User Guide

Immobilon®-E Blotting Sandwiches

PVDF Transfer Membrane and Filter Paper
for Western Blotting and Immunodetection of Proteins

Introduction

Immobilon®-E Blotting Sandwiches consist of one sheet of Immobilon®-E transfer membrane with a sheet of blotting filter paper on either side. The sandwiches are pre-cut and compatible with the most commonly used pre-cast electrophoresis gels.

NOTE: Pink paper separates the sandwiches from one another. Within each sandwich, blue paper protects the Immobilon®-E membrane (Figure 1). Remove the pink and blue papers before performing western blot procedures.

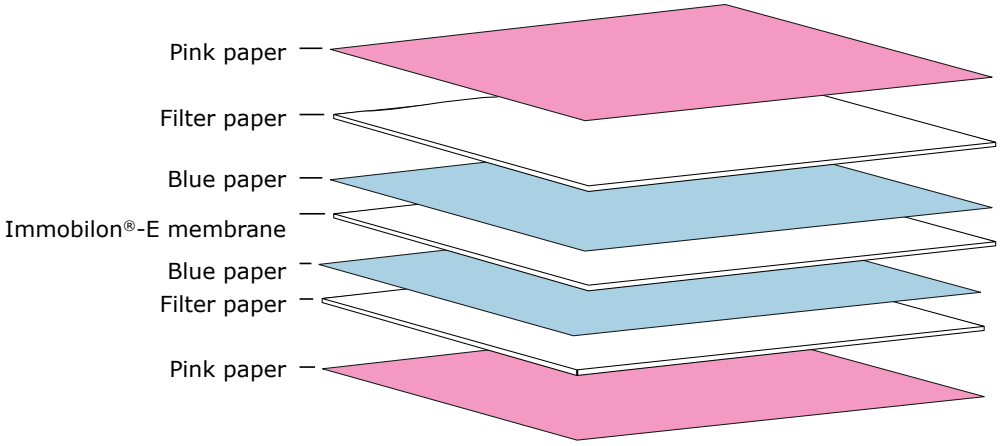
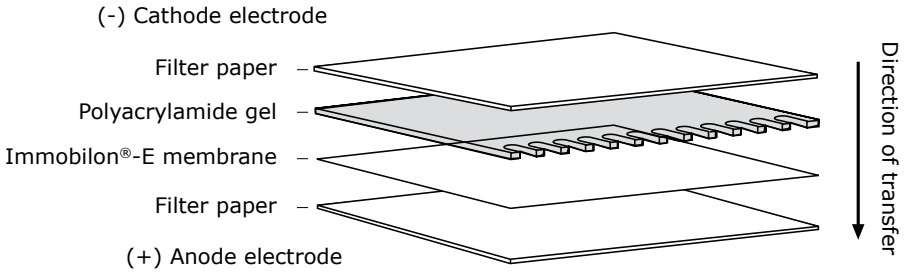


Figure 1. Configuration of Immobilon®-E Blotting Sandwich

Immobilon®-E transfer membrane is a polyvinylidene fluoride (PVDF) microporous membrane for protein blotting applications. Unlike other PVDF transfer membranes, Immobilon®-E membrane does not require an alcohol pre-wet step prior to blotting. It can be wetted with standard transfer buffers. This membrane offers a uniformly controlled pore structure with a high binding capacity for biomolecules. Immobilon®-E membrane has a nominal pore size of 0.45 µm and is useful for blotting proteins with molecular weights greater than 20,000. The membrane and blotting paper are suitable for all chemiluminescent or chromogenic detection methods.

This insert provides a general protocol for immunodetection. The protocol should be optimized for your specific application. For more information, refer to the Protein Blotting Handbook available at www.sigmaaldrich.com/westernhelp.



Materials Recommended for Western Blotting

- Immobilon®-E Blotting Sandwich

  NOTE: Immobilon®-E Blotting Sandwiches can be used in wet-tank and semi-dry electroblotting procedures. Semi-dry transfer requires additional sheets of filter paper.

- Milli-Q® water
- Transfer buffer: 25 mM Tris-base, 192 mM glycine, pH 8.3, 10% alcohol (for tank transfer) or 48 mM Tris, 39 mM glycine, pH 9.2, 10% alcohol (for semi-dry transfer)
- Blocking buffer: Block-CH buffer (cat. no. WBAVDCH01) or 0.5–5% (w/v) blocking agent (bovine serum albumin, casein, nonfat dry milk) in wash buffer
- Wash buffer: Phosphate-buffered saline (PBS) or Tris-buffered saline (TBS) containing 0.05-0.1% Tween® 20 surfactant (PBST or TBST) PBS: 10 mM sodium phosphate, pH 7.2, 0.9% NaCl TBS: 10 mM Tris, pH 7.4, 0.9% NaCl
- Primary antibody (specific for the protein of interest), diluted in blocking buffer or wash buffer
- Secondary antibody (specific for the primary antibody), labeled with a detection enzyme (e.g., horseradish peroxidase [HRP] or alkaline phosphatase [AP]), diluted in blocking buffer or wash buffer.

Protein Transfer

1. Resolve the protein mixture on a 1D or 2D polyacrylamide gel.
2. Immerse the gel in the transfer buffer and allow it to equilibrate for 10–15 minutes.
3. Separate the sheet of Immobilon®-E transfer membrane from the two filter paper sheets. Notch or label one corner of the membrane to correspond to a corner of the gel.
4. Wet the Immobilon®-E membrane in transfer buffer and allow it to equilibrate for 2–3 minutes. The color will change from opaque white to translucent gray. Some variability in the translucency of the membrane is normal.

   CAUTION: To prevent tearing, handle the membrane with care. Once the membrane has been wet out, do not allow it to dry out until the proteins have been transferred to it. If the membrane dries out (turns opaque white) even partially, it must be wetted with alcohol (>50% methanol, ethanol, or isopropanol), rinsed in Milli-Q® water, then equilibrated in transfer buffer for at least 5 minutes.
5. Soak the sheets of filter paper in transfer buffer for at least 30 seconds.
6. Assemble the transfer stack as shown below or according to transfer apparatus manufacturer's instructions.

   CAUTION: To ensure an even transfer, remove air bubbles by carefully rolling a clean pipette or blot roller over the surface of each layer in the stack. Do not apply excessive pressure, as this may damage the gel and membrane.
7. Transfer proteins according to transfer apparatus manufacturer's instructions.
8. Remove the blot from the transfer system and rinse the membrane briefly in Milli-Q® water to remove gel debris. The blot may be used immediately, or air dried for storage.
9. **[Optional]** To visualize all of the transferred proteins, Immobilon®-E membrane may be stained with any reversible blot stain compatible with immunodetection (e.g., Ponceau-S or Sypro® blot stains), or viewed by transillumination using a light box.

Immunodetection

The following is a general protocol for immunodetection with Immobilon®-E membrane. For optimal results, refer to the immunodetection reagent manufacturer's instructions.

1. If the blot was dried, rewet it in alcohol (>50% methanol, ethanol, or isopropanol) for 15 seconds or until it changes from opaque white to translucent gray.
2. Rinse the blot in Milli-Q® water for 1 minute.
3. Place the blot in blocking buffer and incubate for 1 hour with gentle agitation. Prepare primary-antibody solution in wash or blocking buffer.
4. Place the blot in diluted primary-antibody solution and incubate for 1 hour with gentle agitation.
5. Wash the blot with wash buffer 3–5 times for 5 minutes each wash. Prepare secondary-antibody solution in wash or blocking buffer.
6. Place the blot in diluted enzyme-labeled secondary-antibody solution and incubate for 1 hour with gentle agitation.
7. Wash the blot with wash buffer 3–5 times for 5 minutes each wash.
8. If developing with a chromogenic reagent, incubate blot in the developing solution until sufficient signal has been generated for the band of interest. To stop development, transfer the blot to Milli-Q® water or follow the instructions provided with the developing reagent. The developed blot may be dried on filter paper and imaged.
9. If developing with chemiluminescent detection, incubate in developer 1–5 minutes according to detection reagent manufacturer's instructions, and then expose the blot to X-ray film or acquire image using a digital imaging system.

Safety Data Sheet

Safety Data Sheets (SDS) are available at www.sigmaaldrich.com. Enter your catalogue number in the search box.

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Immobilon®-E Blotting Sandwiches (0.45 µm pore size) for general Western blotting applications

Description	Qty/pk	Catalogue No.
7 cm × 8.4 cm	20	IESN07852
8.5 cm × 13.5 cm	20	IESN08132

Immobilon®-E Membrane (0.45 µm pore size) for general Western blotting applications

Description	Qty/pk	Catalogue No.
26.5 cm × 187.5 cm roll	1	IEVH00005
7 cm × 8.4 cm sheet	4	IEVH07804
7 cm × 8.4 cm sheet	50	IEVH07850
8 cm × 10 cm sheet	10	IEVH08100
9 cm × 12 cm sheet	10	IEVH09120
10 cm × 10 cm sheet	10	IEVH10100

Related products for general Western blotting applications

Description	**Catalogue No.**
Block-CH noise cancelling reagent for chemiluminescence detection, 500 mL	WBAVDCH01
Immobilon® blotting filter paper, 7 × 8.4 cm sheet, 100/pk	IBFP0785C
Immobilon® blotting filter paper, 8.5 × 13.5 cm sheet, 100/pk	IBFP0813C
Immobilon® ECL Ultra Western HRP substrate, 100 mL	WBULS0100
Immobilon® Signal Enhancer for immunodetection, 500 mL	WBSH0500
Immobilon® Western HRP substrate, 100 mL	WBKLS0100
Immunoblot Blocking Reagent, 20 g	20-200
Immobilon® Forte Western HRP substrate, 100 mL	WBLUF0100
Immobilon® Crescendo Western HRP substrate, 100 mL	WBLUR0100
Immobilon® Classico Western HRP substrate, 100 mL	WBLUC0100
Phosphate-buffered saline with 3% nonfat milk, pH 7.4, dry powder	P2194
Phosphate-buffered saline with Tween® 20 surfactant, pH 7.4, tablet	08057
Ponceau S solution, 0.1% (w/v) in 5% acetic acid, 1 L	P7170
Re-Blot™ Plus Strong Antibody Stripping solution, 10X, 50 mL	2504
TMB substrate, insoluble (Calbiochem®), 100 mL	613548
Tris-buffered saline with Tween® 20 surfactant, pH 7.6, tablet	91414
Tris-glycine buffer 10X Concentrate, 1 L	T4904-1L
Immobilon GO Device for Simple Immunodetection (10-pack)	IMGDV010