Immobilon® Block Noise Cancelling Reagents

Introduction

Immobilon® Block Noise Cancelling Reagents are designed especially for western blotting applications. This family of three protein-free, ready-to-use buffers has been optimized to reduce background levels when using chemiluminescent or fluorescent detection. Immobilon® Block buffers are ideal for use with Immobilon® PVDF western blotting membranes and the SNAP i.d.® 2.0 system, and are also compatible with standard western blotting techniques.

Immobilon® Block Package Contents

Item number	Volume
Block-CH for HRP chemiluminescent applications	
WBAVDCH01	500 mL
WBAVDCH01-100ML	100 mL
Block-FL for fluorescent applications	
WBAVDFL01	500 mL
WBAVDFL01-100ML	100 mL
Block-PO for use with phosphospecific antibodies, compatible with chemiluminescent and fluorescent detection	
WBAVDP001	500 mL
WBAVDP001-100ML	100 mL

Storage/Shelf Life

Immobilon® Block buffers can be stored at room temperature. Protection from light is not required. Block-PO buffer may become cloudy over time, but this will not affect performance. Refer to bottle label for expiration date.

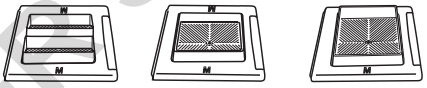
Usage Guidelines

- When used with the SNAP i.d.® 2.0 system, Immobilon® Block buffers are effective in a single 60 second application. Please refer to the SNAP i.d.® user guide for instructions on blot holder assembly and protocol details.
- For optimum results, use Immobilon®-E or Immobilon®-P membrane for chemiluminescent (luminol) or visual detection (e.g. TMB), and Immobilon-FL membrane for fluorescent detection applications.
- When using the traditional western blotting protocol, follow your regular immunodetection protocol and allow at least one hour for the blocking step.
- Optional: primary and secondary antibodies can be diluted in the Block buffer.
- To avoid membrane tearing, always wear gloves and use blunt tip forceps (Millipore cat. no. XX6200006) when handling membrane.
- For optimum results in chemiluminescent applications, use Immobilon® Chemiluminescent HRP Substrates.
- Block-PO buffer has been optimized for phosphospecific antibodies and is compatible with both chemiluminescent and fluorescent detection.

NOTE: Because Immobilon® Block buffers are protein free, membranes may be stained after the immunodetection process to detect protein transfer problems. This step is useful for troubleshooting and can also be used to demonstrate equivalent protein loading in the gel.

- Due to the efficiency of both the SNAP i.d.® 2.0 system and Immobilon® Block buffer solution, the volume during the blocking step can be reduced from 30 mL down to 20 mL for a full size mini blot.
- To ensure efficient membrane blocking, the blot should be entirely covered with Immobilon® Block solution. Final volume used will depend on the size of blot and container. See table below.

SNAP i.d.® 2.0



MultiBlot Frame | **Mini Frame** | **Midi Frame**

	MultiBlot Frame	Mini Frame	Midi Frame
Blocking solution volume	15 mL/well	30 mL	30 mL
Antibody volume	2.5 mL/well	5 mL	10 mL
Wash buffer* volume	4 × 15 mL each	4 × 30 mL each	4 × 30 mL each

**Tris- or phosphate-buffered saline solution, supplemented with 0.1% Tween® 20 surfactant*

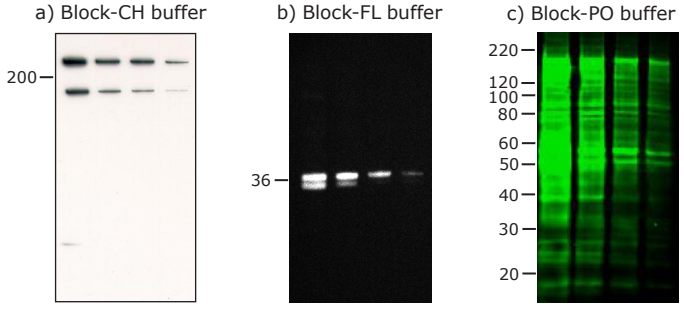
Proof of Performance

Lysate samples were diluted and separated in SDS-PAGE electrophoresis. Gels were transferred to Immobilon®-P (a) and Immobilon®-FL (b and c) membranes and processed in a SNAP i.d.® system.

a) **Detection of BRCA protein in A431 cell lysate.** The blot was blocked with Block-CH buffer and detected by chemiluminescence using Immobilon® Western HRP substrate.

b) **Detection of GAPDH protein in a rat liver lysate.** The blot was probed with anti-mouse Alexa Fluor® 488 dye, blocked with Block-FL buffer, and the image acquired in a Kodak® 4000 MM Image Station.

c) **Tyrosine phosphorylated proteins after probing with Millipore® anti-phosphotyrosine, clone 4G10® antibody in A431 EGF-stimulated cell lysate.** The blot was blocked with Block-PO buffer and detected in the Li-cor® Odyssey® Infrared System using IRDye® 800 infrared dye.



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The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

Ordering Information

This section lists the catalogue numbers for Immobilon® Block Noise Cancelling Reagents and related products. You can purchase these products on-line at www.SigmaAldrich.com/westernblot, or see contact information below.

Immobilon®-E Membrane (0.45 µm pore size) for General Western Blotting Applications

Description	Qty/Pk	Cat. No.
26.5 × 187.5 cm	1	IEVH00005
7 x 8.4 cm sheet	4	IEVH07804
7 x 8.4 cm sheet	50	IEVH07850
8 x 10 cm sheet	10	IEVH08100
9 x 12 cm sheet	10	IEVH09120
10 x 10 cm sheet	10	IEVH10100
Blotting Sandwich 7 x 8.4 cm	20	IESN07852
Blotting Sandwich 8.5 x 13.5 cm	20	IESN08132

Immobilon-FL Membrane (0.45 µm pore size)

26.5 × 375 cm roll	1	IPFL00010
26.5 x 187.5 cm roll	1	IPFL00005
20 x 20 cm sheet	10	IPFL20200
10 x 10 cm sheet	10	IPFL10100
7 x 8.4 cm sheet	10	IPFL07810

Chemiluminescent Detection Substrates for Western Blotting

Description	Qty/Pk	Cat. No.
Immobilon® Western Chemiluminescent HRP Substrate	2 x 25 mL	WBKLS0050
	2 x 250 mL	WBKLS0100
	2 x 250 mL	WBKLS0500
Immobilon® ECL Ultra Western HRP Substrate	2 x 10 mL	WBULS0100-20ML
	2 x 50 mL	WBULS0100
	2 x 250 mL	WBULS0500
Immobilon® Classico Western HRP Substrate	20 mL	WBLUC0020
	100 mL	WBLUC0100
	500 mL	WBLUC0500
Immobilon® Crescendo Western HRP Substrate	20 mL	WBLUR0020
	100 mL	WBLUR0100
	500 mL	WBLUR0500
Immobilon® Forte Western HRP Substrate	20 mL	WBLUF0020
	100 mL	WBLUF0100
	500 mL	WBLUF0500

Related Products for General Western Blotting Applications

Immobilon® Blotting Filter Paper, 7 x 8.4 cm sheet	IBFP0785C
Immobilon® Blotting Filter Paper, 8.5 x 13.5 cm sheet	IBFP0813C
Ponceau S solution, BioReagent, 0.1 % (w/v) in 5% acetic acid	P7170
ReBlot™ Plus Mild Antibody Stripping Solution, 10X, 50 mL	2502
ReBlot™ Plus Strong Antibody Stripping Solution, 10X, 50 mL	2504

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Contact Information

For the location of the office nearest you, go to www.millipore.com/offices.

Technical Assistance

Visit the tech service page on our web site at www.millipore.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at www.millipore.com/terms ("Conditions of Sale").

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