

Immobilon™ Transfer Membranes

For superior protein and nucleic acid blots

Membranes for:

- *Westerns*
- *Dots/slots*
- *Southerns*
- *Northerns*
- *Colony/plaque lifts*

Protein Applications

Immobilon-P Membrane

The original, and most commonly used, PVDF transfer membrane for western blotting. It has a 0.45 µm pore size and is recommended for blotting proteins >20 kDa.

- Compatible with a variety of detection chemistries, including radioactive, chromogenic, and chemiluminescent techniques
- High protein adsorption and retention ensures greater sensitivity
- Won't crack or curl like nitrocellulose. Can be cut without fracturing and reprobed multiple times
- Our Rapid Immunodetection Protocol eliminates the need for blocking and reduces detection times by up to 2 hours

Immoilon-P^{SQ} Membrane

This PVDF membrane has a 0.2 µm pore size. The large internal structure results in higher protein adsorption and sequencing yields than other membranes. Recommended for blotting proteins <20 kDa and sequencing.

- Higher capacity and retention than 0.45 µm membranes
- Prevents blow-through of low molecular weight proteins
- Compatible with a variety of detection chemistries, including radioactive, chromogenic, and chemiluminescent techniques

New!

Immobilon-FL Membrane

The first transfer membrane optimized for fluorescence applications. This PVDF membrane exhibits extremely low background fluorescence.

- Compatible with all commonly used fluorescent dyes
- Can be used at all excitation and emission wavelengths. Ideal for multiplexing
- Quality tested to ensure low background fluorescence in western blotting applications
- Also compatatible with non-fluorescent detection chemistries

Nucleic Acid Applications

Immobilon-Ny+ Membrane

A positively charged nylon membrane optimized for reliable and reproducible transfer, immobilization, hybridization, and subsequent reprobing.

- Provides maximum sensitivity with minimal background due to the density and uniformity of the positively charged surface
- Has exceptional retention and reprobing characteristics. Studies show 50% greater signal than other positively charged nylon membranes—5x greater after 12 reprobes
- Performs well in both chemiluminescent and radioactive detection systems
- Can be used to detect sub-picrogram amounts of DNA and RNA

Immobilon-NC Membranes

An economical alternative for nucleic acid and protein blotting protocols.

- Immobilon-NC (HAHY) membrane is a mixed cellulose esters matrix with surfactants that improve wettability and handling during the transfer process.
- Immobilon-NC (HATF) membrane is a mixed cellulose esters matrix with no surfactants that can interfere with cell wall integrity during cell growth. Recommended for colony lifts.

New!

Blotting Sandwiches with Immobilon-P Membrane

- Immobilon-P membrane interleaved with two sheets of pre-cut blotting paper offers convenience and time savings for high throughput labs. Available in sizes to match most pre-cut gels. See Ordering Information for cut sizes.

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Selecting an Immobilon Membrane for Your Application

	Immobilon-P	Immobilon-P^{SQ}	Immobilon-FL	Immobilon-NY+	Immobilon-NC (HAHY)
Description	The original PVDF membrane for western blotting applications	Small pore structure results in superior binding of proteins with MW <20 kDa	Optimized for fluorescent-based immunodetection applications	Optimized for general nucleic acid applications	Optimized for RNA and DNA blotting applications
Composition	PVDF Blotting sandwiches include pre-cut membrane and two sheets of blotting paper	PVDF	PVDF	Positively charged nylon with polyester reinforcement	Mixed cellulose esters with surfactants to improve wettability and handling
Pore size	0.45 µm	0.2 µm	0.45 µm	0.45 µm	0.45 µm
Phobicity	Hydrophobic	Hydrophobic	Hydrophobic	Hydrophilic	Hydrophilic
Applications	• Western blotting • Binding assays • Amino acid analysis • N-terminal protein sequencing • Dot/slot blotting • Glycoprotein visualization • Lipopolysaccharide analysis • Mass spectrometry	• Low molecular weight western blotting • Amino acid analysis • Mass spectrometry • N-terminal protein sequencing	• Western blotting • Dot/slot blotting • Fluorescence-based detection	• Southern blotting • Northern blotting • Nucleic acid reprobing • Gene arrays • DNA finger-printing • Colony lifts • Plaques lifts • NA dot/slot blots	• Southern blotting • Northern blotting • Western blotting
Detection methods	Chemiluminescent Chromogenic Radioactive	Chemiluminescent Chromogenic Radioactive Fluorescent	Fluorescent Chemifluorescent Chromogenic Chemiluminescent	Chemiluminescent Radioactive	Chemiluminescent Radioactive
Typical protein binding capacity	Insulin: 85 µg/cm^2 BSA: 131 µg/cm^2 Goat IgG: 294 µg/cm^2	Insulin: 262 µg/cm^2 BSA: 340 µg/cm^2 Goat IgG: 448 µg/cm^2	Insulin: 85 µg/cm^2 BSA: 131 µg/cm^2 Goat IgG: 294 µg/cm^2	Not applicable	Not applicable
Compatible stains	Coomassie™ Brilliant Blue Amido black India ink Ponceau-S red Colloidal gold CPTS Toluidine blue Transillumination Sypro® Ruby blot stain	Coomassie Brilliant Blue Amido black India ink Ponceau-S red Colloidal gold CPTS Toluidine blue Transillumination Sypro Ruby blot stain	Sypro Ruby blot stain Coomassie Brilliant Blue Amido black Ponceau-S red CPTS Transillumination	Not applicable	Not applicable

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Performance

Immobilon-P Membrane (0.45 µm)

Optimized for Superior Western Blots (chemiluminescent or chromogenic)

Immobilon-NC (HATF)
Detergent-free membrane for colony and plaque lifts
Mixed cellulose esters without surfactants
0.45 µm
Hydrophilic
• Colony lifts • Plaque lifts
Radioactive
Not applicable
Not applicable

Rapid Immunodetection Method Reduces Blotting Time

Step	Standard Immunodetection	Rapid Immunodetection
1. Block the membrane	1 hr	None
2. Incubate with primary antibody	1 hr	1 hr
3. Wash the membrane	3 x 10 min	3 x 5 min
4. Incubate with secondary antibody	1 hr	30 min
5. Wash the membrane	3 x 10 min	3 x 5 min
6. Add substrate	10 min	10 min
Total time	4 hr 10 min	2 hr 10 min

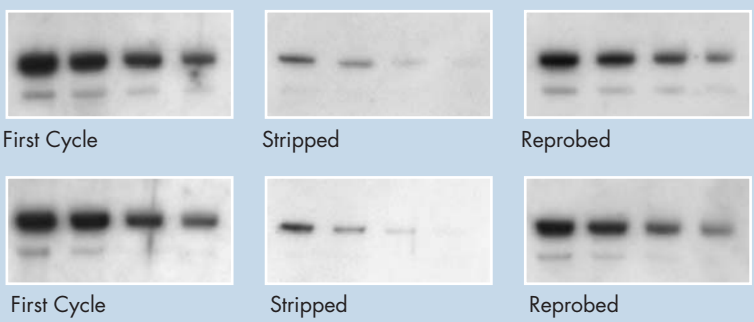
For a detailed protocol, go to www.millipore.com/blottinghandbook.

Compatible with All Commonly Used Stains



Western blots of calf liver proteins detected with (left to right): Ponceau-S red, CPTS, and Coomassie Brilliant Blue total protein blot stains. Lanes (left to right): molecular weight standards, 20, 5, and 1.25 µg of liver proteins.

Superior Reprobing with Detergent and Low pH Methods



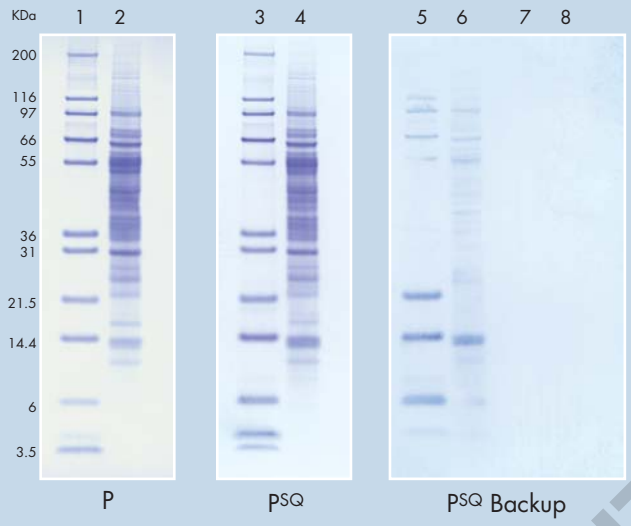
Reprobing Immobilon-P membrane with anti-human transferrin (1:10,000 dilution) and HRP-conjugated rabbit anti-goat IgG (1:20,000) by detergent (top row) and low pH methods (bottom row) using ECL (Amersham) detection reagents. (1) First cycle of detection; (2) stripped membrane detected with the secondary antibody only; (3) second cycle—stripped membrane detected with primary and secondary antibody. Left to right, 5 µL of human serum dilutions 1:12,500, 1:25,000, 1:50,000, and 1:100,000.

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Immobilon-P^{SQ} Membrane (0.2 µm)

Large Internal Surface for Blotting Proteins <20 kDa and for Sequencing

Prevents Small Protein "Blow-Though"

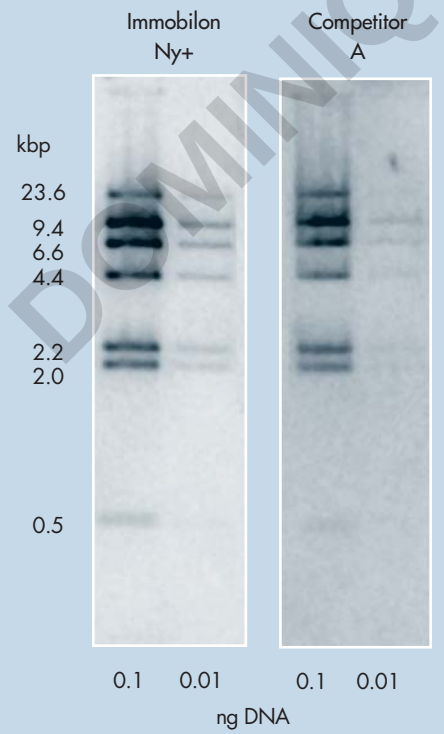


Molecular weight standards (lanes 1, 3, 5, 7) and calf liver lysate (lanes 2, 4, 6, 8) were transferred to Immobilon-P or Immobilon-P^{SQ} membranes. A sheet of Immobilon-P^{SQ} membrane was placed behind the primary membranes to capture proteins that passed through (lanes 5 and 6 behind Immobilon-P membrane; lanes 7 and 8 behind Immobilon-P^{SQ} membrane).

Immobilon-Ny+ Membrane (0.45 µm)

Optimized for Reliable and Reproducible Transfer, Immobilization, Hybridization, and Reprobing

Detect Sub-picogram Amounts of DNA



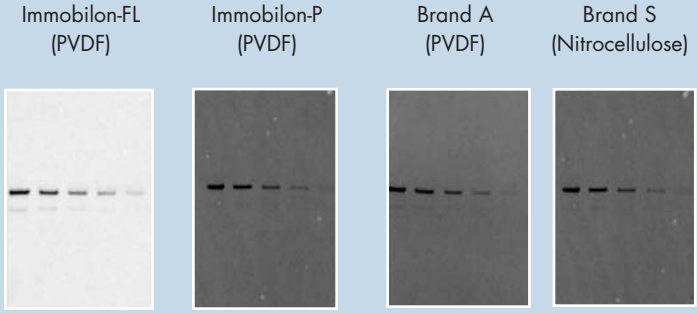
0.01 ng of DNA were loaded onto the gel. The 2.2 and 2.0 kpb bands correspond to 0.48 and 0.42 picograms of DNA. Quantitation showed the signal on the Immobilon-Ny+ membrane was approximately 50% higher than the competitor's positively charged nylon membrane.

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Immobilon-FL Membrane (0.45 µm)

Optimized for Fluorescence-based Immunodetection

Lower Background than Nitrocellulose or Other PVDF Membranes



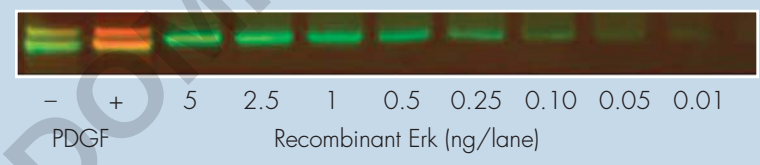
Reverse image showing fluorescent detection of transferrin in human serum using FITC-conjugated second antibody. Serum dilutions: 1:4,000, 1:8,000, 1:16,000, 1:32,000, 1:64,000. All membranes were scanned using a Storm™ 840 imaging system in blue fluorescence mode. Background fluorescence levels of Immobilon-FL membrane are up to 10X less than other blotting membranes.

Ideal for Multiplexing



Actin-tubulin assay on Immobilon-FL membrane. Rabbit muscle actin (red) was detected using rabbit anti-actin 1°AB and QDot® 655 goat anti-rabbit 2°AB. Porcine brain tubulin (green) was detected using mouse anti-tubulin 1°AB and QDot 565 goat anti-mouse 2°AB. Sensitivities down to 1 ng were observed on a Kodak imager. Data provided by Quantum Dot Corporation.

Quantify Both Total and Phosphorylated Protein Expression



MAP kinase assay in PDGF-treated 3T3 cells shows excellent sensitivity and multiplex labeling using Qdot 605 anti-rabbit conjugate (pan Erk) and Qdot 705 anti-mouse conjugate (phospho Erk). Bands are pseudocolored for better visualization. Data provided by Quantum Dot Corporation.

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Ordering Information

Immobilon-P Membrane, PVDF, 0.45 µm pore size

Type	Size	Qty/Pk	Catalogue No.
Sheet	7 x 8.4 cm	50	IPVH 078 50
	8 x 10 cm	10	IPVH 081 00
	8.5 x 13.5	10	IPVH 081 30
	9 x 12 cm	10	IPVH 091 20
	10 x 10 cm	10	IPVH 101 00
	15 x 15 cm	10	IPVH 151 50
	20 x 20 cm	10	IPVH 202 00
	26 x 26 cm	10	IPVH 304 F0
Roll	26.5 cm x 3.75 m	1	IPVH 000 10

Blotting Sandwiches

Type	Size	Qty/Pk	Catalogue No.
Immobilon-P membrane interleaved with blotting paper	7 x 8.4 cm	20	IPSN 078 52
	8.5 x 13.5 cm	20	IPSN 081 32

Immobilon-P^{SQ} Membrane, PVDF, 0.2 µm pore size

Type	Size	Qty/Pk	Catalogue No.
Sheet	7 x 8.4 cm	10	ISEQ 078 50
	8 x 10 cm	10	ISEQ 081 00
	8.5 x 13.5	10	ISEQ 081 30
	9 x 12 cm	10	ISEQ 091 20
	10 x 10 cm	10	ISEQ 101 00
	15 x 15 cm	10	ISEQ 151 50
	20 x 20 cm	10	ISEQ 202 00
	26 x 26 cm	10	ISEQ 262 60
Roll	26.5 cm x 3.75 m	1	ISEQ 000 10

Immobilon-FL Membrane, PVDF, 0.45 µm pore size

Type	Size	Qty/Pk	Catalogue No.
Sheet	10 x 10 cm	10	IPFL 101 00
	20 x 20 cm	10	IPFL 202 00
Roll	26.5 cm x 3.75 m	1	IPFL 000 10

Immobilon-Ny+ Membrane, web-supported positively charged nylon, 0.45 µm pore size

Type	Size	Qty/Pk	Catalogue No.
Disc	82 mm	50	INYC 082 50
	85 mm	50	INYC 085 50
	132 mm	50	INYC 132 50
	137 mm	50	INYC 137 50
Sheet	6 x 11 cm	50	INYC 611 50
	9 x 12 cm	10	INYC 091 20
	10 x 10 cm	10	INYC 101 00
	15 x 15 cm	10	INYC 151 50
	20 x 20 cm	10	INYC 202 00
	22.2 x 22.2 cm	10	INYC 222 20
	22.2 x 22.2 cm	100	INYC 222 2C
	26 x 26 cm	10	INYC 262 60
Roll	30 cm x 3.3 m	1	INYC 000 10

Immobilon-NC (HATF), surfactant-free MCE, 0.45 µm pore size

Type	Size	Qty/Pk	Catalogue No.
Disc	82 mm	50	HATF 082 50
	85 mm	50	HATF 085 50
	132 mm	50	HATF 132 50
	137 mm	50	HATF 137 50

Immobilon-NC (HAHY), MCE, 0.45 µm pore size

Type	Size	Qty/Pk	Catalogue No.
Disc	82 mm	50	HAHY 082 50
	85 mm	50	HAHY 085 50
	137 mm	50	HAHY 137 50
Sheet	30 x 30 cm	50	HAHY 304 F0
Roll	33 cm x 3 m	1	HAHY 000 10

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For Additional Information

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