



# bløk™ Noise Cancelling Reagents

## Introduction

bløk 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, fluorescent, or phosphotyrosine detection. bløk buffers are ideal for use with Immobilon®-P western blotting membranes and the SNAP i.d.® system, and are also compatible with standard western blotting techniques.

## Package Contents

**bløk-CH Buffer, cat. no. WBAVDCH01**  
for HRP chemiluminescent applications, 500 mL

**bløk-FL Buffer, cat. no. WBAVDL01**  
for fluorescent applications, 500 mL

**bløk-PO Buffer, cat. no. WBAVDP001**  
for phosphotyrosine detection (4G10® antibody) applications, compatible with chemiluminescent and fluorescent detection, 500 mL

## Storage/Shelf Life

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

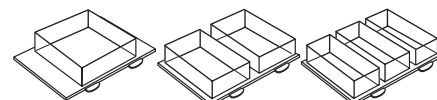
## Usage Guidelines

- When used with the SNAP i.d. system, bløk 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-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 bløk 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.
- bløk-PO buffer has been optimized for detection of phosphotyrosine containing proteins and is compatible with both chemiluminescent and fluorescent detection.

## Usage Guidelines, continued

NOTE: Because bløk buffer is 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. system and bløk 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 bløk solution. Final volume used will depend of the size of blot and container. See table below.

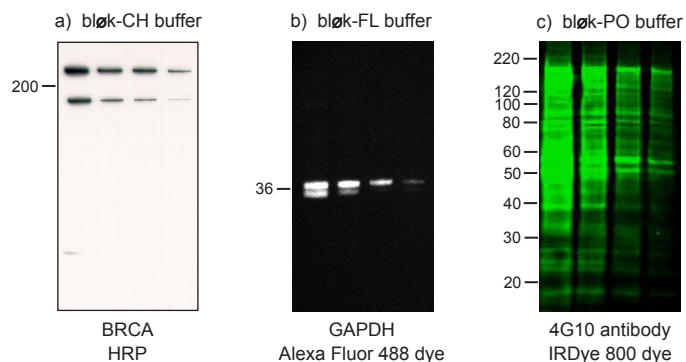


Snap i.d. Blot Holder	Single well	Double well	Triple well
Blocking step	20–30 mL/well	10–15 mL/well	5–10 mL/well
Primary antibody dilution	3 mL/well	1.5 mL/well	1.0 mL/well
Secondary antibody dilution	3 mL/well	1.5 mL/well	1.0 mL/well
<b>Total bløk solution used per blot</b>	<b>26–36 mL</b>	<b>13–18 mL/well</b>	<b>7–12 mL/well</b>

## 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.

- Detection of BRCA protein in A431 cell lysate.** The blot was blocked with bløk-CH buffer and detected by chemiluminescence using Immobilon Western HRP substrate.
- Detection of GAPDH protein in a rat liver lysate.** The blot was probed with anti-mouse Alexa Fluor® 488 dye, blocked with bløk-FL buffer, and the image acquired in a Kodak® 4000 MM Image Station.
- Tyrosine phosphorylated proteins after probing with Millipore anti-phosphotyrosine, clone 4G10 antibody in A431 EGF-stimulated cell lysate.** The blot was blocked with bløk-PO buffer and detected in the Li-cor® Odyssey® Infrared System using IRDye® 800 infrared dye.



## Ordering Information

This section lists the catalogue numbers for bløk buffers and related products. See the Technical Assistance Section for information about contacting Millipore. You can also purchase Millipore products on-line at [www.millipore.com/products](http://www.millipore.com/products).

### bløk Noise Cancelling Reagents

Description	Qty	Cat. No.
bløk-CH Buffer	500 mL	WBAVDCH01
bløk-FL Buffer	500 mL	WBAVDL01
bløk-PO Buffer	500 mL	WBAVDP001

### Immobilon Chemiluminescent Substrates for Western Blotting Applications

Description	Qty	Cat. No.
HRP Substrate	50 mL	WBKLS0050
	100 mL	WBKLS0100
	500 mL	WBKLS0500
AP Substrate	25 mL	WBKDS0025
	100 mL	WBKDS0100

### SNAP i.d. Protein Detection System and Components

Description	Qty	Cat. No.
SNAP i.d. Protein Detection System	1	WBAVDDBASE
SNAP i.d. Triple Well Blot Holder	20	WBAVDDBH03
SNAP i.d. Double Well Blot Holder	30	WBAVDDBH02
SNAP i.d. Single Well Blot Holder	30	WBAVDDBH01

### Immobilon-P PVDF Membrane (0.45 µm pore size) for Western Blotting Applications

Description	Dimensions (cm)	Qty	Cat. No.
Roll	26.5 × 375	1	IPVH00010
Cut Sheet	26 × 26	10	IPVH304F0
	20 × 20	10	IPVH20200
	15 × 15	10	IPVH15150
	10 × 10	10	IPVH10100
	9 × 12	10	IPVH09120
	8.5 × 13.5	10	IPVH08130
	8 × 10	10	IPVH08100
	7 × 8.4	50	IPVH07850

## Ordering Information, continued

### Immobilon-FL PVDF Membrane (0.45 µm pore size) for Western Blotting Applications

Description	Dimensions (cm)	Qty	Cat. No.
Cut sheet	20 × 20	10	IPFL20200
	10 × 10	10	IPFL10100
	7 × 8.4	10	IPFL07810

## Technical Assistance

For more information, contact the Millipore office nearest you. In the U.S., call 1-800-MILLIPORE (1-800-645-5476). Outside the U.S., see your Millipore catalogue for the phone number of the office nearest you or go to our web site at [www.millipore.com/offices](http://www.millipore.com/offices) for up-to-date worldwide contact information. You can also visit the tech service page on our web site at [www.millipore.com/techservice](http://www.millipore.com/techservice).

Material Safety Data Sheets (MSDS) are available on our web site. Go to [www.millipore.com](http://www.millipore.com) and enter your catalogue number in the search box.

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