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

PVDF Western Blotting Membranes

Version: 10
Content Version: April 2021

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

Store the product at +15 to +25°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	PVDF Western Blotting Membranes	▪ Microporous polyvinylidene difluoride (PVDF) membrane. ▪ Surface properties: electrostatic, hydrophobic ▪ Pore size 0.2 µm	1 roll, 30 cm × 3.00 m

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +15 to +25°C, the product is stable through the expiry date printed on the label.

Vial / bottle	Label	Storage
1	PVDF Western Blotting Membranes	Store at +15 to +25°C.

1.3. Additional Equipment and Reagent required

For pre-wetting of membrane

- Methanol
- Double-distilled water
- Transfer buffer

For protein staining

- Coomassie blue, Amido black, India ink, or Ponceau S
- Methanol
- Double-distilled water

For immunostaining

- Blocking reagent, such as BSA, non-fat dry milk, or Western Blocking Reagent*
- Methanol
- Double-distilled water
- Transfer buffer
- Buffered saline solution containing 0.5% Tween 20* (optional)

1.4. Application

The PVDF membrane is an ideal medium for western blotting and is an excellent solid support for other analytical techniques:

- Dot/slot blotting
- Blotting from 2D gels
- Protein sequencing
- HIV detection
- Hybridoma screening
- Cell blotting
- 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.*

2. How to Use this Product

2.1. Before you Begin

General Considerations

Membrane handling

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

Protein binding capacity

Protein	Binding Capacity [µg/cm²]
Goat IgG	294
BSA	131
Insulin	85

2.2. Protocols

Pre-wetting of membrane

⚠ ***The membrane must not be allowed to dry (becomes opaque) during any of the steps. If any drying occurs, repeat Steps 1 to 3.***

1. Moisten the membrane in methanol for 1 to 3 seconds.
   – The color will change from an opaque white to a uniform translucent gray.

2. Incubate the membrane in water for 1 to 2 minutes to elute the methanol.

3. Soak the membrane in transfer buffer for a few minutes to displace the water.
   – The membrane is now ready for blotting.

Electrophoresis and blotting

1. Perform electrophoresis according to standard protocols.
   – Incubate gels for 15 to 20 minutes in transfer buffer.
   ℹ *The transfer buffer may contain up to 20% methanol.*

2. Place the membrane in contact with the gel.
   – Carefully remove air bubbles from between the gel and membrane.

3. Place the membrane/gel into the electroblotting device.

4. Determine the ideal transfer conditions for each protein system.
   ℹ *Protein transfer is influenced by, for example, the pH of the transfer buffer, amount of methanol in the transfer buffer, amount of current used to transfer the protein, and the amount of time the current is applied.*
   ⚠ ***The transfer of high molecular weight proteins works best without methanol, while transfer of low molecular weight proteins works best with methanol.***

Protein staining

Proteins can be stained with dyes, such as Coomassie blue, Amido black, India ink, or Ponceau S. If the membrane is not stained immediately, it can be stored dry. Re-wetting 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-distilled water, and incubate it in the solution used for staining.

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

Immunostaining

1. To prevent nonspecific binding of antibody, incubate the membrane with a suitable blocking reagent, such as bovine serum albumin, non-fat dry milk, or Western Blocking Reagent*.
   – If the membrane is not immediately immunostained, it can be air dried before or after blocking.

2. Before staining, moisten the PVDF membrane in methanol for several seconds, wash in double-distilled, water, then soak with transfer buffer for 3 minutes.
   – Alternatively, re-wet the membrane by incubating it in buffered saline solution* containing 0.5% Tween 20* (v/v) for 15 to 30 minutes.
   For more detailed information, see the Instructions for Use of the corresponding chemiluminescence or chromogenic western blotting kits.*

3. Supplementary Information

3.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

3.2. Changes to previous version

Layout changes.
Editorial changes.

3.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Tween 20	50 ml, 5 x 10 ml	11 332 465 001
Western Blocking Reagent, Solution	100 ml, 10 blots, 100 cm^2	11 921 673 001
	6 x 100 ml, 60 blots, 100 cm^2	11 921 681 001
Lumi-Light Western Blotting Substrate	1 kit, 4,000 cm^2 membrane, 400 blots with 10 x 10 cm	12 015 200 001
BM Chemiluminescence Western Blotting Substrate (POD)	1 set, 1,000 cm^2 membrane (trays), 6,250 cm^2 membrane (transparent plastic bags)	11 500 708 001
	1 set, 4,000 cm^2 membrane (trays), 25,000 cm^2 membrane (transparent plastic bags)	11 500 694 001
BM Chemiluminescence Western Blotting Kit (Mouse/Rabbit)	1 kit, 2,000 cm^2 membrane (trays), 12,500 cm^2 membrane (transparent plastic bags)	11 520 709 001
BM Purple	100 ml	11 442 074 001

3.4. Trademarks

All product names and trademarks are the property of their respective owners.

3.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

3.6. Regulatory Disclaimer

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

3.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

3.8. Contact and Support

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

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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