Instructions for use

ROTI®PVDF and ROTI®Fluoro PVDF

Transfer Membrane

T830, 8989 and 2803 / 2831

ROTI®PVDF, ROTI®PVDF 0.2 and ROTI®Fluoro PVDF are hydrophobic, microporous polyvinylidene membranes, free from adjuvants and detergents. These membranes enable efficient transfer of proteins over a wide molecular weight range.

When compared to a nitrocellulose membrane, ROTI®PVDF, ROTI®PVDF 0.2 and ROTI®Fluoro PVDF show improved handling characteristics, staining capabilities and increased solvent resistance. They provide you with an ideal substrate for immunodetection.

ROTI®PVDF has a pore size of 0.45 µm, ROTI®PVDF 0.2 and ROTI®Fluoro PVDF have a pore size of 0.2 µm. The mechanically rigid transfer membranes can easily be cut to the desired gel size and remain flexible and tear-proof even when dry.

Please note: The two sides of the membranes may deviate slightly in appearance as a result of the production process. This, however, is of no significance to and in no way impairs blot efficiency. The binding properties are identical on both sides of the membranes.

Applications:
Western blot and Far-Western analysis, Dot blot, lipo-polysaccharide- and amino acid analysis, binding assays, immunoblotting, N-terminal peptide sequencing, analysis of small peptides

Detection systems:

ROTI®PVDF (T830):	Radioactivity, chemoluminescence, colour
ROTI®PVDF 0.2 (8989):	Radioactivity, chemoluminescence, colour
ROTI®Fluoro PVDF (2803/2831):	Fluorescence, radioactivity, colour (limited)

Transfer protocols

Semi-Dry Transfer

- Place the gel in cathode buffer (ROTI®Blot K or transfer buffer) and agitate gently for 15 min.
  ROTI®Blot A+K are available as a kit named ROTI®Blot1 (L509.1).
- Cut blotting paper (A125.1) and membrane to the exact size of the gel to be blotted. You will need 8 sheets of paper and one sheet of the membrane for the blot.
- Take four sheets of blotting paper and soak in the cathode buffer (ROTI®Blot K or transfer buffer).
- Take four sheets blotting paper and soak in the anode buffer (ROTI®Blot A or transfer buffer).
  ROTI®Blot K and ROTI®Blot A can be purchased as a set: ROTIBlot1 (L509.1).
- Wet the PVDF membrane in 100 % methanol for approx. 15 seconds. Make sure that the membrane is completely wetted and does not show any dry spots.
  Please note, very important: FluoroPVDF membranes must not be kept in 100% methanol for longer than 20 s. Incubation in methanol for longer than 20 s will damage these specially coated membranes, and they will develop a high and unwanted autofluorescence.
- Transfer the membrane into dd$H_2$0 for 1-2 minutes.
- Equilibrate the membrane for at least 3-5 minutes in anode buffer (ROTI®Blot A or transfer buffer).
- Place the anode buffer-soaked blotting papers onto the anode plate of the blotting unit. Make sure that all the pieces are lined up thoroughly.
- Place the blotting membrane on top of the blotting papers.

- Remove the gel from the blotting buffer and place on top of the blotting membrane, being careful not to introduce any air bubbles.
- Finally, place the cathode buffer-soaked blotting paper on top of the gel.
- Again, make sure that all the pieces are lined up squarely.
- Using a roller gently remove any air bubbles from the stack.
- Place the cathode plate on top of the stack.
- Connect the power leads.
- The blotter should run at 0.8 mA per centimeter squared of gel, e.g. an 8 x 10 cm gel will run at 64 mA for 1 to 2 hours. The actual run time will depend on the size of the molecules to be transferred and the transfer unit used. Large proteins and long nucleic acids will need at least 2 hours, smaller molecules less.
- Follow the instructions given by the manufacturer of you transfer unit.

Tank Transfer

- Cool (and degas) the appropriate volume of transfer buffer (see below).
- Half fill the tank with transfer buffer.
- Pre-equilibrate the gel for 15-30 minutes in cool transfer buffer to remove SDS and salts. This serves to prevent the gel changing size during transfer.
- Cut blotting paper (A125.1) and membrane to the exact size of the gel to be blotted. You will need 2 sheets of paper and one sheet of the membrane for the blot.
- Soak the blotting papers in transfer buffer.
- Wet the PVDF membrane in 100 % methanol for approx. 15 seconds.
- Please note: FluoroPVDF membranes must not be kept in 100% methanol for longer than 20 s. Incubation in methanol for longer than 20 s will damage these specially coated membranes, and they will develop a high and unwanted autofluorescence.
- Wash membrane in ddH_20 for 1-2 minutes.
- Equilibrate the membrane for at least 3-5 minutes in the transfer buffer.
- Soak the fiber pads in transfer buffer.
- The complete assembly of the sandwich should be done in a tray filled with transfer buffer. This helps to avoid trapped air bubbles. Carefully align and overlay the transfer membrane onto the gel in one smooth action. Work from the centre and let the ends progressively roll down. Try to avoid repositioning the membrane as some transfer may occur on contact. Use a clean glass rod wetted in transfer buffer to roll out any trapped bubbles.
- Assemble in the order (in the tray):

  Cathode side
  1 pre-soaked foam pad.
  1 pre-soaked piece of blotting paper.
  Gel
  Membrane (ROTI®PVDF/Fluoro PVDF)
  1 pre-soaked piece of blotting paper.
  1 pre-soaked foam pad.
  Anode side
- Finally, gently squeeze the cassette sides together and fix them.
- Insert the cassette into the tank with the transfer membrane on the anode side.
- Add further transfer buffer until the top loops of the platinum coils and the cassettes are covered.
- Follow the instructions given by the manufacturer of your transfer unit.
- Monitor the temperature as the run proceeds.

Blotting buffers

The most commonly used transfer buffers are:

25 mM Tris, 192 mM glycine, 0-10 % methanol, pH approx. 8.3 (Acc. to Towbin *et al.*, 1979)
48 mM Tris, 39 mM glycine, 0-10 % methanol, pH approx. 9.2 (Acc. to Bjerrum and Schafer-Nielsen, 1986)
10 mM $NaHCO_3$, 3 mM $NaCO_3$, 0-10 % methanol, pH approx. 9.9 (Acc. to Dunn, 1986)

Check, but do **not alter pH values** by adding HCl or NaOH. Addition of acid or base to the buffer will result in higher conductivity, thus often leading to increased formation of heat as well as ions and, subsequently resulting in brown, “burnt” looking blotting paper as well as damage to the electrode plates.

Please note:
Transfer of proteins onto PVDF membranes eliminates the need for alcohol in the buffer used. Presence of methanol is only recommended for keeping the size of the gel constant during transfer, an effect that is particularly important if gradient gels are blotted. We recommend use of 10 % methanol in maximum for PVDF membranes, since methanol may reduce protein movement from the gel by decreasing the gel pore size. PVDF membranes need SDS in order to guarantee optimal absorption quality. The buffers mentioned should be supplemented with SDS in a final concentration of 0.01-0.1 %. Standard concentration is 0.04 % SDS.
Electrophoretical transfer parameters depend on the buffers and units used. Please see instructions given by the manufacturers of the blotters.
For further data on transfer buffers and technical tips for protein transfer to membranes we recommend our **Technical Information Brochure** “Transfer buffers for tank and semi-dry blotting”

Speedy immune detection

Following protocol describes a speedy immune detection method which was developed specially for the PVDF-membrane (Art. No. T830.1). Detecting dry membrane eliminates the need for the blocking step and part of the washing steps, so that analysis is complete in 2.5 hours. Suitable for chromogen and chemiluminescent detection.
Preparation: Dry membrane after blot at 37 °C (approx. 1 hour) or at room temperature (RT) (approx. 2 hours). The membrane must be completely dry.

- Incubate in primary antibody (diluted in blocking solution, e.g. 1 x ROTI®Block, Art. No. A151.1) while gently shaking, 1 hour RT. Attention: The antibody solution must cover the membrane.
- Wash 3 times in PBS* for 5 minutes each time, RT
- Incubate in secondary antibody (diluted in blocking solution) while gently shaking, 30 minutes, RT
- Wash 3 times in PBS* for 5 minutes, RT
- Detection of immuno-complexes with chromogen (NBT, Art. No. 4421.1, BCIP Art. No. 6368.1) or chemoluminescent detection (ROTI-Lumin, Art. No. P078.1).

The protocol is not recommended for the detection of very low protein quantities.
*Phosphate Buffered Saline: ROTI®Stock 10 x PBS (Art. Nr. 1058.1), 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4)

Staining methods

CAUTION: If Coomassie brilliant blue R, amido black, or Ponceau S stain will be used to visualize the proteins and the blot is dry, re-wet it in 100% methanol before staining it.

Ponceau S

Staining with Ponceau S is reversible and does not interfere with subsequent immunodetection methods.
A) Staining of NC membranes: 0.2-2 % Ponceau S in 3 % trichloroacetic acid (Art. No. 8789.1) + 3 % sulphosalicylic acid (Art. No. 4119.1), incubate for 1-5 mins. at room temperature. Enhance contrast by incubation in water acidified by a few drops acetic acid.
B) Staining of PVDF membranes: 0.1-1 % Ponceau S in 10 % acetic acid (Art. No. 6755.1), incubate 1-5 mins. at room temperature. Ponceau S stained bands on PVDF only appear bright and distinct after drying, drying, however, is not recommended if Western blotting shall be performed.
Prior to Western blot analysis decolourise in PBS (Art. No. 1058.1) or TBS (Art. No. 1060) for 10-20 mins. at room temperature.

Coomassie brilliant blue

NOTE: Staining with ROTI®Blue or Brilliant Blue G, respectively, is not reversible. For reversible staining please use staining with ROTIPHORESE® Blue R (Brilliant Blue R) or Ponceau S.

- ✔ Incubate the blot in a solution of 0.1 % Coomassie brilliant blue R, 50 % methanol and 7 % acetic acid for 2 minutes. We recommend use of ROTI®Blue for irreversible and ROTIPHORESE® Blue R for reversible staining of membranes.
- ✔ Destain the blot as necessary in 50 % methanol and 7 % acetic acid for 10 minutes.

- Incubate the blot in 90 % methanol and 10 % acetic acid for 10 minutes to completely destain the background.

Amido black

CAUTION: This stain is not reversible. Follow the steps in the "Ponceau S" section for a reversible technique.

- Incubate the blot in a solution of 0.1 % amido black, 25 % isopropanol and 10 % acetic acid for 10 minutes.
- Destain the blot in the same buffer without amido black for 5 to 10 minutes.

Additionally Recommended Products:

For further package sizes and products please see our catalogue or online at www.carl.roth.com

ROTI®Blot 1 10 x Transfer Buffer for Semi-Dry Blotting		L509.1
Tris, Blotting Grade		0188.1
Glycin, Blotting Grade		0079.1
Methanol, Blotting Grade		0082.1
SDS, Blotting Grade		0183.1
ROTI®Stock 10 x PBS, 1 Litre		1058.1
ROTI®Stock 10 x PBST, 1 Litre		1059.1
ROTI®Stock 10 x TBS, 1 Litre		1060.1
ROTI®Stock 10 x TBST, 1 Litre		1061.1
ROTIPHORESE®- Blue R, 2 x concentrated		3074.1
ROTIPHORESE®- Blue, 5 x conc.		A152.1
Brilliant Blue R 250		3862.1
Brilliant Blue G 250		9598.1
Ponceau S (C.I. 27195)		5938.1
Amido black 10 B (C.I. 20470)		9590.1
ROTIlabo®Blotting paper		
0.18 mm	10 x 13 cm, 100 sheets	CL68.1
	20 x 20 cm, 100 sheets	CL69.1
	46 x 57 cm, 100 sheets	CL70.1
	58 x 60 cm, 100 sheets	CL71.1
0.36 mm	10 x 13 cm, 100 sheets	CL64.1
	20 x 20 cm, 100 sheets	CL65.1
	46 x 57 cm, 100 sheets	CL66.1
	58 x 60 cm, 100 sheets	CL67.1
0.75 mm	10 x 13 cm, 100 sheets	0942.1
	20 x 20 cm, 100 sheets	0943.1
	58 x 60 cm, 100 sheets	0945.1
1.0 mm	15 x 15 cm, 25 sheets	CL72.1
	20 x 20 cm, 25 sheets	CL73.1
	58 x 60 cm, 25 sheets	CL74.1
1.5 mm	58 x 60 cm, 25 sheets	CL75.1

ROTI®PVDF	Roll (26.5 cm x 3.75 m)	**T830.1**
ROTI®PVDF 0.2	Roll (30 cm x 3 m)	**8989.1**
ROTI®Fluoro PVDF	Roll (26.0 cm x 3.30 m)	**2803.1**
	2 sheets (20 cm x 13 cm)	**2831.1**

Carl Roth GmbH + Co. KG
Schoemperlenstraße 3-5 • 76185 Karlsruhe
P.O. Box 100121 • 76231 Karlsruhe
Phone: +49 (0) 721/ 5606-0
Fax: +49 (0) 721/ 5606-149
info@carlroth.com • www.carlroth.com
gh 02/2021