Data Sheet

Bløk™ Noise-Cancelling Reagents

Protein-free blocking reagents for Western blotting

In Western blotting, blocking of unbound membrane sites is necessary to prevent non-specific binding of the antibodies which otherwise would lead to a high background on the blot. The traditional milk blocker can work well; however, inadvertent variations in its composition can lead to irreproducible results. Such variations include the milk's concentration, fat content, solubility, detergent quality, and numerous other factors.

Bløk reagents are a family of protein-free, noise-cancelling reagents that reduce background for consistent, quality results. They are available in three room temperature-stable, ready-to-use formulations for:

1. Chemiluminescence and chromogenic detection
2. Fluorescence detection
3. Phosphoprotein immunodetection

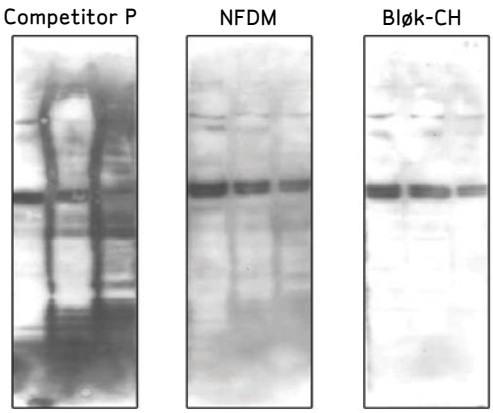
The protein-free nature of Bløk reagents allows for membranes to be stained with chromogenic reagents, such as Ponceau S or Coomassie™ blue, after blocking or immunodetection.

Bløk reagents have been tested and validated for Western blot, dot blot, and ELISA applications.

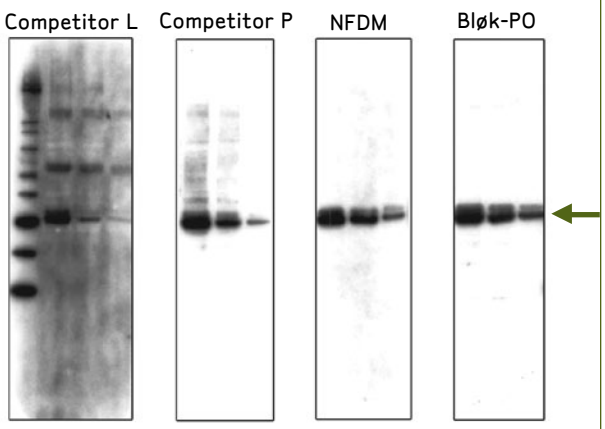
Advantages of Bløk reagents:

- Reduced background for better signal to noise
- Prediluted with detergents for immediate use
- More stable diluent for antibodies than milk
- Stable at room temperature for 1 year
- Allows staining of membranes after immunodetection
- Designed for efficient reagent flow through SNAP i.d. blot holders

Bløk-CH and Bløk-PO Reagents:
Optimal background reduction while preserving phosphorylation state

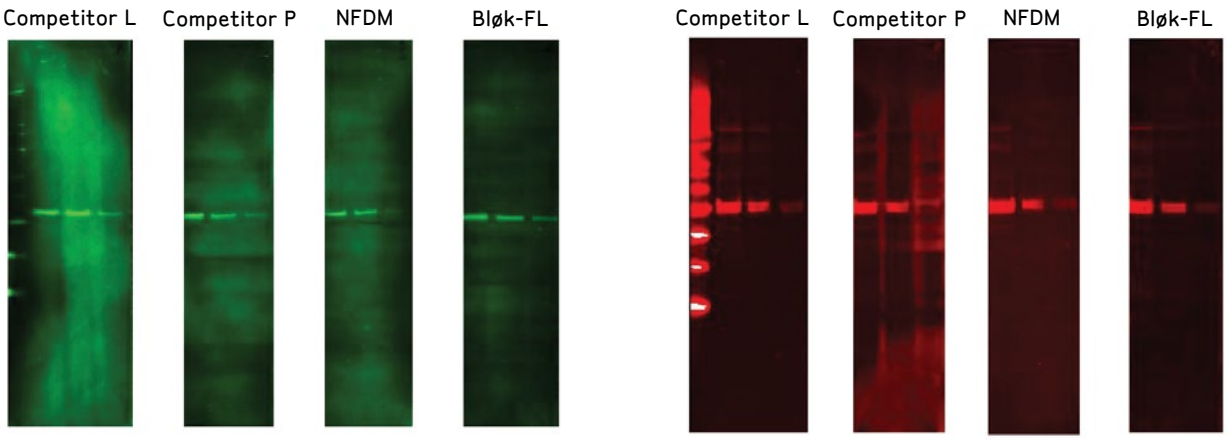


Chemiluminescence detection of p53 in EGF-stimulated A431 lysate (10 - 2.5 µg/lane, Millipore cat. no. 12-110). Blots were blocked with Bløk-CH reagent, then probed with anti-p53 antibody (1:1,000, Millipore cat. no. AB565) diluted in Bløk-CH reagent. Bands were detected using Luminata™ Forte Western HRP substrate (Millipore cat. no. WBLUF0500). NFDM = Non-fat dry milk.



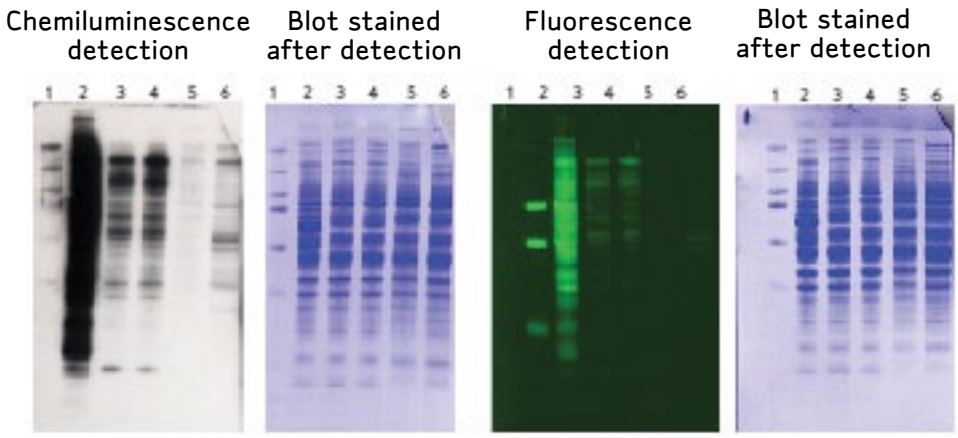
Chemiluminescence detection of pERK in EGF-stimulated A431 lysate (10 – 2.5 µg/lane, Millipore cat. no. 12-110). Blots were blocked with Bløk-PO reagent, then probed with anti-pERK antibody (1:10,000, Millipore cat. no. 05-797R) diluted in Bløk-PO reagent. Bands were detected using Luminata Forte Western HRP substrate (Millipore cat. no. WBLUF0500). NFDM = Non-fat dry milk.

Bløk-FL Reagent:
Low background for fluorescence detection at 680 and 800 nm



Fluorescence detection of actin in EGF-stimulated A431 lysates (1.0 - 25 µg/lane, Millipore cat. no. 12-110). Lysates were resolved by SDS-PAGE and transferred onto Immobilon® FL transfer membrane (Millipore cat. no. IPFL00010). The membranes were blocked and probed with anti-actin antibody (1:400, Millipore cat. no. MAB1501) diluted in the respective blockers. Blots were then incubated with anti-mouse IgG IRDye® 800 (left panel) or 680 (right panel) conjugated (1:1,000, LI-COR cat. no. 926-32210), and scanned on the Odyssey® system after vacuum drying for 1 hr.

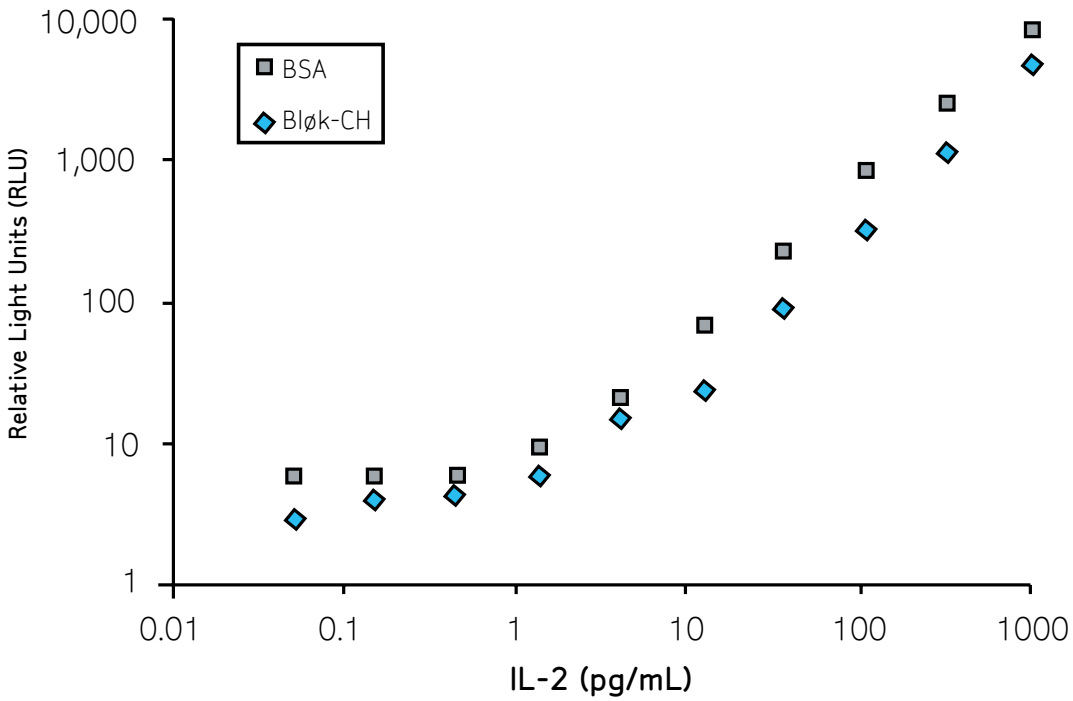
Bløk reagents allow for chromogenic staining of the blots following immunodetection



Lane 1: Molecular weight marker; **Lane 2:** pervanadate-stimulated A431; **Lane 3:**, non-stimulated A431 control; **Lane 4**: EGFR-stimulated A431; **Lane 5:** non-stimulated A431 control; **Lane 6:** EGFR- stimulated A431

Two blots containing different samples of A431 cell lysate, some freshly prepared (lanes 2 - 4) and some old samples (5 - 6), were normalized to 10 µg of total protein per lane. The blots were probed with anti-phosphotyrosine, clone 4G10, and detected by chemiluminescence (left two blots) and by fluorescence (right two blots). Lanes 5 and 6 showed significantly lower signal than lanes 3 and 4 in both detection methods. Staining with Coomassie blue right after immunodetection ruled out the possibilities of loading and transfer errors.

Bløk reagents are effective ELISA blockers



A dose response curve of an IL-2 sandwich ELISA demonstrating the performance of Bløk-CH reagent relative to BSA. ELISA wells were coated with anti-IL-2 capture antibody overnight with gentle shaking at 4 °C. They were washed and blocked with Bløk-CH reagent, then incubated with IL-2 standards for 2 hr at room temperature. Plates were washed with Bløk-CH reagent, then incubated with biotinylated anti-IL-2 antibody followed by HRP-conjugated streptavidin for 1 hr at room temperature. Signal was detected using Luminata Forte ELISA HRP substrate (Millipore cat. no. ELLUF0100).

ORDERING INFORMATION

Bløk Noise-Cancelling Reagents

Description	Detection Reagent Compatibility	Quantity	Catalogue No.
Bløk-CH Reagent	Chemiluminescence Detection	500 mL	WBAVDCH01
Bløk-FL Reagent	Fluorescence Detection	500 mL	WBAVDFL01
Bløk-PO Reagent	Phosphorylated Protein Detection	500 mL	WBAVDP001

SNAP i.d. Protein Detection System

Product Description		Quantity	Catalogue No.
SNAP i.d. Protein Detection System			WBAVDBASE
SNAP i.d. Consumables and Accessories	Single Blot Holder	30/pk	WBAVDBH01
	Double Blot Holder	30/pk	WBAVDBH02
	Triple Blot Holder	20/pk	WBAVDBH03
	Antibody Collection Tray	20/pk	WBAVDABTR
	SNAP i.d. Blot Roller		WBAVDROLL

Immobilon Transfer Membranes

Product Description	Size	Quantity	Catalogue No.
Immobilon-P: PVDF 0.45 µm	7 x 8.4 cm	50/pk	IPVH07850
	26.5 cm x 3.75 m	1 roll	IPVH00010
Immobilon-FL: PVDF 0.45 µm	7 x 8.4 cm	10/pk	IPFL07810
	26.5 cm x 3.75 m	1 roll	IPFL00010
Immobilon-P^{SQ}: PVDF 0.2 µm	7 x 8.4 cm	50/pk	ISEQ07850
	26.5 cm x 3.75 m	1 roll	ISEQ00010

Luminata Western HRP Substrates

Product Description	Quantity	Catalogue No.
Luminata Classico Western HRP Substrates	100 mL	WBLUC0100
Luminata Classico Western HRP Substrates	500 mL	WBLUC0500
Luminata Crescendo Western HRP Substrates	100 mL	WBLUR0100
Luminata Crescendo Western HRP Substrates	500 mL	WBLUR0500
Luminata Forte Western HRP Substrates	100 mL	WBLUF0100
Luminata Forte Western HRP Substrates	500 mL	WBLUF0500

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