invitrogen™

Nitrocellulose/Filter Paper Sandwich (0.45 μm and 0.2 μm)

Catalog nos. LC2006 (0.45 μm), LC2009 (0.2 μm) **Store at room temperature**

Instructions for Use

Description

The Nitrocellulose/Filter Paper Sandwich includes a high quality membrane ideal for blotting proteins from E-PAGE™ Gels or similar dimension gels.

The major features of the Nitrocellulose/Filter Paper Sandwich are:

- Composed of 100% pure nitrocellulose to provide high-quality transfer with low background
- Contains no support fabric or detergents
- Compatible with commonly used transfer conditions and detection methods such as staining, immunodetection, fluorescence, or radiolabeling
- Supplied in a pre-cut, pre-assembled membrane/filter paper sandwich to fit E-PAGE™ gels

Brief instructions are included in this manual to perform semi-dry blotting of E-PAGE™ *48* and *96* gels using the Nitrocellulose/Filter Paper Sandwich. For details and other blotting protocols, refer to the E-PAGE™ Technical Guide available for downloading from www.invitrogen.com or contact Technical Service.

Specifications

Quantity:	16 membrane/filter paper sandwiches (sufficient for 16 transfers)
Pore Size:	0.45 μm (efficient transfer of >20 kDa proteins)
	0.2 μm (efficient transfer of <20 kDa proteins)
Membrane Dimensions:	8.6 cm x 13.5 cm (Filter paper: 8.6 cm x 13. 5 cm)
Membrane Media:	100% pure nitrocellulose
Binding Capacity:	80 μg/cm^2 of proteins
Binding Interaction:	Hydrophobic and electrostatic
Re-probe Characteristics:	No

Materials Needed

- NuPAGE® Transfer Buffer (20X) (cat. no. NP0006)
- NuPAGE® Antioxidant (cat. no. NP0005)
- Semi-dry blotter that can accommodate an E-PAGE™ Gel
- E-PAGE™ Blotting Pad (Supplied with E-PAGE™ Gels or available separately from Invitrogen: cat. no. LC2101)
- 6 pieces of Blotting Filter paper (cat. no. LC2008 or equivalent)
- Blotting Roller (cat no. LC2100)
- Methanol

Preparing 2X Transfer Buffer

We recommend using the NuPAGE® Transfer Buffer with 15% methanol and NuPAGE® Antioxidant for optimal transfer of proteins from E-PAGE™ Gels.

Prepare 500 ml of 2X NuPAGE® Transfer Buffer as follows:

NuPAGE® Transfer Buffer (20X)	50 ml
NuPAGE® Antioxidant	0.5 ml
Methanol	75 ml
Deionized Water	to 500 ml

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Instructions for Use, Continued

Equilibrating the Gel

Equilibration of the gel in transfer buffer results in removal of salts that may increase conductivity and heat during transfer. Perform equilibration for the recommended time, as longer equilibration results in protein diffusion.

1. After electrophoresis, remove the gel from the cassette as described in the E-PAGE™ manual.
2. Using a gel knife, trim off the top and bottom electrode areas of the gel.
3. Equilibrate the E-PAGE™ Gel in 200 ml 2X NuPAGE® Transfer Buffer (see previous page) for 30 minutes with shaking.

Preparing Blotting Membrane

Nitrocellulose Membranes (0.45 µm and 0.2 µm)

In a clean container, soak the membrane in 2X NuPAGE® Transfer Buffer (see previous page) for several minutes.

Semi-Dry Blotting Protocol

1. In a clean container, soak 6 pieces of 2.5 mm Blotting Filter paper (8.6 cm x 13.5 cm) in 2X NuPAGE® Transfer Buffer (see previous page). Remove any air bubbles trapped between filter paper sheets using the Blotting Roller while the paper is still submerged in buffer.
2. In a clean container, soak the E-PAGE™ Blotting Pad in 2X NuPAGE® Transfer Buffer (see previous page). Press the pad to ensure the elimination of any visible air bubbles. Inspect both sides of the Blotting Pad for air bubbles before use.
3. Place 3 pieces of pre-soaked Blotting Filter paper from Step 1 on the anode plate of a semi-dry blotting apparatus. Ensure that all filter paper sheets are aligned properly and remove any air bubbles with the Blotting Roller.
4. Place the pre-soaked blotting membrane on top of the filter paper stack and remove any air bubbles with the Blotting Roller.
5. Remove the gel from the transfer buffer. Gently rub a gloved finger over the well side of the gel to remove small gel pieces from the well area. Submerge the gel in transfer buffer to remove any gel pieces away from the gel, as they can cause air bubbles and field distortion during transfer.
6. Place the flat side of the gel on top of the blotting membrane such that the well side of the gel is facing up and remove any air bubbles with the Blotting Roller. Fill the wells of the gel with 2X NuPAGE® Transfer Buffer (see previous page).
7. Place the pre-soaked E-PAGE™ Blotting Pad on the gel and gently roll out air bubbles using the Blotting Roller.
8. Place 3 pieces of 2.5 mm Blotting Filter Paper from Step 1 on top of the Blotting Pad. Ensure that all filter paper sheets are aligned properly and flush with the gel/membrane sandwich. Remove any air bubbles with the Blotting Roller.
9. Place the cathode plate on the stack without disturbing the blot sandwich. Follow the manufacturer's instructions to further assemble the semi-dry blotting apparatus.
10. Transfer at 25 V for 1 h (~14 V/cm). You may need to optimize the transfer conditions for your specific proteins or semi-dry blot apparatus.

Quality Control

Product qualification is described in the Certificate of Analysis (CofA), available on our website by product lot number at www.invitrogen.com/cofa.