Western Blotting Tools

What is your Western blot telling you about your research?

EMD Millipore Corp. is a subsidiary of Merck KGaA, Darmstadt, Germany

Rethink Western blotting.

Explore our products designed to improve each step of the Western blotting workflow.

Western blotting is one of the most commonly used techniques in the lab, yet difficulties persist in obtaining consistent, quality results. At EMD Millipore, we've been helping scientists perform their Western blots for decades, with continued innovation and steadfast technical support.

Explore our expanded portfolio of products, including optimized reagents for chemiluminescent and fluorescent Westerns, as well as the SNAP i.d.® 2.0 system, which reduces blocking, washing and antibody incubation time from hours to minutes.

Access to our Western blotting expertise is easy—flip to our troubleshooting section at the end of this brochure, or contact our experienced technical service team at: **www.emdmillipore.com/techservice**

Western Blotting Workflow Solution

Protein extraction & preparation	Electrophoresis & transfer	Blocking	Antibody incubation, washing	Detection
Gentle protein extraction kits, pg. 2	High protein binding with Immobilon® membranes, pg. 4	30 minute blot processing with the SNAP i.d.® 2.0 system, pg. 7	SNAP i.d.® 2.0 system, pg. 7	Premixed Luminata™ Western HRP substrates for stronger signals, pg. 14
Rapid protein isolation with PureProteome™ magnetic beads, pg. 3		Protein-free Bløk® noise-cancelling reagents, pg. 10	Optimized antibody conditions for the SNAP i.d.® 2.0 system, pg. 9	
Fast, effective concentration with Amicon® Ultra centrifugal filters, pg. 3				

Table of Contents

Protein extraction & sample preparation

Protein extraction and purification represent the first of many challenges in obtaining a quality lysate or purified protein sample that delivers publication-quality Western blot results. EMD Millipore's quality reagents unite superior performance with speed to reduce exposure of proteins to unfavorable conditions, leading to more stable, intact proteins for downstream analysis.

Extraction kits and protease inhibitors

Protein stability is fundamental to all aspects of protein research, including analysis by Western blotting. Combine our gentle protein extraction kits with protease inhibitors to obtain stabilized, intact and active proteins.

Description	Catalogue No.
BugBuster® Protein Extraction Reagent (for bacterial lysis)	70584
BugBuster® Plus Benzonase® Nuclease (nucleic acid degradation for more efficient lysis and less viscous lysate)	70750
BugBuster® Master Mix	71456
YeastBuster® Protein Extraction Reagent (for yeast cell lysis)	71186
CytoBuster® Protein Extraction Reagent (for mammalian cell lysis)	71009
ProteoExtract® Subcellular Proteome Extraction Kit	539790
ProteoExtract® Complete Mammalian Proteome Extraction Kit	539779
ProteoExtract® Native Membrane Protein Extraction Kit	444810
ProteoExtract® Transmembrane Protein Extraction Kit	71772
Nuclear Extraction Kit	2900
RIPA Lysis Buffer, 10X, 100 mL	20-188
Calbiochem® Protease Inhibitor Cocktail Set III, EDTA-Free	539134-1SET
Pepstatin A, 100 mg	516481
Chymostatin, 100 mg	EI6
Leupeptin, 100 mg	EI8

For a complete list of our inhibitors and inhibitor cocktail product offerings, please visit: **www.emdmillipore.com/inhibitors**

Affinity purification

EMD Millipore offers a wide range of tools for protein purification, including affinity magnetic beads, affinity agarose resins and Amicon® Pro purification system.

- PureProteome™ magnetic beads are ideal for small volume affinity purification assays, such as immunoprecipitation and serum depletion or enrichment.
- Affinity agarose portfolio for larger volume applications, such as antibody purification and recombinant protein purification.
- Amicon® Pro purification system is ideal for small volume affinity purification assays followed by buffer exchange and/or concentration.

Description	Catalogue No.
PureProteome™ Protein A Magnetic Beads, 10 mL	LSKMAGA10
PureProteome™ Protein G Magnetic Beads, 10 mL	LSKMAGG10
Protein A Agarose, fast flow, 10 mL	16-156
Protein G Agarose, fast flow, 10 mL	16-266
Protein A/G Mix, 10 mL	IP10-10ML

Learn more at: **www.emdmillipore.com/purity**

Choose an Amicon® Pro device:

To choose the appropriate Amicon® Pro device, determine the molecular weight cut-off (MWCO) of your protein of interest.

	MWCO				
Amicon® Pro Purification System	**3,000**	**10,000**	**30,000**	**50,000**	**100,000**
Amicon® Pro Purification System 12/pk	ACS500312	ACS501012	ACS503012	ACS505012	ACS510012
Amicon® Pro Purification System 24/pk	ACS500324	ACS501024	ACS503024	ACS505024	ACS510024

Learn more at: **www.emdmillipore.com/amiconpro**

Buffer exchange and concentration

Simultaneously concentrate and desalt your samples with Amicon® Ultra centrifugal filters. Their unparalleled rapid and reproducible performance minimizes protein exposure to harsh buffers. For fast and easy dialysis, use D-Tube™ Dialyzers, which provide 89% recovery and 99.9% desalting in as little as two to five hours.

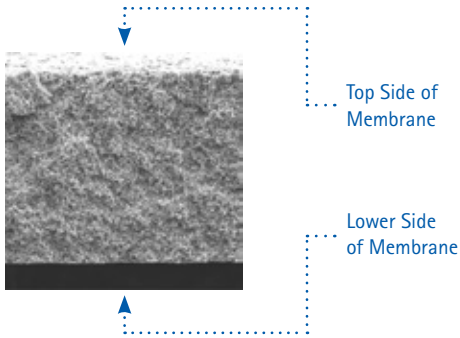
Description	Catalogue No.
Amicon® Ultra – 0.5 mL Filters*, 24/pk	UFC501024
Amicon® Ultra - 2 mL Filters*, 24/pk	UFC201024
Amicon® Ultra – 4 mL Filters*, 24/pk	UFC801024
Amicon® Ultra – 15 mL Filters*, 24/pk	UFC901024
D-Tube™ Mini (10 to 250 µL), 96-well, 7,000 NMWCO**	71712-3
D-Tube™ Midi (50 to 800 µL), 10/pk, 7,000 NMWCO**	71507-3
D-Tube™ Maxi (100 µL to 3 mL), 10/pk, 7,000 NMWCO**	71509-3
D-Tube™ Mega (3 to 10 mL), 10/pk, 7,000 NMWCO**	71740-3
D-Tube™ Mega (10 to 15 mL), 10/pk, 7,000 NMWCO**	71743-4
D-Tube™ Mega (15 to 20 mL), 10/pk, 7,000 NMWCO**	71746-3

* Find the right filter to concentrate your sample. Search with our Amicon® Ultra Selector Tool for access to all Molecular Weight Cut-Off (MWCO) and pack size options: **www.emdmillipore.com/amicon**

** For complete D-Tube™ ordering information, visit: **www.emdmillipore.com/psp**

Electrophoresis & transfer

Immobilon® Western blotting transfer membranes



Membranes are 3-dimensional structures full of microscopic pores (Scanning electron microscope image of a cross-section of Immobilon®-P, Magnification: 500x).

Publications citing Immobilon®: ~52,000

This family of trusted, quality transfer membranes includes Immobilon®-P, the first and most commonly used PVDF membrane for Western transfers.

How do Immobilon® membranes work?

Membranes bind biomolecules through hydrophobic (polyvinylidene (PVDF)) or electrostatic (cellulose-based membranes) interactions. Membrane pores increase the surface binding area while restricting sizes of bound proteins.

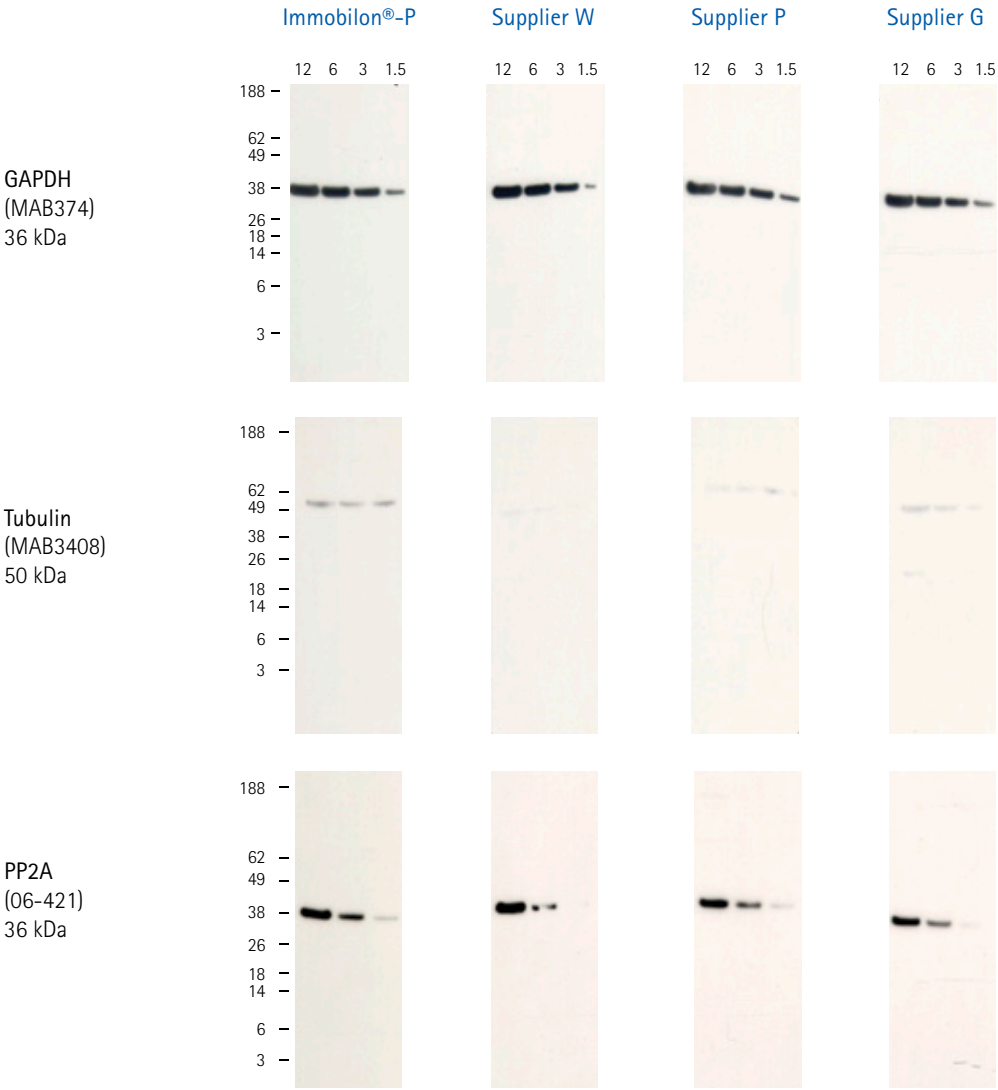
Key Benefits

- Stronger protein signals due to high protein adsorption & retention
- Prolonged shelf life due to higher tensile strength (will not crack or curl like pure nitrocellulose)
- Easier stripping & reprobing with PVDF membranes
- Variety of pore sizes provide optimal protein retention

Comparison of various Immobilon® membranes

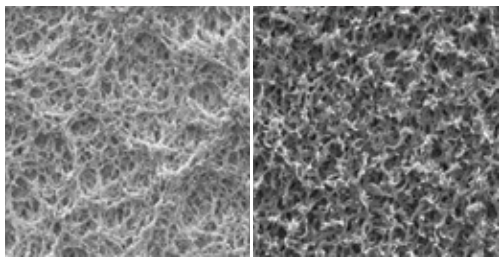
	Immobilon®-P	Immobilon®-P^{SQ}
Best used for	Most protein transfers for any gel matrix	Small proteins (<20kDa), lysates or difficult Westerns
Composition	PVDF	PVDF
Hydrophilicity	Hydrophobic	Hydrophobic
Pore size	0.45 µm	0.2 µm
Detection method	Chemiluminescence	Chemiluminescence Fluorescence
Protein binding capacity	Insulin: 160 µg/cm^2 BSA: 215 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

Membrane performance

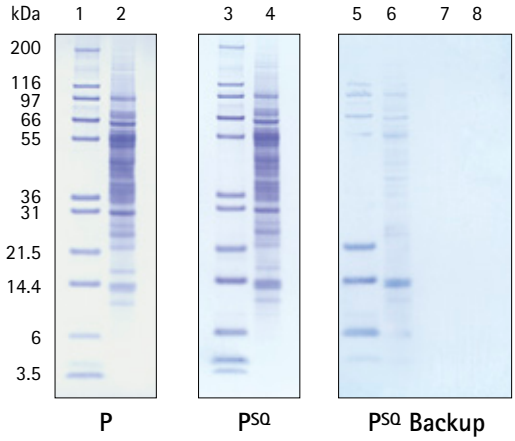


Description	Qty	Catalogue No.
Immobilon®-P PVDF Transfer Membrane, 0.45 µm		
26.5 cm x 3.75 m	1 roll	IPVH00010
7 x 8.4 cm	50/pk	IPVH07850
8.5 x 13.5 cm	10/pk	IPVH08130
20 x 20 cm	10/pk	IPVH20200

Immobilon®-P^{SQ} transfer membrane for smaller proteins



Scanning electron microscopy images (3000x magnification) showing the smaller & more uniform pores in the Immobilon®-P^{SQ} membrane (right) relative to Immobilon®-P membrane (left).



Immobilon®-P^{SQ} membrane prevents the proteins from blowing through the membrane, increasing protein signal. Molecular weight standards (lanes 1 and 3) and calf liver lysate (lanes 2 and 4) 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).

Publications citing Immobilon®-P^{SQ}: ~750

How do Immobilon®-P^{SQ} membranes work?

This PVDF membrane has a 0.2 µm pore size with a thickness of ~200 µm. Because it has smaller pores and approximately three times the internal surface area of most membranes, Immobilon®-P^{SQ} membrane has higher protein binding capacity, improving retention of small proteins.

Key Benefits

- Higher binding capacity and retention resulting in stronger signals
- Prevents blow-through of low molecular weight proteins (<20 kDa)
- Compatible with chemiluminescent and fluorescence detection techniques

Ideal for:

1. Westerns involving lysates or small proteins (<20 kDa), such as histones
2. Difficult Westerns due to:
   - Low-abundance target proteins
   - Low-affinity antibodies

Description	Qty	Catalogue No.
Immobilon®-P^{SQ} PVDF Transfer Membrane, 0.2 µm		
26.5 cm x 3.75 m	1 roll	ISEQ00010
7 x 8.4 cm	50/pk	ISEQ07850
8.5 x 13.5 cm	10/pk	ISEQ08130
20 x 20 cm	10/pk	ISEQ20200

Blocking & antibody addition

SNAP i.d.® 2.0 System

Take protein detection to new dimensions.

The SNAP i .d.® 2.0 system is the second generation in rapid immunodetection technology from EMD Millipore. This fast, versatile system now includes exciting new capabilities to better optimize your Western blotting conditions.

Key Benefits

- Faster results
- Faster testing of different antibodies
- Higher throughput of Western blots each day

How does the SNAP i.d.® 2.0 system work?

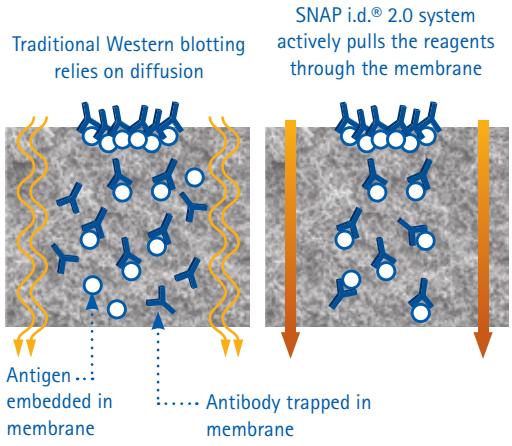
The vacuum-driven SNAP i.d.® 2.0 system fully exploits three-dimensional reagent distribution and decreases the immunodetection time from hours to minutes using the following mechanisms:

1. The system increases local antibody concentrations at binding sites by using vacuum filtration, driving the antibody-antigen binding reaction forward and shortening incubation times.
2. Vacuum pulls any residual, unbound antibody out of the membrane, lowering background signal.

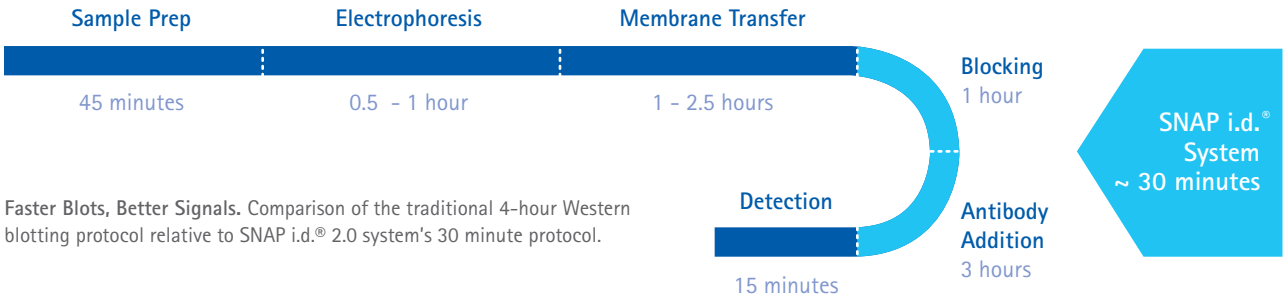
Key Features

- Fastest immunodetection on the market
- Increased antibody-antigen binding
- Superior washes for lower background
- Antibody recollection

How does the SNAP i.d.® 2.0 system Lower Background?
Traditional immunodetection relies on the slow diffusion of reagents into and out of the blot, leading to long incubation times and possible high background. The SNAP i.d.® 2.0 system actively pulls the antibodies through the membrane for maximum interaction with the antigens without a residual high background.



SNAP i.d.® 2.0 system in the Western blotting workflow



Faster Blots, Better Signals. Comparison of the traditional 4-hour Western blotting protocol relative to SNAP i.d.® 2.0 system's 30 minute protocol.

Midi Blot Holder

Mini Blot Holder

MultiBlot Holder

SNAP i.d.® 2.0 systems

Description	Catalogue No.
SNAP i.d.® 2.0 System - Mini (7.5 x 8.4 cm)	SNAP2MINI
SNAP i.d.® 2.0 System - Midi (8.5 x 13.5 cm)	SNAP2MIDI
SNAP i.d.® 2.0 System - MultiBlot (4.5 x 8.4 cm)	SNAP2MB3
SNAP i.d.® 2.0 System - Mini and Midi (7.5 x 8.4 cm and 8.5 x 13.5 cm)	SNAP2MM
SNAP i.d.® 2.0 System - Mini and MultiBlot (7.5 x 8.4 cm and 4.5 x 8.4 cm)	SNAP2MB1
SNAP i.d.® 2.0 System - Midi and MultiBlot (8.5 x 13.5 cm and 4.5 x 8.4 cm)	SNAP2MB2

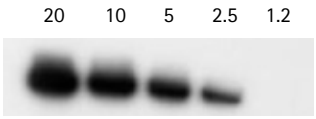
SNAP i.d.® 2.0 consumables

Description	Qty	Catalogue No.
SNAP i.d.® 2.0 Mini Blot Holders (7.5 x 8.4 cm)	100/pk	SNAP2BHMN0100
SNAP i.d.® 2.0 Midi Blot Holders (8.5 x 13.5 cm)	100/pk	SNAP2BHMD0100
SNAP i.d.® 2.0 MultiBlot Holders (4.5 x 8.4 cm)	50/pk	SNAP2BHMB050

SNAP i.d.® 2.0 accessories

Description	Qty	Catalogue No.
SNAP i.d.® 2.0 Antibody Collection Tray	20/pk	SNAPABTR
SNAP i.d.® 2.0 Blot Roller	1/pk	SNAP2RL
SNAP i.d.® 2.0 Mini Blot Holding Frames (double pack)	2/pk	SNAP2FRMN02
SNAP i.d.® 2.0 Midi Blot Holding Frames (double pack)	2/pk	SNAP2FRMD02
SNAP i.d.® 2.0 Mini Blot Holding Frame (single pack)	1/pk	SNAP2FRMN01
SNAP i.d.® 2.0 Midi Blot Holding Frame (single pack)	1/pk	SNAP2FRMD01
SNAP i.d.® 2.0 MultiBlot Frame (single pack)	1/pk	SNAP2FRMB01

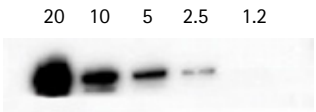
SNAP i.d.® Analysis



Anti-Huntingtin Protein (Catalogue No. MAB2166)

1:400 dilution of this antibody detected Huntingtin protein in rat brain lysate (20 - 1.2 µg).

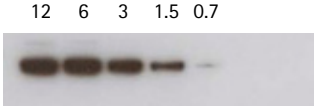
Proteins were detected using Luminata™ Forte HRP detection reagent. Exposure of the blots to X-ray film time varies from 20 sec. to 30 min.



Anti-Metabotropic Glutamate Receptor 5 (Catalogue No. AB5675)

1:200 dilution of this antibody detected Metabotropic Glutamate Receptor 5 in rat brain lysate (20 - 1.2 µg).

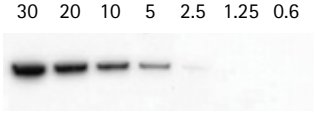
Proteins were detected using Luminata™ Forte HRP detection reagent. Exposure of the blots to X-ray film time varies from 20 sec. to 30 min.



Anti-erbB2 (intracellular domain) (Catalogue No. 04-291)

1:200 dilution of this antibody detected erbB2 in A431 lysate (12 - 0.7 µg).

Proteins were detected using Luminata™ Forte HRP detection reagent and blots. Exposure of the blots to X-ray film varies from 20 sec. to 30 min.



Anti-Pyk2 (Catalogue No. 06-559)

1:200 dilution of this antibody detected Pyk2 protein in rat brain lysate (30 - 0.6 µg).

Proteins were detected using Luminata™ Forte HRP detection reagent and blots. Exposure of the blots to X-ray film varies from 20 sec. to 30 min.

Resources for the SNAP i.d.® 2.0 system

Optimized antibody conditions for the SNAP i.d.® 2.0 system

Obtain fast, reproducible results using optimized dilutions, blocking, and incubation conditions for the SNAP i.d.® 2.0 system.

For a complete listing, visit the SNAP i.d.® 2.0 System Antibody Optimization Reference Guide at: **www.emdmillipore.com/snapab**

All the proteins below were detected using Luminata forte HRP detection reagent and blots, exposure of the blots to X-ray film varies from 20 sec to 30 min.

Target Protein	Dilution used in the SNAP i.d.® system	Catalogue No.
Akt/pkB	1 to 400	05-796
CREB	1 to 200	06-863
Caspase-3	1 to 400	AB3623
Cyclin D1	1 to 200	04-1151
EGF receptor	1 to 200	05-104
MAP Kinase ErK1/2	1 to 500	06-182
anti-erbB2	1 to 200	04-291
anti-GAPDH	1 to 10,000	MAB374
anti-GST M1 (mu)	1 to 200	ABN19
anti-Glyal	1 to 400	MAB3402
anti-Glutamate receptor 1	1 to 400	AB1504
anti-Huntigton protein	1 to 400	MAB2166
anti-Integrin	1 to 1000	AB1928
anti-mGluR5	1 to 200	AB5675
anti-NFkB p52	1 to 400	06-413
anti-NMDR-1	1 to 400	AB9864
anti-P53 (N-term)	1 to 200	04-083
anti-α PAN Cadherin	1 to 400	ABT35
anti-PP2 (serin/threonine phosphatase)	1 to 1000	05-321
anti-PTEN	1 to 200	04-035
anti-PyK-2	1 to 200	06-559
anti-RAS clone Ras 10	1 to 500	05-516
anti-Rac 1 clone 23A8	1 to 200	05-389
anti-STAT-1	1 to 100	05-987
anti-Tau 1	1 to 400	MAB3420
anti-β Tubulin	1 to 1000	MAB3408

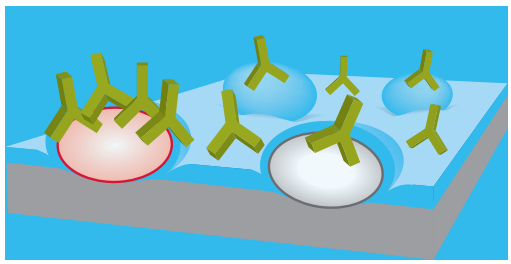
Publications citing the SNAP i.d.® system: 600+

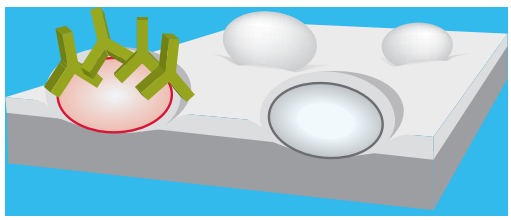
Join the Community of Published SNAP i.d.® Users:

For a sampling of the peer-reviewed publications citing the SNAP i.d.® system, visit: **www.emdmillipore.com/snappub**

Bløk®

Noise cancelling reagents





Milk can leave a thick protein deposit, resulting in non-specific binding of the antibody to the entire blot (top panel). Bløk® reagent coats the blot with a thin chemical layer that does not bind antibodies (bottom panel), leading to less non-specific binding by the antibodies and a lower background.

In Western blotting, blocking of unbound membrane sites is necessary to prevent non-specific binding of the antibodies, which leads to high backgrounds. Traditional milk/protein-blockers can leave a thick layer of sticky proteins that:

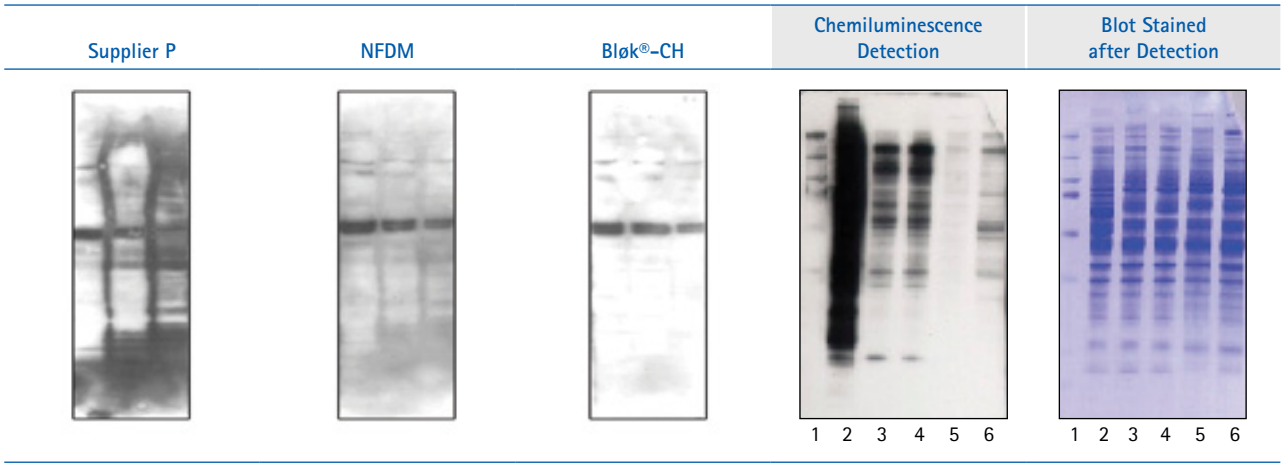
1. Reduce the sensitivity or detection by masking the signal.
2. Are not compatible with detection of protein phosphorylation due to the presence of phosphoproteins in milk.

How does Bløk®-CH reagent improve results?

This chemical-based, protein-free blocker decreases background caused by non-specific antibody binding.

Key Benefits

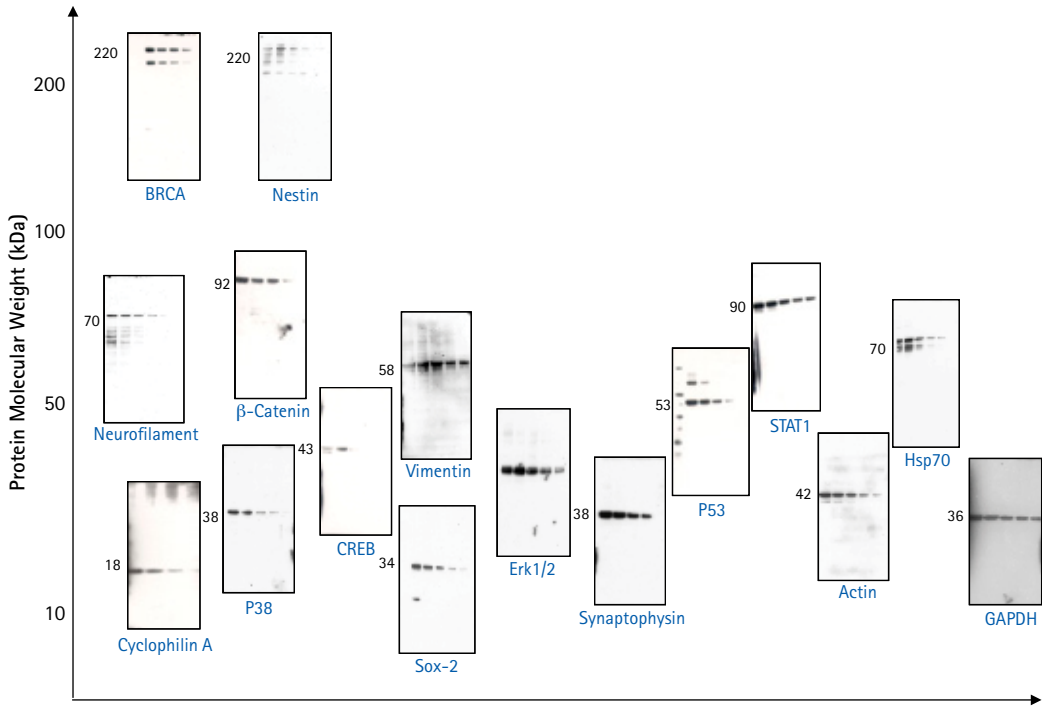
- Reduced background for better protein detection
- No need to run a second gel for Coomassie staining
- Stable at room temperature for 2 years
- Ready to use, no mixing required



Bløk® reagents provide better signal-to-noise ratios compared to NFDM or blocking reagents from Supplier P. Chemiluminescence detection of p53 in EGF-stimulated A431 lysate (10 - 2.5 µg/lane). Blocking reagents used during the blocking and antibody incubation steps are indicated on top. NFDM = nonfat dry milk.

Bløk® reagents enable Coomassie blue staining of membrane after immunodetection. A blot containing freshly prepared samples of A431 cell lysates (lanes 2 - 4) and old samples (lanes 5 - 6), normalized to 10 µg of total protein per lane. The blot was blocked with Bløk®-CH probed with anti-phosphotyrosine, clone 4G10, and detected by chemiluminescence (left panel). Lanes 5 and 6 showed significantly lower signal than lanes 3 and 4. Staining the membrane with Coomassie blue right after immunodetection ruled out the possibilities of loading and transfer errors.

Bløk® reagents perform well with diverse antibodies and lysates



All primary and secondary antibodies were diluted in Bløk®-CH

Description	Detection Method	Qty	Catalogue No.
Bløk®-CH Reagent	Chemiluminescence detection	500 mL/bottle	WBAVDCH01
Bløk®-FL Reagent	Fluorescence detection	500 mL/bottle	WBAVDFL01
Bløk®-PO Reagent	Phosphoprotein detection	500 mL/bottle	WBAVDP001

Antibodies for Western blotting

EMD Millipore offers an extensive, focused portfolio of antibodies and immunoassays. With the expertise of Upstate®, Chemicon® and Calbiochem®, EMD Millipore provides validated products with breadth and depth, backed by excellent service and support, in major research areas:

- Cell Signaling
- Cell Structure and Migration
- Neuroscience
- Stem Cell Biology
- Epigenetics and Gene Regulation
- Cancer
- Toxicity
- Metabolism
- Inflammation and Immunology

Browse our entire selection of antibodies and assays at:
www.emdmillipore.com/antibodies

Ordering information for select highly published antibodies*

Research Focus Area	Antibody Description	Catalogue No.
Neuroscience	Anti-Glutamate Receptor 2, extracellular, clone 6C4	MAB397
Cancer	Anti-p62 (Sequestosome-1), clone 11C9.2	MABC32
Epigenetics	Anti-acetyl-Histone H3	06-599
Cell Structure	Anti-Actin, clone C4	MAB1501
Signaling	Anti-IRS1, clone 4.2.2	05-1085

*View complete antibody portfolio at: **www.emdmillipore.com/antibodies**

Detection: Chemiluminescent Westerns

SignalBoost™ immunoreaction enhancer

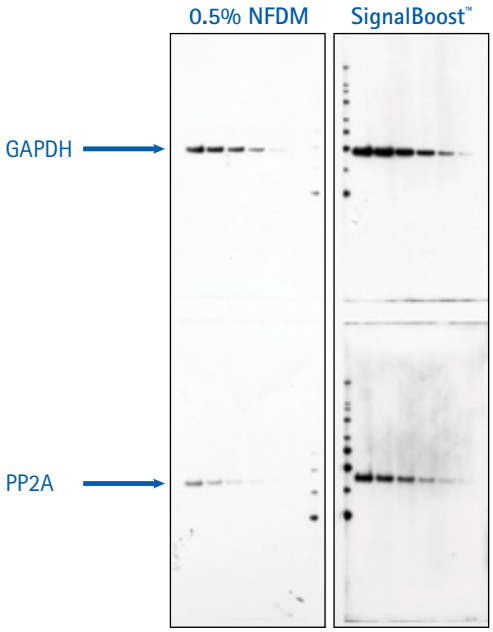
Detecting proteins in a Western can be difficult for multiple reasons (low protein abundance, low affinity antibody, epitope availability, etc.). SignalBoost™ Immunoreaction Enhancer can amplify your signals so you can get your data more quickly and spend less time troubleshooting.

How does SignalBoost™ enhancer work?

When added during the primary and secondary antibody incubations steps of Western blotting, the enhancer increases the binding efficiency of the antibodies to their target epitopes, increasing the signal intensity on the Western blot.

Key Benefits

- Enhanced signals in immunoblots or dot blots
- Cost savings of antibodies. Use only 10% of the antibody used for a typical Western blot and achieve comparable signal intensity
- Works well for any detection method: chemiluminescent, fluorescent or colorimetric



Same blot, stronger signals with SignalBoost™ Enhancer. Two-fold dilutions of A431 cell lysate were resolved & transferred onto Immobilon®-P membrane. Following blocking with 0.5% non-fat dry milk on the SNAP i.d.® system, blots were probed with either anti-GAPDH (top panel, 1:10,000 dilution, Catalogue No. MAB374) or anti-PP2A (bottom panel, 1:200, Catalogue No. 05-421). The antibodies were diluted in either 0.5% non-fat dry milk or SignalBoost™ Immunoreaction Enhancer. After 10 min, the blots were washed with TBST & probed with an appropriate secondary antibody diluted in the indicated diluent. Blots were visualized with Luminata™ Forte Western HRP Substrate (Catalogue No. WBLUF0500). NFDM: Non-fat dry milk; TBST: Tris-buffered saline with Tween-20.

Peer-Reviewed Publications Citing SignalBoost™ Immunoreaction Enhancer

1. Sones W.R., et al., (2010), Cholesterol Depletion Alters Amplitude and Pharmacology of Vascular Calcium-activated Chloride Channels, *Cardiovasc Res., 87(3),* 476-84.
2. Kadota Y., et al., (2009) Involvement of Mesoderm-specific Transcript in Cell Growth of 3T3-L1 Preadipocytes, *Journal of Health Science, 55(5),* 814-19.
3. Lo S.Z., et al., Tumor Necrosis factor-α Promotes Survival in Methotrexate-exposed Macrophages by an NF-κB-dependent pathway, *Arthritis Res Ther., 13(1),* R24.

Description	Catalogue No.
SignalBoost™ Immunoreaction Enhancer Kit	407207

Learn more at: **www.emdmillipore.com/western**

Luminata™ Western chemiluminescent HRP substrates

Chemiluminescent HRP* substrates (also known as ECL reagents) are the most sensitive reagents used in the detection of Western blots.

The Luminata™ Western HRP Substrates are a family of three premixed HRP substrates, which offer several advantages over other detection reagents.

* horseradish peroxidase

Key Benefits

- Broad range of sensitivities
- Premixed for more reproducible signals
- Most sensitive substrates in their class

	Luminata™ Classico	Luminata™ Crescendo	Luminata™ Forte
			
Unique Feature	Premixed	Premixed	Premixed
Best used for	Blots where the primary antibody is incubated for ~1 hr	Blot where the primary antibody is incubated > 2 hrs	Blots with overnight primary antibody incubation, or detection of PTM** proteins
Detection Range	~6 pg	~1–3 pg	~400 fg
Signal Duration	1 hr	3 hr	3 hr
Stock Solution Stability	1 yr at 4 °C	1 yr at 4 °C	1 yr at room temperature

**PTM - Post-translationally modified.

Classification of chemiluminescent HRP substrates

Approximate Detection Limit*	~ 6 pg	~ 1 - 3 pg	~ 400 fg	~ 0.1 pg
EMD Millipore	**Luminata™ Classico**	**Luminata™ Crescendo**	**Luminata™ Forte**	**Visualizer™ Western Blot Kit**
Pierce	Pierce ECL	SuperSignal® Pico	SuperSignal® Dura	SuperSignal® Femto
GE Healthcare	ECL	ECL Plus		ECL Advance
Bio-Rad	Immun-Star™			
Invitrogen	Novex®			
PerkinElmer	Western Lightning® ECL	Western Lightning® ECL Plus		

*Detection limits obtained from suppliers' published specifications.

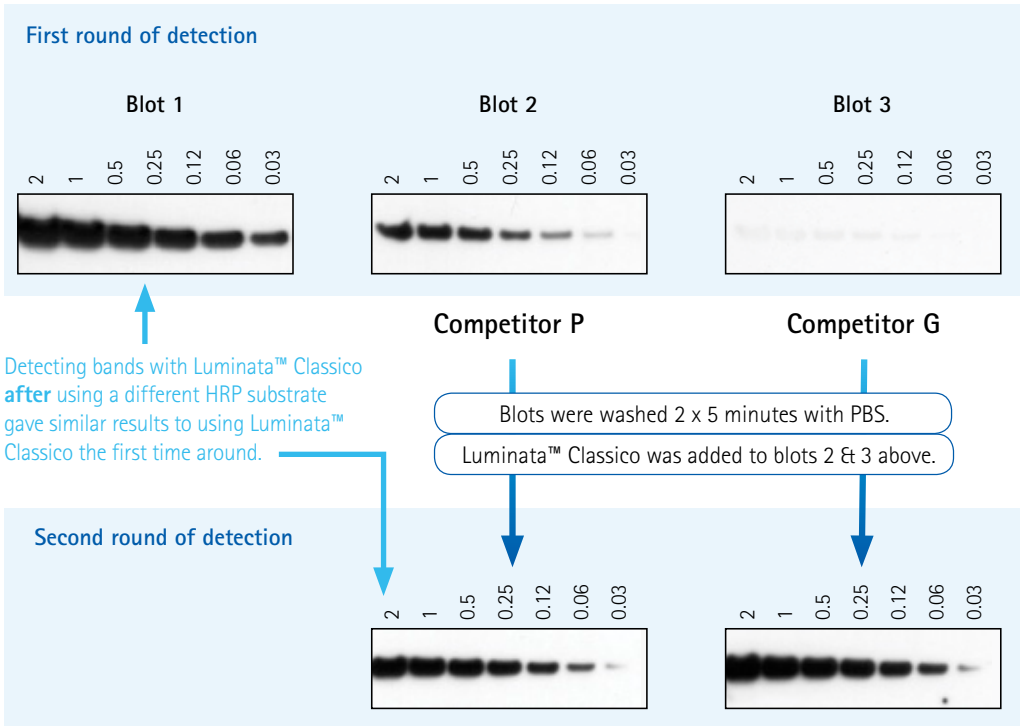
Test Luminata™ substrates AFTER your regular HRP substrate

We've tested the Luminata™ substrates after using other commercial HRP substrates on the same blot and found no significant differences in band intensity compared to first detecting with Luminata™ substrates. Try it and you may detect bands you were not able to visualize previously.

Obtain the best Western blots possible using Luminata™ Western HRP substrates

When no bands were detected with Luminata™ Classico Western HRP substrate (boxed blot), two choices were available:

1. Test a more sensitive reagent, such as Luminata™ Crescendo or Forte substrate
2. Increase antibody concentration from 1:10,000 up to 1:1,000



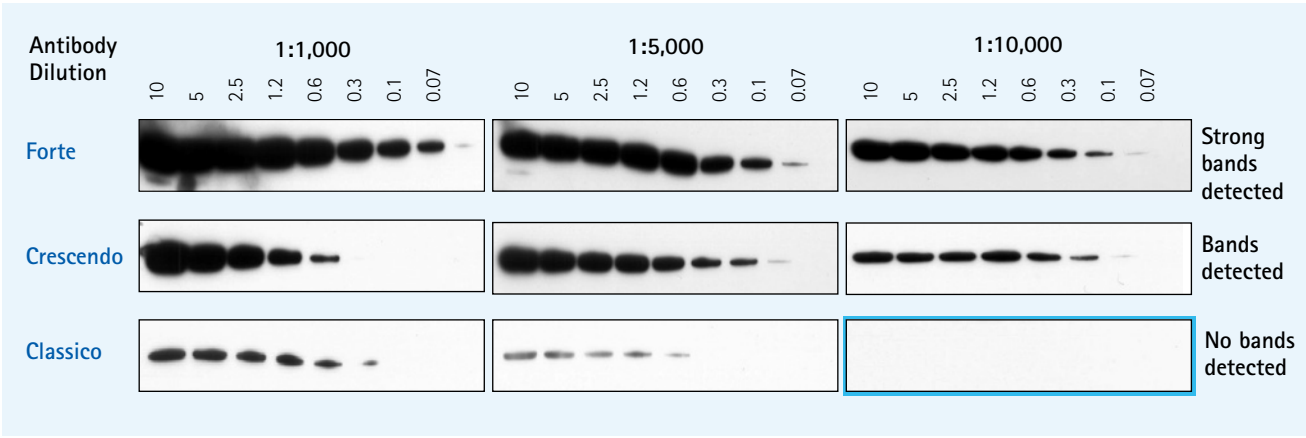
Re-detection of GAPDH. Three Western blots containing a 2-fold dilution series of A431 extract (ranging from 2–0.03 µg) were probed with 1:1000 dilution of anti-GAPDH (Catalogue No. MAB374) and 1:1000 dilution of anti-mouse HRP-conjugated secondary antibody (Catalogue No. AP124P). They were first visualized with the indicated HRP substrate, then washed and re-visualized with Luminata™ Classico substrate. Blots were exposed to X-ray film for 1 minute.

Selected publications citing Luminata™ substrates

1. Vanderperre B., et al., (2011, April 8), An Overlapping Reading Frame In the PRNP Gene Encodes a Novel Polypeptide Distinct From the Prion Protein. *FASEB J.*
2. Texada M.J., et al., (2011, February 15), Tropomyosin is an Interaction Partner of the Drosophila Coiled Coil Protein yuri gagarin, *Exp Cell Res., 317(4)*, 474-87.
3. Xu S., et al., (2011, March 10), Cell Density Regulates *In Vitro* Activation of Heart Valve Interstitial Cells, *Cardiovasc Pathol.*
4. Quentien M.H., et al., (2010, December 21), Truncation of PITX2 Differentially Affects its Activity on Physiological Targets, *J Mol Endocrinol, 46(1)*, 9-19.
5. Fujimori K., Amano F., (2011, April), Niacin Promotes Adipogenesis by Reducing Production of Anti-adipogenic PGF(2α) Through Suppression of C/EBPβ-activated COX-2 Expression, *Prostaglandins Other Lipid Mediat., 94(3-4)*, 96-103.

Using higher sensitivity HRP substrates produced the best results and was advantageous in three respects:

1. **Better results**: It produced stronger bands for a more quantitative blot (compare the increase in band intensities for Luminata™ Crescendo & Forte substrates at 1:10,000 dilution).
2. **Faster**: It took only 10 minutes to wash blot and add a new substrate relative to the 2.5 hours required to repeat antibody incubations.
3. **Cheaper**: The HRP substrates are much cheaper than the price of antibodies.



Immunoblots containing the indicated amounts of A431 lysate were probed with different concentrations of anti-GAPDH antibody (Catalogue No. MAB374) indicated in the top row, followed by an appropriate secondary antibody. Bands were visualized using the indicated Luminata™ HRP substrate and exposed to x-ray film for 5 minutes.

Description	Qty	Catalogue No.
Luminata™ Classico Western HRP Substrate	100 mL	WBLUC0100
	500 mL	WBLUC0500
Luminata™ Crescendo Western HRP Substrate	100 mL	WBLUR0100
	500 mL	WBLUR0500
Luminata™ Forte Western HRP Substrate	100 mL	WBLUF0100
	500 mL	WBLUF0500
Visualizer™ Western Blot Detection Kit, Mouse	250 cm^2 membrane	64-201SP
	1000 cm^2 membrane	64-201
Visualizer™ Western Blot Detection Kit, Rabbit	250 cm^2 membrane	64-202SP
	1000 cm^2 membrane	64-202

ReBlot™ Plus
Western blot recycling kit

Publications citing ReBlot™ Plus: ~2,900

This quick stripping reagent is the product of choice for regenerating Western blots.

What is ReBlot™ Plus?

ReBlot™ Plus reagents efficiently strip probed blots of bound antibodies. ReBlot™ Plus reagents are available in two formulations, "Mild" and "Strong".

- Re-Blot™ Plus Mild Stripping Solution - Provides good results on both nitrocellulose and PVDF membranes.
- Re-Blot™ Plus Strong Stripping Solution - Performs when membranes with high signal are to be stripped, or use when Re-Blot™ Plus Mild treatment is not sufficient.

Key Benefits

- β-Mercaptoethanol-free to avoid pungent smells
- Room temperature stripping in only 15 minutes
- Fast reuse of blots for multiple antibody probings
- Non-acidic, for less risk of protein degradation (such as in Edman degradation)

ReBlot™ efficiently strips blots on (right column) or off the SNAP i.d.® system (left column) to allow for fast reprobing with different antibodies.

Two-fold dilutions of A431 lysate were resolved by SDS-PAGE & transferred onto Immobilon®-P. The blots were probed with HSP70 (1:8,000, Catalogue No. MAB374, top row) using either the traditional Western (left column) or SNAP i.d.® system (right column). Following stripping using ReBlot™ Plus Strong for 15 minutes (middle row), the blots were reprobed with anti-actin antibody (1:8,000, Catalogue No. MAB1501 bottom row).

ReBlot's™ ability to efficiently strip the blot led to a clean actin blot, even though both primary antibodies share the same anti-mouse secondary antibody.

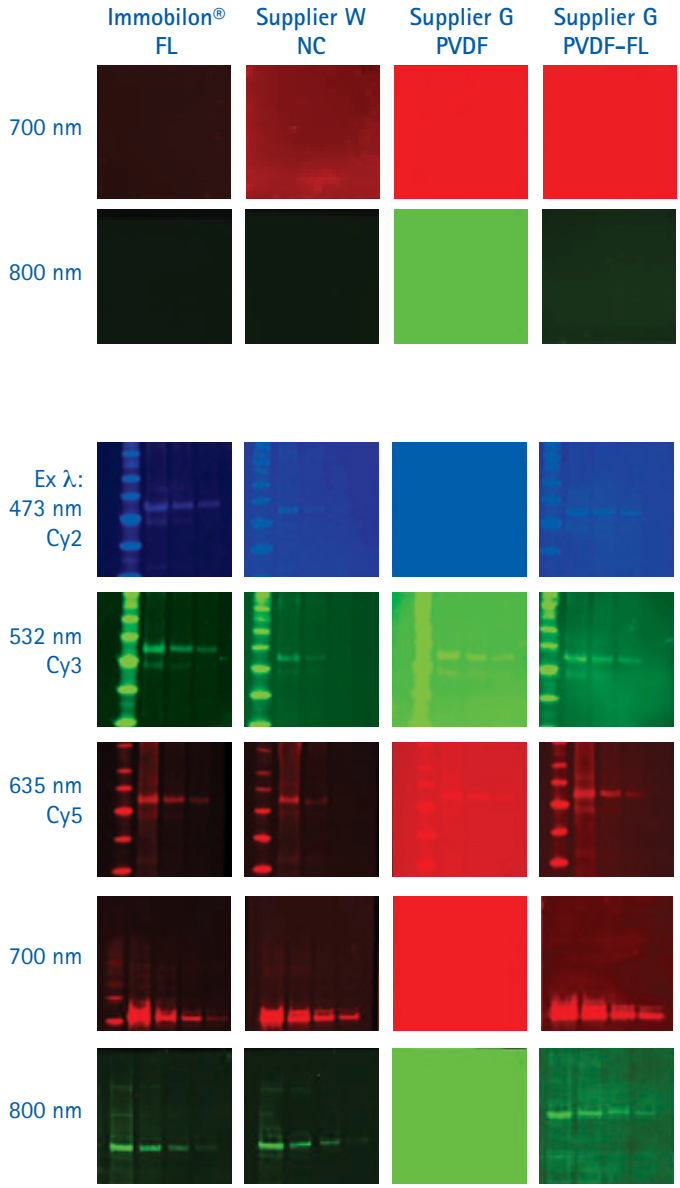
Description	Qty	Catalogue No.
ReBlot™ Plus Mild Antibody Stripping Solution, 10x	50 mL	2502
ReBlot™ Plus Strong Antibody Stripping Solution, 10x	50 mL	2504

Detection: Fluorescent Westerns

Fluorescence-based detection of Western blots, while increasing in popularity due to multiplex detection capabilities, requires specialized tools to obtain optimal results. The reagents presented here have been optimized to work together for fast, reproducible fluorescent Westerns.
For more information, visit: **www.emdmillipore.com/flwestern**

TECHNIQUE SPOTLIGHT

Immobilon®-FL transfer membrane



Publications citing Immobilon®-FL: ~9,000

How does Immobilon®-FL membrane work?

This 0.45 µm membrane is the first transfer membrane specifically optimized for fluorescence-based detection of Western blots. Its extremely low background autofluorescence improves sensitivity of all fluorescence detection protocols.

Key Benefits

- The only membrane that works at near-infrared wavelengths (700-800 nm)
- Strong signals due to higher protein adsorption & retention on the membrane
- Low background to detect even faint bands
- High tensile strength for multiple stripping and reprobing cycles

For more information, visit:
www.emdmillipore.com/flwestern

Bløk®-FL noise cancelling reagent

Blocking the non-specific binding sites on a membrane is critical to avoiding a high background. Protein-based blocking reagents, such as non-fat dry milk, form a layer on the membrane surface that itself can mediate non-specific antibody binding. Furthermore, these blockers can go bad over time either because of blocking protein degradation or microbial growth.

Howdoes Bløk®-FL reagent improve results?

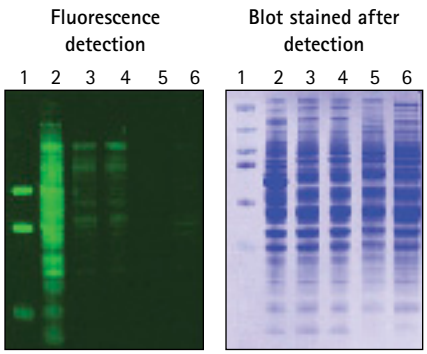
This chemical-based, protein-free blocker decreases background caused by non-specific antibody binding without leaving a thick, sticky layer similar to milk.

Key Benefits

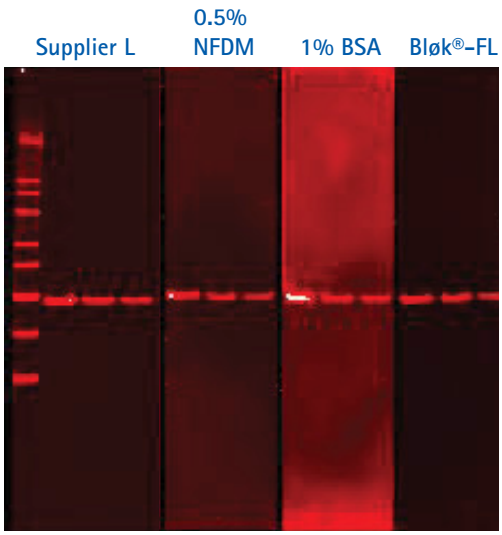
- Specially formulated for reduced background on fluorescent Westerns
- Ready to use straight from the bottle
- Stable at room temperature for 2 years
- Enables colorimetric staining of the blots after immunodetection

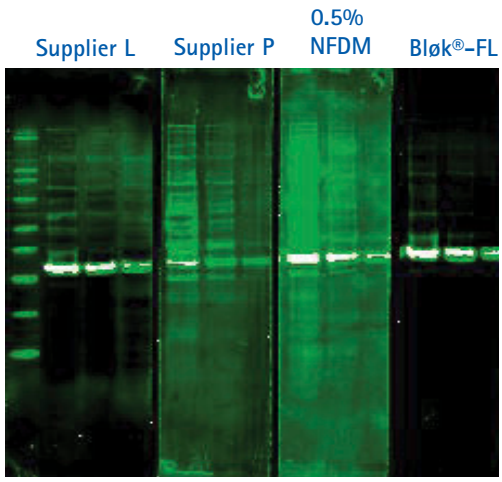
Avoid running a gel just for Coomassie staining

The combination of Bløk® Noise Cancelling Reagents and Immobilon®-PVDF membranes enable membrane staining after immunodetection.



A blot 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 (left panel). The blot was blocked with Bløk®-FL and probed with anti-phosphotyrosine, clone 4G10®, and detected by fluorescence. 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.



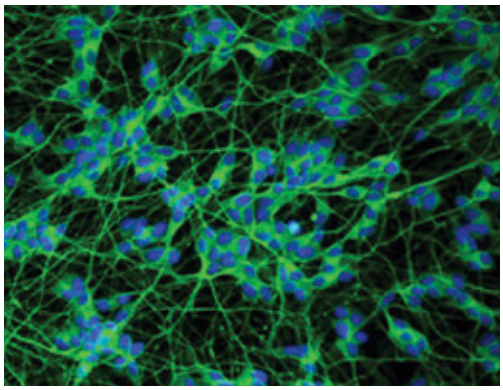


Bløk®-FL reagent provides the best signal-to-noise results. Two Immobilon®-FL blots with dilution series of EGF-stimulated A431 lysate (2-0.5 µg/lane,12-110) were blocked with indicated blocker and probed with either anti-GAPDH antibody (A)1:10,000, Catalogue No. MAB374) or anti-Actin antibody (B)(1:2,000, Catalogue No. MAB1501) diluted in the indicated blocker. Following probing with secondary anti-mouse IgG antibody IRDye680 (A) or IRDye800 (B) , the blots were scanned on the Odyssey® scanner (LI-COR) after vacuum drying for 1 hour.

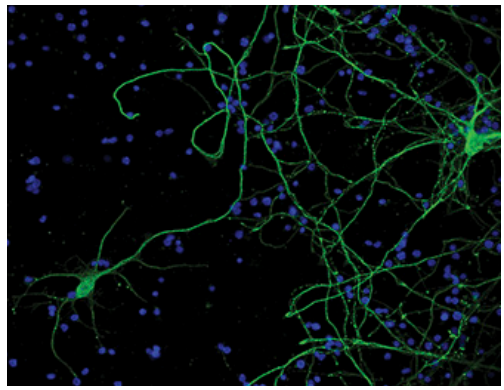
Description	Qty	Catalogue No.
Bløk®-FL Reagent for fluorescence detection	500 mL	WBAVDFL01
Immobilon®-FL Membrane, 0.45 µm		
26.5 cm x 3.75 m	1 roll	IPFL0010
7 x 8.4 cm	10/pk	IPFL07810
10 x 10 cm	10/pk	IPFL10100

Fluorescent conjugated antibodies

EMD Millipore offers a wide range of fluorescent secondary antibodies with demonstrated performance in detection applications as immunofluorescence (IF), immunohistochemistry (IH), Western blot (WB), and flow cytometry. With specificity for whole Ig molecules or antibody fragments such as the Fc or Fab regions, these antibodies are available in a variety of fluorophores, including FITC, DyLight®, Cy dyes, and rhodamine (TRITC). For a complete list of our secondary antibodies and isotype controls, visit: **www.emdmillipore.com/antibodies**



Merged images of differentiated SH-SY5Y cells stained with with Hoechst HCS Nuclear Stain (blue) and Anti-βIII tubulin (Catalogue No. 05-559)/Donkey anti-Mouse FITC conjugated (Catalogue No. AP191F) antibodies (green).



Merged images of rat cortex primary neurons (E18) stained with DAPI (blue) and Pan Neuronal Marker ((Catalogue No. MAB2300)/Goat Anti-Mouse FITC-conjugated (Catalogue No. AP181F) Antibodies (green)).

Ordering Information for select secondary antibodies

Description	Qty	Catalogue No.
Goat anti-Mouse, FITC conjugate	2 mg	AP124F
Donkey anti-Rabbit, Cy3 conjugate	500 µg	AP182C
Donkey anti-Mouse, Cy3 conjugate	500 µg	AP192C
Donkey anti-Rabbit, Biotin conjugate	500 µL	AP182B
Goat anti-Mouse IgG, DyLight® 649 conjugate	500 µg	AP181SD
Donkey anti-Mouse IgG, DyLight® 649 conjugate	500 µg	AP192SD
Goat anti-Rabbit IgG, DyLight® 488 conjugate	2 mg	AP132JD
Goat anti-Rabbit, FITC conjugate	2 mg	AP132F
Donkey anti-Mouse, FITC conjugate	500 µg	AP192F
Goat anti-Rabbit, Cy3 conjugate	2 mg	AP132C
Donkey anti-Rabbit, FITC conjugate	500 µg	AP182F
Donkey anti-Goat, Cy3 conjugate	500 µg	AP180C
Goat anti-Mouse, Cy3 conjugate	500 µg	AP124C
Goat anti-Rabbit, FITC conjugate	1 mL	AP307F
Goat anti-mouse, FITC conjugate	1 mL	AP308F
Donkey anti-Guinea Pig, HRP conjugate	500 µL	AP193P
Rabbit anti-Sheep, HRP conjugate	1.5 mL	AP147P

Detection: Phosphorylated Proteins

Protein phosphorylation is a reversible, post-translational modification that serves to transmit signals through the cell. Detecting phosphorylated proteins via Western blotting is an important step in discovering the upstream regulation, downstream function, crosstalk and feedback mechanisms in most signaling pathways. EMD Millipore provides reagents specifically designed for accurate, sensitive phosphoprotein detection.

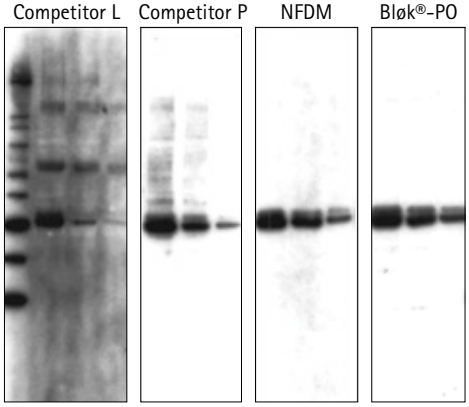
TECHNIQUE SPOTLIGHT

Bløk®-PO noise cancelling reagent

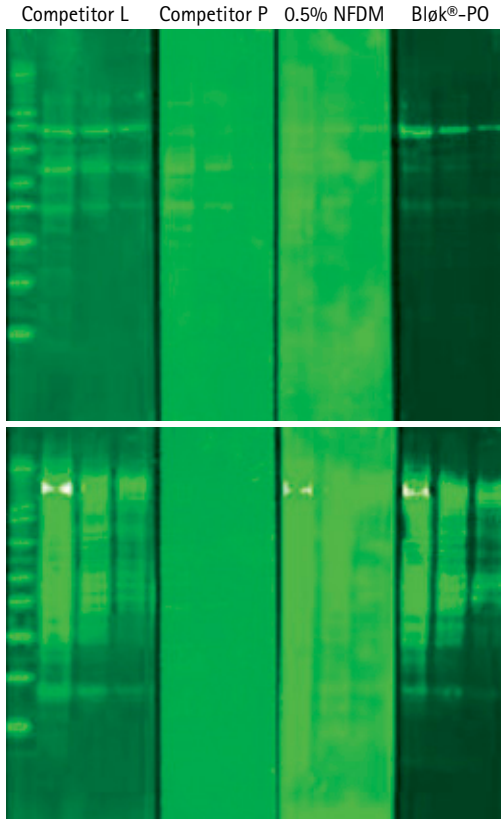
Blocking of non-specific protein binding sites on a blot is essential to decreasing the background and obtaining meaningful results. Although milk is the most commonly used blocker, the presence of phosphorylated mammalian proteins in milk can results in a very high background. For that reason, non-protein based blockers are ideal for immunoblotting for phosphorylated proteins.

How does Bløk®-PO reagent improve results?

This chemical-based blocker contains phosphatase inhibitors to preserve the phosphorylation state of the blotted proteins.



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



Bløk®-PO reagent works best for detection of phosphoproteins.

Fluorescence detection: Dilution series of EGF-stimulated A431 lysate (20-2.5 µg/lane,Catalogue No. 12-110) were resolved by SDS-PAGE and transferred onto Immobilon®-FL membranes. The blots were blocked with respective blocker, probed with either anti-phosphoserine antibody, clone 4A4 (1:400, Catalogue No. 05-1000) (upper panel) or anti-phosphotyrosine antibody, clone 4G10 (1:400, Catalogue No. 05-321) (lower panel), diluted with respective blocker, followed by anti-mouse IgG antibody IRDye800 conjugated (1:1,000, Catalogue No. 926-32210, LI-COR). The blots were scanned on the Odyssey® scanner (LI-COR) after vacuum drying for 1 hour.

Key Benefits

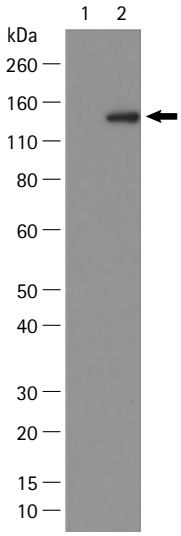
- Protein-free for reduced background and better detection
- Contains phosphatase inhibitors to keep phosphorylated sites intact
- No need to run a second gel for Coomassie staining.
- Stable at room temperature for 1 year
- Formulated for immediate use

Phosphospecific antibodies

EMD Millipore's extensive portfolio of antibodies includes over 600 validated, phosphospecific antibodies. These antibodies are excellent tools to explore biological pathways and signals that involve phosphorylation.

Anti-phospho-MYPT1 (Thr696) (Catalogue No. ABS45)

Myosin phosphatase target subunit 1 (MYPT1) regulates the interaction of actin and myosin downstream of the guanosine triphosphatase Rho, which inhibits myosin phosphatase via Rho-kinase. Inhibition of myosin light chain phosphatase, via phosphorylation of MYPT1, results in Ca^{2+} sensitization of smooth muscle contraction. MYPT1 is localized on stress fibers, and is distributed close to the cell membrane and at cell-cell contacts to regulate myosin phosphatase activity.

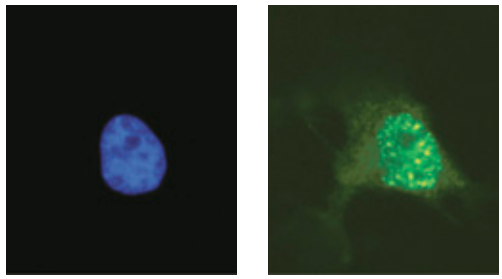


Western blot detection of phospho-MYPT1. Lysates of NIH3T3 cells +/- calyculin/okadaic acid were resolved by electrophoresis, transferred to PVDF membranes and probed with Anti-Phospho-MYPT1 (Thr696) (1:1,000) on the SNAP i.d.® system. Proteins were visualized using a Donkey anti-Rbt IgG:HRP conjugate and visualized using chemiluminescence detection.

Arrow indicates Phospho-MYPT1 (Thr696) (~130 kDa).

Anti-phospho-Histone H2A.X (Ser139), clone JBW301 (Catalogue No. 05-636)

Phosphorylation of histone H2A.X on Ser139 is an early event in cellular response to DNA damage. Phosphorylated H2A.X helps recruit DNA repair machinery to double-strand breaks, eventually recruiting p53, which causes the cell cycle to pause so repair can be completed.



Detection of phospho-Histone H2A.X in cells undergoing DNA damage. Jurkat cells were treated with the cytotoxic agent, etoposide, and stained with Anti-phospho-Histone H2A.X (Ser139, Catalogue No. 05-636), clone JBW301 (green, right panel); DNA stained with DAPI (left panel).

Ordering information for select phosphospecific antibodies

Description	Qty	Catalogue No.
Anti-Phosphotyrosine, clone 4G10	100 µg	05-321
Anti-phospho-Histone H2A.X (Ser139)	200 µg	05-636
Anti-phospho-CREB (Ser133)	100 µL	06-519
Anti-phospho-Smad2, (Ser465/467)	100 µL	AB3849
Anti-phosphoserine, clone 4A4	100 µg	05-1000
Anti-phospho-ACK1 (Tyr284)	100 µL	09-142
Anti-phospho-ATM (Ser1981), clone 10H11.E12	200 µg	05-740
Anti-phospho-MYPT1 (Thr696)	200 µg	ABS45
Anti-phospho-Src (Tyr416), clone 9A6	100 µg	05-677
Anti-phospho-GluR1 (Ser845), clone EPR2148	100 µL	04-1073

Troubleshooting Western blots

As your Western blotting partner, our technical support team is ready to help you anytime.
Troubleshoot your Westerns using the reference guide below, or for customized assistance, visit:
www.emdmillipore.com/techservice

Immunodetection

Symptom	Possible Cause	Remedy
Weak signal	Improper blocking reagent	The blocking agent may have an affinity for the protein of interest and thus obscure the protein from detection. Try a different blocking agent and/or reduce both the amount or exposure time of the blocking agent.
	Insufficient antibody reaction time	Increase the incubation time.
	Antibody concentration is too low or antibody is inactive	Multiple freeze-thaws or bacterial contamination of antibody solution can change antibody titer or activity. Increase antibody concentration or prepare it fresh.
	Outdated detection reagents	Use fresh substrate and store properly. Outdated substrate can reduce sensitivity.
	Protein transfer problems	Optimize protein transfer.
	Dried blot in chromogenic detection	If there is poor contrast using a chromogenic detection system, the blot may have dried. Try rewetting the blot in water to maximize the contrast.
	Tap water inactivates chromogenic detection reagents	Use Milli-Q® water for reagent preparation.
	Azide inhibits HRP	Do not use azide in the blotting solutions.
	Antigen concentration is too low	Load more antigen on the gel prior to the blotting.
No signal	Antibody concentration too low	Increase concentration of primary and secondary antibodies.
	HRP inhibition	HRP-labeled antibodies should not be used in solutions containing sodium azide.
	Primary antibody was raised against native protein	Separate proteins in non-denaturing gel or use antibody raised against denatured antigen.
Uneven blot	Fingerprints, fold marks or forceps imprints on the blot	Avoid touching or folding membrane; use gloves and blunt end forceps.
Speckled background	Aggregates in the blocking reagent	Filter blocking reagent solution through 0.2 µm or 0.45 µm Millex® syringe filter unit.
	Aggregates in HRP-conjugated secondary antibody	Filter secondary antibody solution through 0.2 µm or 0.45 µm Millex® syringe filter unit.
High background	Insufficient washes	Increase washing volumes and times. Pre-filter all of your solutions including the transfer buffer using Millex® syringe filter units or Steriflip™ filter units.
	Secondary (enzyme conjugated) antibody concentration is too high	Increase antibody dilution.
	Protein-protein interactions	Use Tween-20 (0.05%) in the wash and detection solutions to minimize protein-protein interactions and increase the signal to noise ratio.
	Immunodetection on Immobilon®-P^{SQ} transfer membrane	Increase the concentration or volume of the blocking agent used to compensate for the greater surface area of the membrane. Persistent background can be reduced by adding up to 0.5M NaCl and up to 0.2% SDS to the wash buffer and extending the wash time to 2 hours.
	Poor quality reagents	Use high quality reagents and Milli-Q® water.
	Crossreactivity between blocking reagent and antibody	Use different blocking agent or use Tween-20 detergent in the washing buffer.
	Film overexposure	Shorten exposure time.
	Membrane drying during incubation process	Use volumes sufficient to cover the membrane during incubation.
	Poor quality antibodies	Use high quality affinity purified antibodies.
	Excess detection reagents	Drain blots completely before exposure.

Symptom	Possible Cause	Remedy
Persistent background	Non-specific binding	Use High Salt Wash. (PBS or TBS supplemented with 0.5% NaC1 and 0.2% SDS)
High background (rapid immunodetection)	Membrane wets out during rapid immunodetection	Reduce the Tween-20 (<0.04%) detergent in the antibody diluent.
		Use gentler agitation during incubations.
		Rinse the blot in Milli-Q® water after electrotransfer to remove any residual SDS carried over from the gel. Be sure to dry the blot completely prior to starting any detection protocol.
	Membrane was wet in methanol prior to the immunodetection	Do not pre-wet the membrane.
	Membrane wasn't completely dry prior to the immunodetection	Make sure the membrane is completely dry prior to starting the procedure.
Non-specific binding	Primary antibody concentration too high	Increase primary antibody dilution.
	Secondary antibody concentration too high	Increase secondary antibody dilution.
	Antigen concentration too high	Decrease amount of protein loaded on the gel.
Reverse images on film (white bands on dark background)	Too much HRP-conjugated secondary antibody	Reduce concentration of secondary HRP-conjugated antibody.
Poor detection of small proteins	Small proteins are masked by large blocking molecules such as BSA	Consider casein or a low molecular weight polyvinylpyrrolidone (PVP).
		Surfactants such as Tween and Triton X-100 may have to be minimized.
		Avoid excessive incubation times with antibody and wash solution.

Fluorescent detection

Symptom	Possible Cause	Remedy
High overall background	High background fluorescence from the blotting membrane	Use Immobilon®-FL PVDF blotting membrane.
Multiplexing problems	Experimental design	The two antibodies must be derived from different host species so that they can be differentiated by secondary antibodies of different specificities. Before combining the two primary antibodies, test the banding pattern on separate blots to determine where bands will appear. Use cross-adsorbed secondary antibodies in two-color detection.
Speckled background	Dust/powder particles on the surface of the blot	Handle blots with powder-free gloves and clean surface of the scanner.
Low signal	Wet blot	Drying the blot may enhance signal strength. The blot can be scanned after re-wetting. Do not wrap the blot in plastic/Saran wrap while scanning.
	Blot photo-bleached	While fluorescent dyes usually provide long-lasting stable signal, some fluorescent dyes can be easily photo-bleached. To prevent photo-bleaching, protect the membrane from light during secondary antibody incubations and washes, and until the membrane is ready to be scanned. Store developed blots in the dark for subsequent imaging.
	Wrong excitation wavelength or emission filter	Follow dye manufacturers instructions for blot imaging.

Related products: Western blotting recipes

2X Sample Buffer (2105)

Component	Catalogue No.
130 mM Tris HCl pH 8.0	9310
20% (v/v) Glycerol	4750
4.6% (w/v) SDS	7910
0.02% Bromophenol blue	2830
2% DTT	3860

8X Resolving Gel Buffer: 100 mL

Component	Catalogue No.
0.8 g SDS (add last)	7910
36.3 g Tris base (=3 M)	9210

Adjust pH to 8.8 with concentrated HCl

4X Stacking Gel Buffer: 100 mL

Component	Catalogue No.
0.4 g SDS (add last)	7910
6.05 g Tris base (=0.5 M)	9210

Adjust pH to 6.8

10X Running Buffer: 1 L

Component	Catalogue No.
30.3 g Tris base (=0.25 M)	9210
144 g Glycine(=1.92 M)	4810
10 g SDS (=1%, add last)	7910

Do not adjust pH!

10X Transfer Buffer: 1 L (Catalogue No. 9000, ready to use)

Component	Catalogue No.
30.3 g Tris base (=0.25 M)	9210
144 g Glycine(=1.92 M)	4810

pH should be 8.3, do not adjust

Wash Buffer

Component	Catalogue No.
OmniPur® 10X PBS, Premixed Powder	6508

Immobilon® transfer membranes

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

SNAP i.d.® 2.0 systems

Description	Catalogue No.
SNAP i.d.® 2.0 System - Mini (7.5 x 8.4 cm)	SNAP2MINI
SNAP i.d.® 2.0 System - Midi (8.5 x 13.5 cm)	SNAP2MIDI
SNAP i.d.® 2.0 System - MultiBlot (4.5 x 8.4 cm)	SNAP2MB3
SNAP i.d.® 2.0 System - Mini and Midi (7.5 x 8.4 cm and 8.5 x 13.5 cm)	SNAP2MM
SNAP i.d.® 2.0 System - Mini and MultiBlot (7.5 x 8.4 cm and 4.5 x 8.4 cm)	SNAP2MB1
SNAP i.d.® 2.0 System - Midi and MultiBlot (8.5 x 13.5 cm and 4.5 x 8.4 cm)	SNAP2MB2
SNAP i.d.® 2.0 System - Single IHC	SNAP2IHC
SNAP i.d.® 2.0 System - Double IHC	SNAP2IHC2

SNAP i.d.® 2.0 consumables

Description	Qty	Catalogue No.
SNAP i.d.® 2.0 Mini Blot Holders (7.5 x 8.4 cm)	100/pk	SNAP2BHMN0100
SNAP i.d.® 2.0 Midi Blot Holders (8.5 x 13.5 cm)	100/pk	SNAP2BHMD0100
SNAP i.d.® 2.0 MultiBlot Holders (4.5 x 8.4 cm)	50/pk	SNAP2BHM050
SNAP i.d.® 2.0 IHC Slide Holders	24/pk	SNAP2SH

SNAP i.d.® 2.0 accessories

Description	Qty	Catalogue No.
SNAP i.d.® 2.0 Antibody Collection Tray	20/pk	SNAPABTR
SNAP i.d.® 2.0 Blot Roller	1/pk	SNAP2RL
SNAP i.d.® 2.0 Mini Blot Holding Frames (double pack)	2/pk	SNAP2FRMN02
SNAP i.d.® 2.0 Midi Blot Holding Frames (double pack)	2/pk	SNAP2FRMD02
SNAP i.d.® 2.0 Mini Blot Holding Frame (single pack)	1/pk	SNAP2FRMN01
SNAP i.d.® 2.0 Midi Blot Holding Frame (single pack)	1/pk	SNAP2FRMD01
SNAP i.d.® 2.0 MultiBlot Holding Frame (single pack)	1/pk	SNAP2BHMB050
SNAP i.d.® 2.0 IHC Frame	1/pk	SNAP2FRIHC

Bløk® noise cancelling reagents

Description	Detection Method	Qty	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

Luminata™ Western HRP substrates

Description	Qty	Catalogue No.
Luminata™ Classico Western HRP Substrates	500 mL	WBLUC0500
Luminata™ Crescendo Western HRP Substrates	500 mL	WBLUR0500
Luminata™ Forte Western HRP Substrates	500 mL	WBLUF0500

Western blotting enhancing reagents

Description	Qty	Catalogue No.
SignalBoost™ Immunoreaction Enhancer Kit	400 mL	407207

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