml (60 blots, 100 cm^2)	11 921 673 001 11 921 681 001
Lumi-Light Western Blotting Substrate	400 ml (4,000 cm^2 membrane)	12 015 200 001
Lumi-Light^{PLUS} Western Blotting Substrate	100 ml (1,000 cm^2 membrane)	12 015 196 001
Lumi-Light^{PLUS} Western Blotting Kit (Mouse/Rabbit)	1 kit (1,000 cm^2 membrane)	12 015 218 001

3.4 Trademarks

3.5 Disclaimer

For life science research only. Not for use in diagnostic procedures.

Contact and Support

To ask questions, solve problems, suggest enhancements and report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit the Roche Applied Science homepage, **www.roche-applied-science.com**, and select your home country. Country-specific contact information will be displayed.

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