EMD MILLIPORE

Protein Research Tools

What is your Western blot telling you?

EMD Millipore is a division of Merck KGaA, Darmstadt, Germany

Fisher Scientific

Improve your results, and they'll tell you a story you can publish.

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 publish 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.® 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.millipore.com/techservice**

Western Blotting Workflow Solution

Protein Extraction & Preparation	Electrophoresis & Transfer	Blocking	Antibody Incubation, Washing	Detection
Rapid protein isolation with PureProteome™ magnetic beads, pg. 2	High protein binding with Immobilon® membranes, pg. 4	22-minute immunodetection with SNAP i.d.® protein detection system, pg. 7	SNAP i.d.® protein detection system, pg. 7	Premixed Luminata™ Western HRP substrates for stronger signals, pg. 13
Fast, effective concentration with Amicon® Ultra centrifugal filters, pg. 3		Protein-free Bløk® noise-cancelling reagents, pg. 10	Optimized antibody conditions for the SNAP i.d.® detection system, pg. 9	

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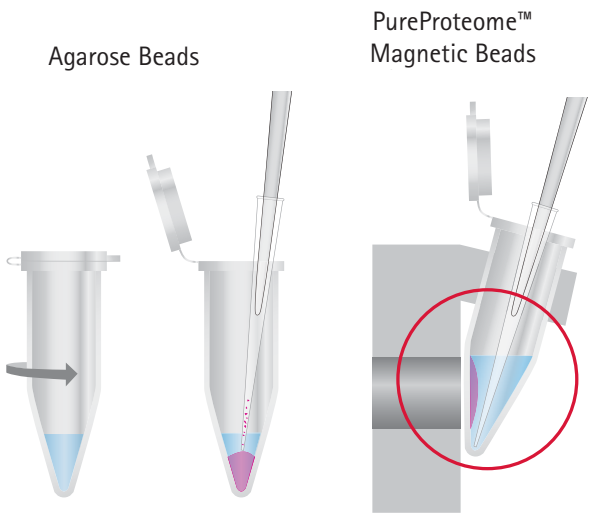
Protein Extraction & Sample Preparation

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.

Affinity Purification

For extremely fast and easy protein purification, trust our magnetic bead purification systems, which feature low non-specific binding and minimal sample loss. Conventional purification methods require centrifugation to pellet, followed by careful aspiration to avoid losing sample. Magnetic beads are isolated using a magnetic stand, enabling total removal of buffers and complete recovery of beads with no sample loss.

Affinity Purification:
Why choose magnetic beads over agarose?



	Agarose Beads	PureProteome™ Magnetic Beads
Minimum beads required:	> 25 µL	2.5 µL

>25 µL of Agarose is required because of practical handling purposes, which is tenfold more than PureProteome™ magnetic beads.

When you do the calculation— PureProteome™ magnetic beads are more economical than agarose beads.

Affinity Purification with PureProteome™ Magnetic Beads

FEATURED PRODUCTS

PureProteome™ Nickel Magnetic Beads

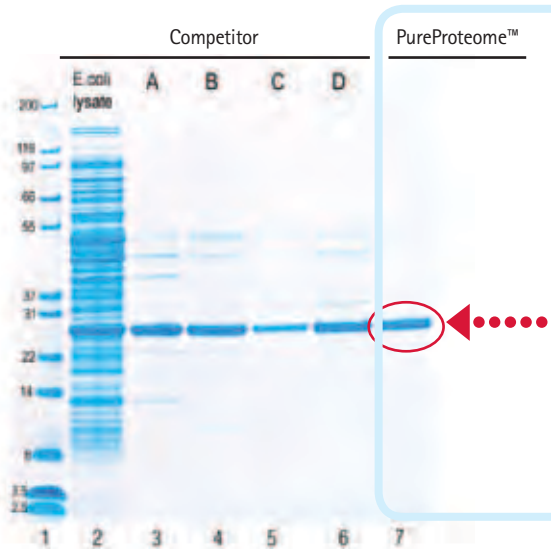
Bind, wash and elute His-tagged proteins 30 minutes faster than using agarose spin column methods.

Trust EMD Millipore to develop a superior magnetic bead system optimized to advance your research. Compared to other magnetic purification systems, PureProteome™ beads have a higher binding capacity and are captured more efficiently by our unique magnetic stands.

Advantages of PureProteome™ Nickel Magnetic Beads:

- **Be efficient with high capacity beads**: bind 28 mg His-tagged protein/mL settled beads
- **High affinity beads give you peace of mind**: proteins bind beads tightly in buffers containing EDTA
- **Achieve high purity and yields**: low binding of untagged proteins yields highly pure His-tagged proteins
- **Faster**: shorten your protocol by 15 to 30 minutes
- **Easier to use**: clearly visible beads enable you to monitor each step

PureProteome™ Beads vs. Other Magnetic Beads



LEFT: Polyhistidine-tagged 24 kDa protein purified from 1 mL *E. coli* culture with PureProteome™ beads (lane 7) and non-EMD Millipore magnetic beads (lanes 3-6). Coomassie blue-stained SDS-PAGE gel also shows MW standards (lane 1) and starting lysate (lane 2).

PureProteome™ Beads vs. Agarose Beads

RIGHT: Purification of 6X-His-tagged C-RP expressed in *E. coli*. 25 µL of settled beads (PureProteome™: 100 µL 20% slurry; Competitor G: 40 µL 50 % slurry) were washed in 10 mL binding buffer, then incubated with 500 µL *E. coli* lysate for 30 minutes at RT with end-over-end mixing. Beads were then washed three times with wash buffer containing 20 mM imidazole, then eluted with two fractions of 100 µL elution buffer containing either 250 mM imidazole (PureProteome™) or 500 mM imidazole (Competitor G). 10 µL of each sample was loaded.

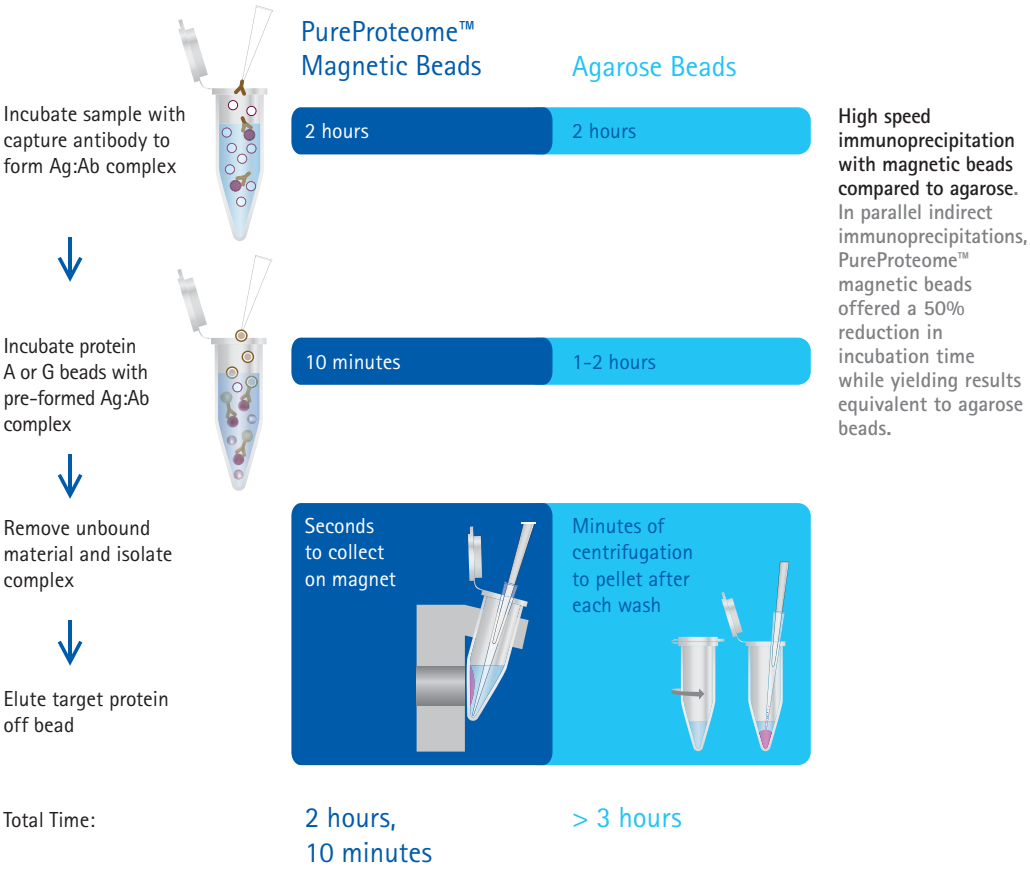
PureProteome™ Protein A & G Beads

Fast and easy immunoprecipitation

Traditional methods require hours of incubation time and minutes of harsh centrifugation to isolate sample. In contrast, PureProteome™ magnetic beads enhance binding equilibrium, enabling faster, gentler processing. The beads are easily resuspended for fast mixing and efficient interaction between the beads and protein.

Advantages of PureProteome™ Immunoprecipitation:

- **Be efficient with high capacity beads**: increased surface area allows for significantly greater binding capacity than non-EMD Millipore beads
- **Achieve high purity**: low non-specific binding of other proteins
- **Save time with fast sample processing:** enhanced binding equilibrium decreases incubation times by > 50%



High speed immunoprecipitation with magnetic beads compared to agarose. In parallel indirect immunoprecipitations, PureProteome™ magnetic beads offered a 50% reduction in incubation time while yielding results equivalent to agarose beads.

Relative Affinity of Protein A and Protein G

Key code for relative affinity of protein A and G for respective antibodies:

- Strong affinity
- Moderate/slight affinity
- Requires evaluation
- No affinity

Description	Catalogue No.
PureProteome™ Nickel Magnetic Beads	LSKMAGH10
PureProteome™ Protein A Magnetic Beads	LSKMAGA10
PureProteome™ Protein G Magnetic Beads	LSKMAGG10
PureProteome™ Albumin Magnetic Beads	LSKMAGL10
PureProteome™ Albumin/IgG Depletion Kit	LSKMAGD12
PureProteome™ Streptavidin Magnetic Beads	LSKMAGT10
PureProteome™ NHS FlexiBind Magnetic Beads	LSKMAGN04
PureProteome™ 8 place Magnetic Stand	LSKMAGS08
PureProteome™ 2 place Magnetic Stand	LSKMAGS15

Buffer Exchange and Concentration

FEATURED PRODUCTS

Amicon® Ultra Centrifugal Filters

Fast and easy protein concentration

Amicon® Ultra Centrifugal filters provide fast sample processing and promote high sample recoveries, even in dilute samples, through ultrafiltration. The unique features of the Amicon® Ultra centrifugal filters give you the fastest, most efficient concentration for sensitive downstream applications.

Amicon® Ultra Centrifugal Filter Advantages:

Maximize Concentration with Highest Protein Recovery

True Dead Stop

- Avoids spinning to dryness
- Provides a predictable concentration factor
- No need to calibrate for several samples to run in parallel

Reverse Spin Recovery

- Reverse spin devices enable you to maximize protein recovery without introducing pipetting errors
- Low binding membrane and polypropylene housing for >90% sample recovery

Fast an Without Compromise

Vertical membranes

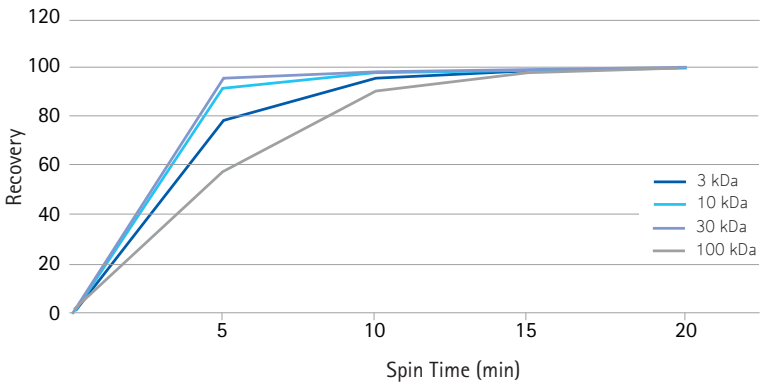
- Aligned with filtrate rather than perpendicular for less clogging, less waste and faster filtration
- Ultra-fast sample processing achieving concentration in as little as 10 minutes
- 25- to 80-fold concentration in a single step

Broad Chemical Compatibility

- Heat-sealed membrane eliminates adhesives and downstream extractables
- Large spectrum of compatibility
- Compatible with pH 1 to 9

Reliable Samples

- Spin precious samples with confidence in one robust, sleek unit that prevents leakage

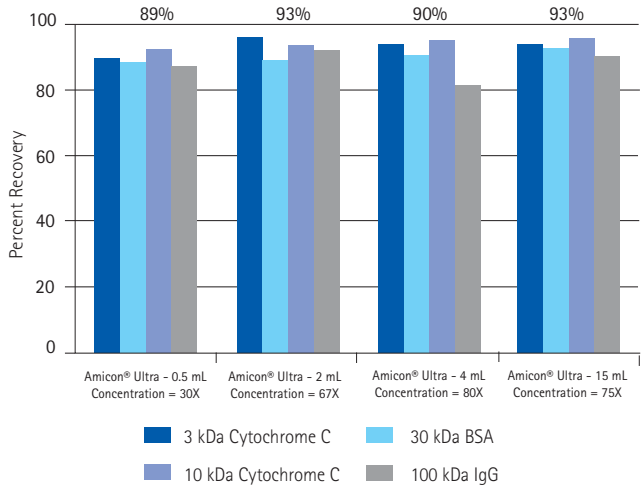


Amicon® Ultra 4 mL Filters—Fast Spin Times with Excellent Recovery

Average spin time for Amicon® Ultra-4 mL Filters: Four different proteins (3 kDa Cytochrome C, 10 kDa Cytochrome C, 30 kDa BSA, and 100 kDa IgG) were tested on the Amicon® Ultra-4mL Filters for percent recovery and spin time. The data show that greater than 95% of all protein was recovered in 15 minutes or less.

Consistently high recovery of diverse proteins with Amicon® Ultra filters

Concentration and percent recovery using Amicon® Ultra Filters: 4 different devices (Amicon® Ultra-0.5 mL, Amicon® Ultra-2 mL, Amicon® Ultra-4 mL, Amicon® Ultra-15 mL), were tested with four different proteins (3 kDa Cytochrome C, 10 kDa Cytochrome C, 30 kDa BSA and 100 kDa IgG) to determine percent recovery and concentration factor.



To select an Amicon® Ultra Centrifugal Filter, identify the starting volume, molecular weight of protein or nucleic acid being concentrated, final volume and concentration factor.

Then consult the product selection chart below to choose the Amicon® Ultra filter with the right molecular weight cutoff (MWCO).

	Amicon® Ultra-0.5	Amicon® Ultra-2	Amicon® Ultra-4	Amicon® Ultra-15
Starting Volume	< 0.5 mL	< 2 mL	< 4 mL	< 15 mL
MOLECULAR WEIGHT (MW)				
Proteins				
6 < MW < 20 k	3,000	3,000	3,000	3,000
20 < MW < 60 k	10,000	10,000	10,000	10,000
60 < MW < 100 k	30,000	30,000	30,000	30,000
100 < MW < 200 k	50,000	50,000	50,000	50,000
200 k < MW	100,000	100,000	100,000	100,000
LENGTH				
Single-Stranded and Double-Stranded Nucleic Acids				
137-1159 bp	30,000	30,000	30,000	30,000
PARTICLE DIAMETER (DIA)				
Nanoparticles				
1.5 < dia < 3 nm	3,000	3,000	3,000	3,000
3 < dia < 5 nm	10,000	10,000	10,000	10,000
5 < dia < 7 nm	30,000	30,000	30,000	30,000
7 < dia < 10 nm	50,000	50,000	50,000	50,000
10 nm < dia	100,000	100,000	100,000	100,000

MWCO: Molecular Weight Cut Off

10,000 MWCO Amicon® Ultra-4 and -15 filters are both CE marked for *in vitro* diagnostic use.

Once you've chosen the right Amicon® Ultra filter for your needs, choose your rotor, G force and spinning time for concentrating your molecule.

Designed as standard 1.5 mL, 15 mL conical or 50 mL conical tubes, Amicon® Ultra filters fit all stardard rotor types.

CHOOSE A ROTOR AND G FORCE

CONCENTRATION FACTOR

ADJUST SPINNING TIME

	Amicon® Ultra-0.5	Amicon® Ultra-2	Amicon® Ultra-4	Amicon® Ultra-15
				
Starting Volume	< 0.5 mL	< 2 mL	< 4 mL	< 15 mL
Final Volume	15–20 µL	15–70 µL	50 µL	200 µL
Design of the Device	Standard 1.5 mL	Standard 15 mL	Standard 15 mL	Standard 50 mL
Fixed-Angle (35 °) Rotor	14,000 g 1,000 g reverse spin	7,500 g 1,000 g reverse spin	5,000 g for 100,000 7,500 g for all other MWCO	5,000 g
Swinging Bucket Rotor	N/A	4,000 g 1,000 g reverse spin	4,000 g	4,000 g

Final Volume	15–20 µL with reverse spin	15–70 µL with reverse spin	50 µL	200 µL
Concentration Factor	X25–X30	X14–X67	X80	X75

For Proteins and Nanoparticles

3,000	30 min.	60 min.	40 min.	40 min.
10,000	15 min.	40 min.	15 min.	20 min.
30,000	10 min.	20 min.	10 min.	20 min.
50,000	10 min.	15 min.	10 min.	15 min.
100,000	10 min.	30 min.	10 min.	15 min.

Single-Stranded and Double-Stranded Nucleic Acids

30,000	10 min.	15 min., fixed angle 40 min., swinging rotor	10 min., 5,000 g, fixed angle	10 min., 5,000 g, fixed angle

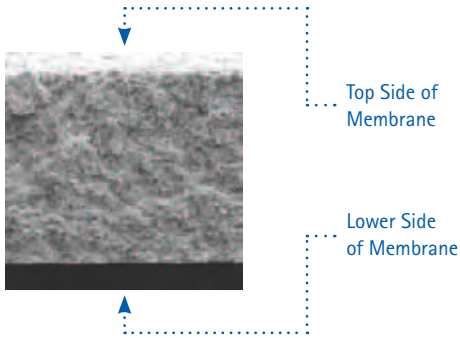
Amicon® Ultra Centrifugal Filters

	Product	Amicon® Ultra-0.5	Amicon® Ultra-2	Amicon® Ultra-4	Amicon® Ultra-15
	Maximum initial sample volume (mL)	0.5	2	4	15
	Final concentrate (retentate) volume (µL)	15–20	15–70	30–70	150–300
MWCO	**Qty/Pk**				
3,000 MWCO	8	UFC500308		UFC800308	UFC900308
	24	UFC500324	UFC200324PL	UFC800324	UFC900324
	96	UFC500396		UFC800396	UFC900396
	500	UFC5003BK			
10,000 MWCO	8	UFC501008		UFC801008	UFC901008
	24	UFC501024	UFC201024PL	UFC801024	UFC901024
	96	UFC501096		UFC801096	UFC901096
	500	UFC5010BK			
30,000 MWCO	8	UFC503008		UFC803008	UFC903008
	24	UFC503024	UFC203024PL	UFC803024	UFC903024
	96	UFC503096		UFC803096	UFC903096
	500	UFC5030BK			
50,000 MWCO	8	UFC505008		UFC805008	UFC905008
	24	UFC505024	UFC205024PL	UFC805024	UFC905024
	96	UFC505096		UFC805096	UFC905096
	500	UFC5050BK			
100,000 MWCO	8	UFC510008		UFC810008	UFC910008
	24	UFC510024	UFC210024PL	UFC810024	UFC910024
	96	UFC510096		UFC810096	UFC910096
	500	UFC5100BK			

To use the online Amicon® selector tool to choose the perfect filter and view protocols visit: www.millipore.com/AmiconSelect

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.

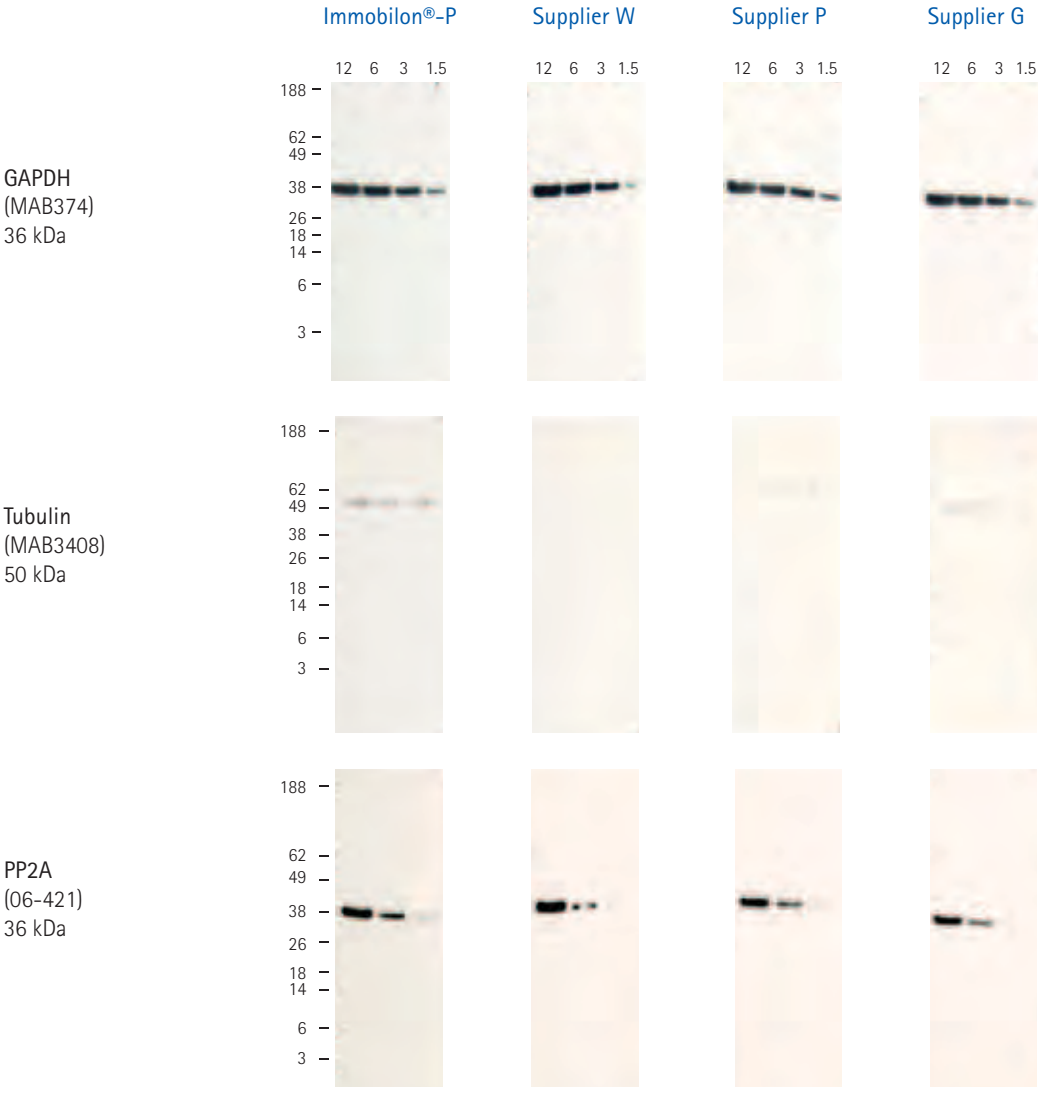
Key Benefits

- 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

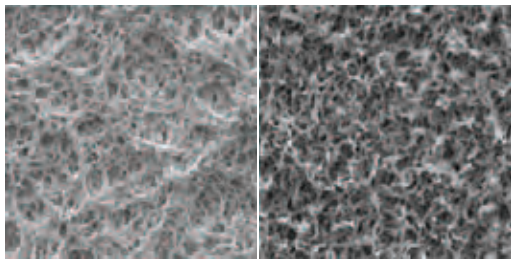
	Immobilon® NC	Immobilon®-P,	Immobilon®-P^{SQ}
Best used for	Transfers requiring hydrophilic membrane	Most protein transfers for any gel matrix	Small proteins (<20kDa), lysates or difficult Westerns
Composition	Mixed cellulose esters (MCE)	PVDF	PVDF
Hydrophilicity	Hydrophilic	Hydrophobic	Hydrophobic
Pore size	0.45 µm	0.45 µm	0.2 µm
Detection method	Chemiluminescence Fluorescence	Chemiluminescence	Chemiluminescence Fluorescence
Protein binding capacity	Insulin: 117 µg/cm^2 BSA: 160 µg/cm^2 Goat IgG: 259 µg/cm^2	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

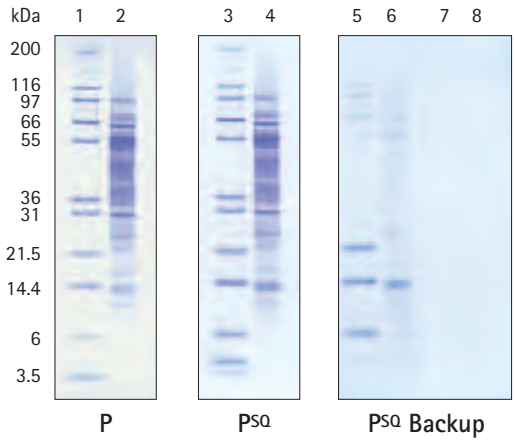


Description	Qty	Catalogue No.
Immobilon®-P PVDF Transfer Membrane, 0.45 µm		
26.5 cm x 3.75 m	1 roll	IPVH00010
7 x 8.4 cm	50/pk	IPVH07850
8.5 x 13.5 cm	10/pk	IPVH08130
20 x 20 cm	10/pk	IPVH20200

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} 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, 0.2um		
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.® Protein Detection System

Rapid Immunodetection in minutes

Publications citing the SNAP i.d.® system: ~125

The SNAP i.d.® system is quickly becoming the new gold standard for the immunodetection phase of Western blotting.

How Does the SNAP i.d.® System Work?

The vacuum-driven SNAP i.d.® Protein Detection System 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 as well as decreased antibody volumes, 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.

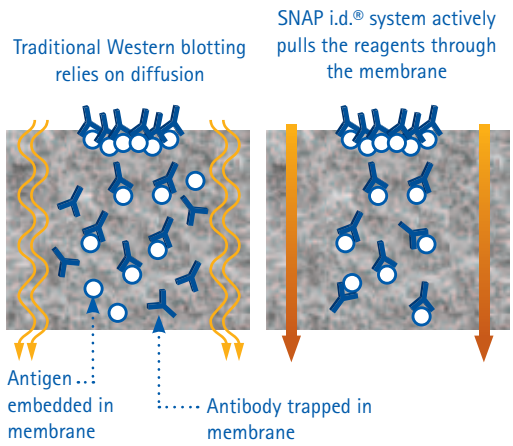
Key Benefits

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

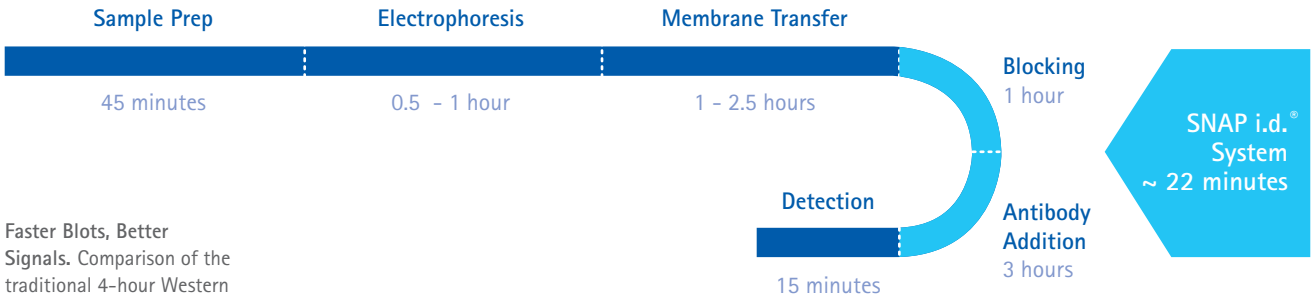
Key Features

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

How does SNAP i.d.® 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.® system actively pulls the antibodies through the membrane for maximum interaction with the antigens without a residual high background.



SNAP i.d.® in the Western blotting workflow



Faster Blots, Better Signals. Comparison of the traditional 4-hour Western blotting protocol relative to SNAP i.d® system's 22-minute protocol.

SNAP i.d® Protein Detection System

Description	Qty	Catalogue No.
SNAP i.d.® Protein Detection System	1	WBAVDBASE

SNAP i.d® Consumables and Accessories

Description	Qty	Catalogue No.
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	1/pk	WBAVDROLL

Traditional	SNAP i.d.®	Protein
		PTEN
		NFκB
		GST mu
		ERK 1/2
		Synaptophysin
		STAT1
		Src
		CREB
		PP2
		TGFB1

Resources for the SNAP i.d.® System

Optimized Antibody Conditions for the SNAP i.d.® System

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

For a complete listing, visit the SNAP i.d.® Antibody Optimization Reference Guide at:
www.millipore.com/SNAPab

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

For a complete list of the 125 (and counting!) peer-reviewed publications citing the SNAP i.d.® system, visit **www.millipore.com/snappub**. Below are three sample references from the growing list:

1. Mihrshahi R., Barclay A.N., Brown M.H., (2009, October 15), Essential roles for Dok2 and RasGAP in CD200 receptor-mediated regulation of human myeloid cells, *J Immunol., 183(8)*, 4879-86.
2. Fujimori K., Ueno T., et al. (2010, March 19), Suppression of Adipocyte differentiation by aldo-keto reductase 1B3 acting as prostaglandin F2α synthase, *J Biol Chem., 285(12)*, 8880-6.
3. Sakane A., Honda K., Sasaki T., (2010, February), Rab13 regulates neurite outgrowth in PC12 cells through its effector protein, *Mol Cell Biol., 30(4)*, 1077-87.

Bløk®

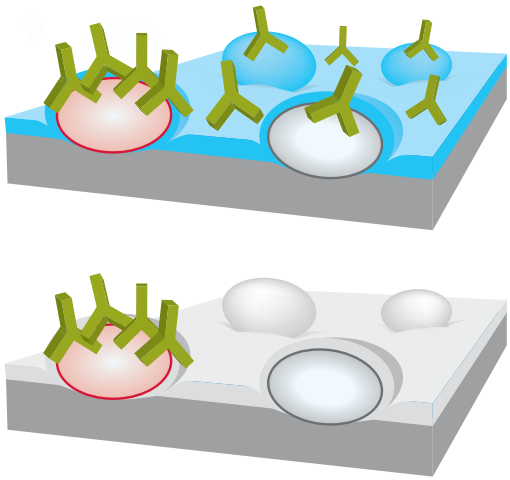
Noise Cancelling Reagents

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. Promotes non-specific interactions with antibodies, leading to an increased background.
2. Are not compatible with detection of protein phosphorylation due to the presence of phosphoproteins in milk.

How Does Bløk® Reagent Improve Results?

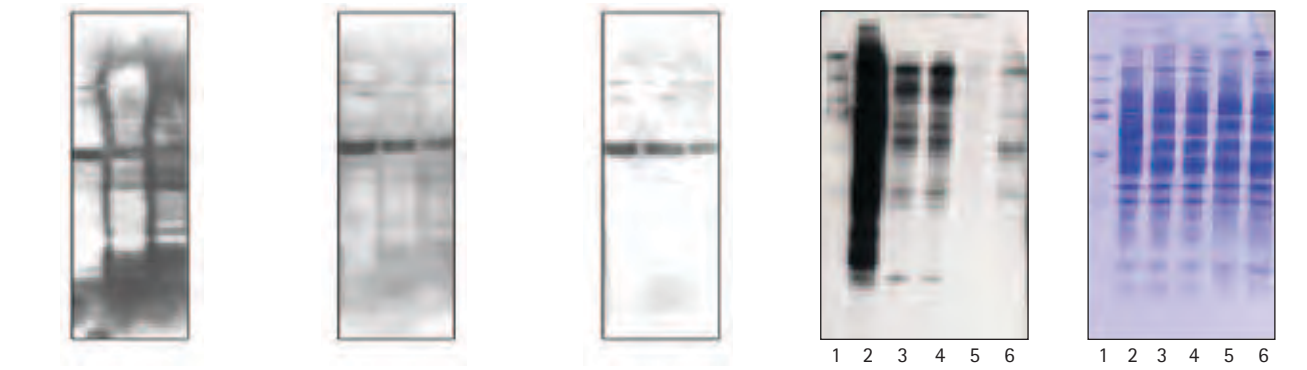
This chemical-based, protein-free blocker decreases background caused by non-specific antibody binding without leaving a thick, sticky layer similar to milk.



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.

Key Benefits

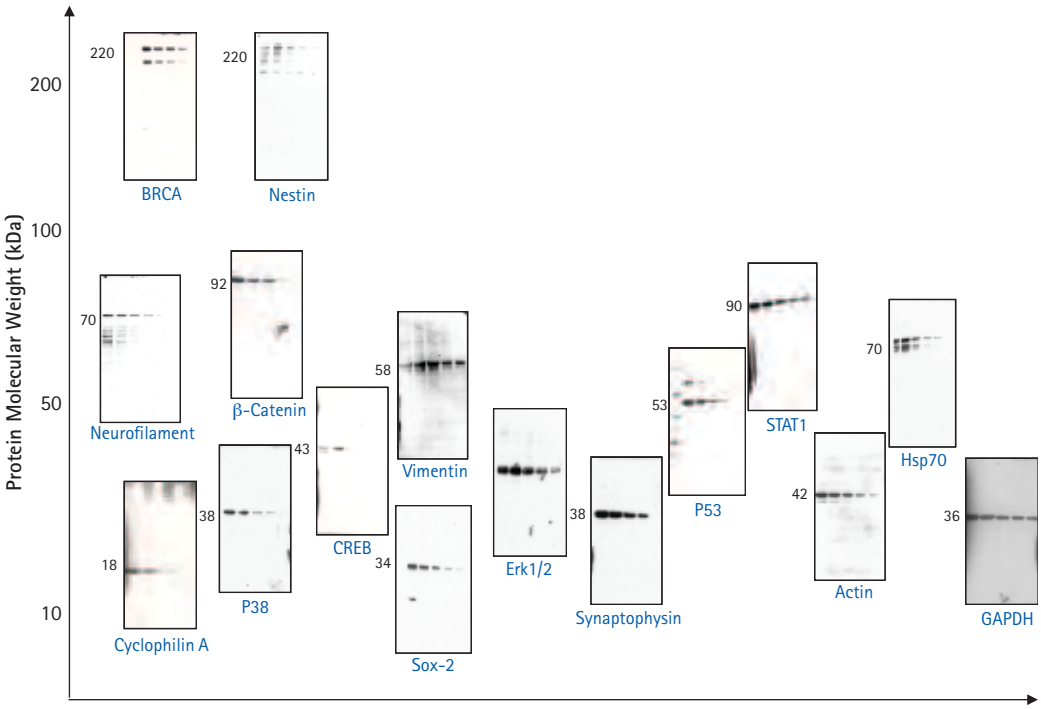
- Reduced background for better protein detection
- No need to run a second gel for Coomassie staining
- Stable at room temperature for 2 years
- Ready to use, no mixing required



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

Detection: Chemiluminescent Westerns

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*	~ 10 pg	~ 1 pg	~ 0.5 – 0.25 pg	~ 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.

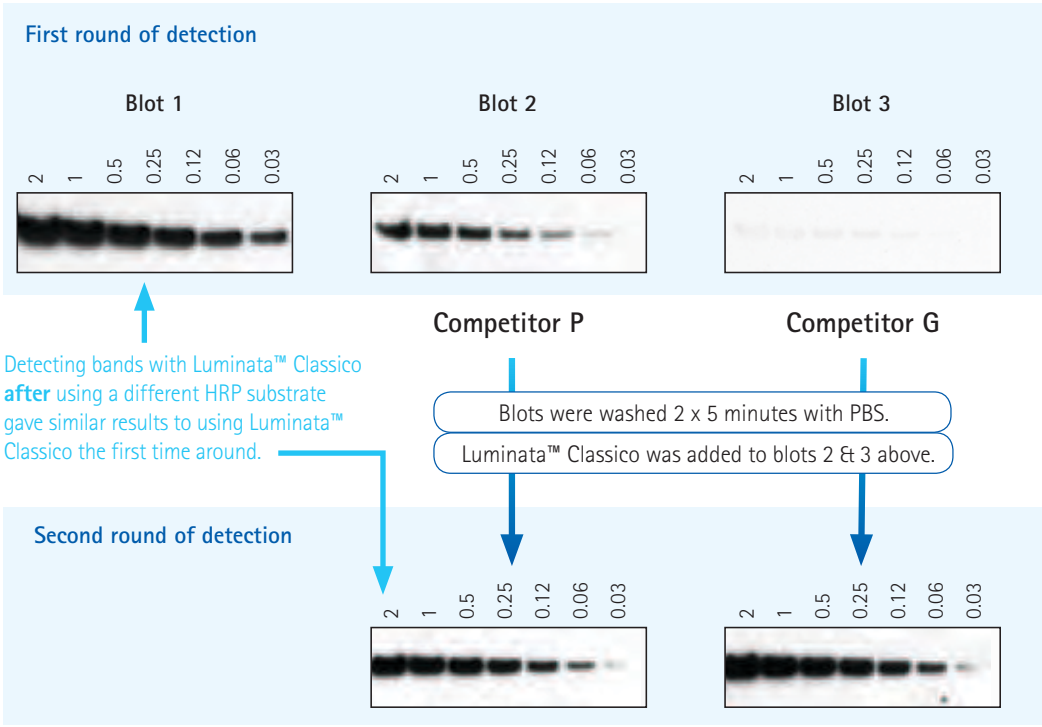
Test Luminata™ Substrates AFTER Your Regular HRP Substrate

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



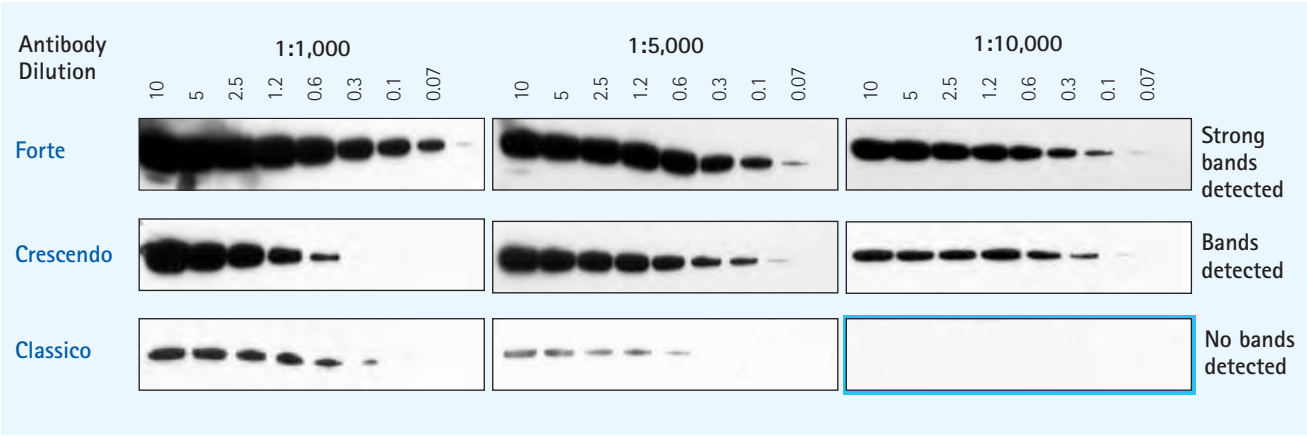
Re-detection of GAPDH. Three Western blots containing a 2-fold dilution series of A431 extract (ranging from 2–0.03 µg) were probed with 1:1000 dilution of anti-GAPDH (Catalogue No. MAB374) and 1:1000 dilution of anti-mouse HRP-conjugated secondary antibody (Catalogue No. AP124P). They were first visualized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

Peer-Reviewed 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

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 efficienty strip the blot led to a clean actin blot, even though both primary antibodies share the same anti-mouse secondary antibody.

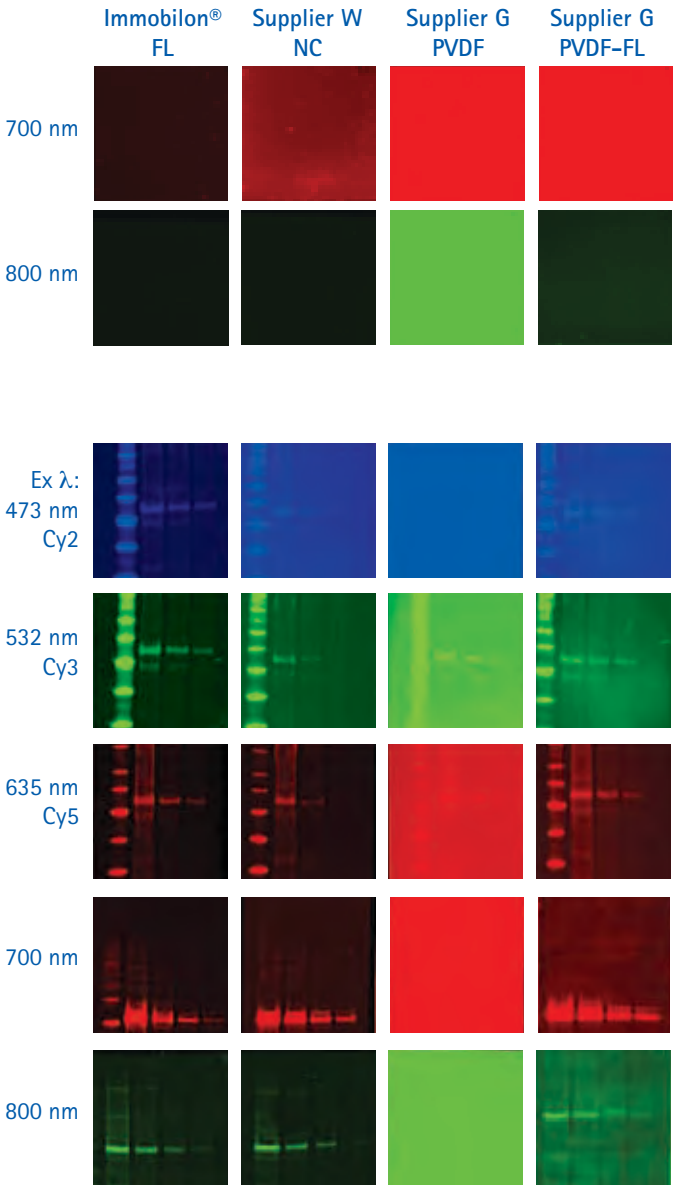
Description	Qty	Catalogue No.
ReBlot™ Plus Mild Antibody Stripping Solution, 10x	50 mL	2502
ReBlot™ Plus Strong Antibody Stripping Solution, 10x	50 mL	2504

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.
Visit **www.millipore.com/FLWestern** for more information.

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 (700-800nm)
- 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

Visit **www.millipore.com/FLWestern** for more information.

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.

How Does Bløk®-FL Reagent Improve Results?

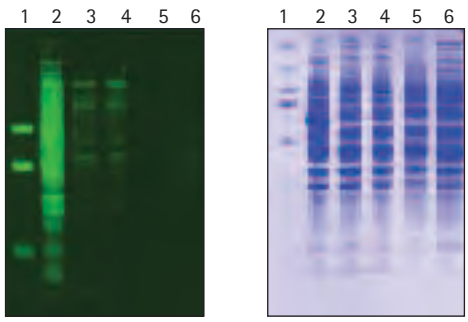
This chemical-based, protein-free blocker decreases background caused by non-specific antibody binding without leaving a thick, sticky layer similar to milk.

Key Benefits

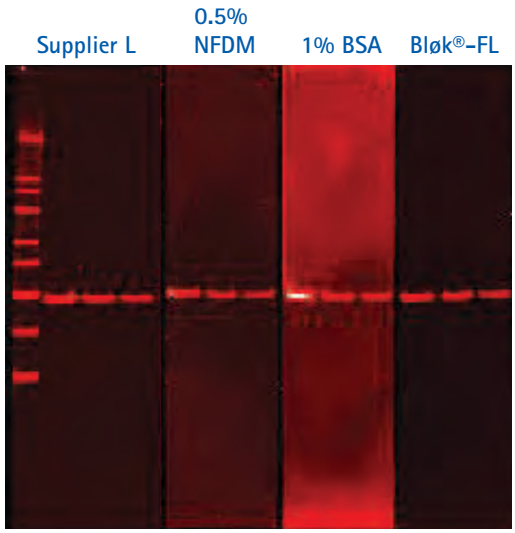
- Specially formulated for reduced background on fluorescent Westerns
- Ready to use straight from the bottle
- Stable at room temperature for 2 years
- Enables colorimetric staining of the blots after immunodetection

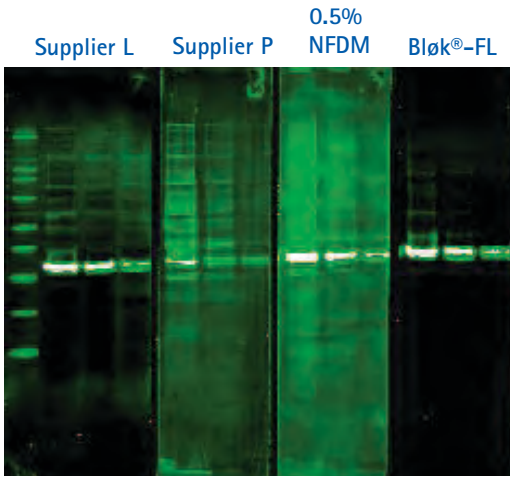
Avoid running a gel just for Coomassie staining

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.

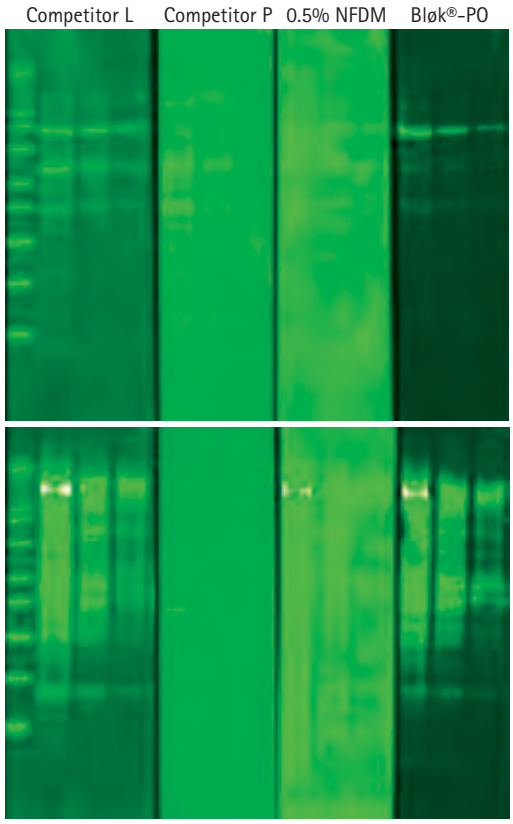
Description	Qty	Catalogue No.
Bløk®-FL Reagent for fluorescence detection	500 mL	WBAVDFL01
Immobilon®-FL Membrane, 0.45 µm		
26.5 cm x 3.75 m	1 roll	IPFL00010
7 x 8.4 cm	10/pk	IPFL07810
10 x 10 cm	10/pk	IPFL10100

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



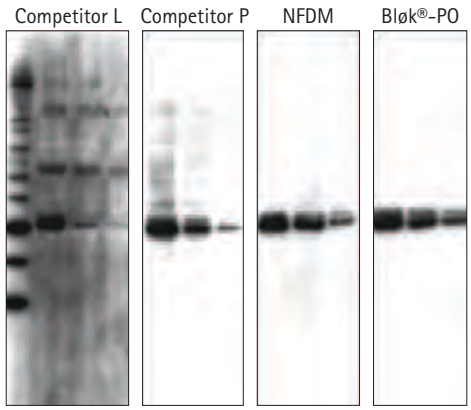
Bløk®-PO reagent works best for detection of phosphoproteins.

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.

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.

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

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 visit **www.millipore.com/techservic**e for customized assistance.

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.

Western Blotting Recipes

2X Sample Buffer

Component
130 mM Tris HCl pH 8.0
20% (v/v) Glycerol
4.6% (w/v) SDS
0.02% Bromophenol blue
2% DTT

8X Resolving Gel Buffer: 100 mL

Component
0.8·g SDS (add last)
36.3 g Tris base (=3M)

Adjust pH to 8.8 with concentrated HCl

4X Stacking Gel Buffer: 100 mL

Component
0.4g SDS (add last)
6.05 g Tris base (='0.5M)

Adjust pH to 6.8

10X Running Buffer: 1 L

Component
30.3 g Tris base (=0.25M)
144 g Glycine(=1.92 M)
10 g SDS (=1%, add last)

Do not adjust pH!

10X Transfer Buffer: 1 L

Component
30.3 g Tris base (=0.25M)
144 g Glycine(=1.92 M)

pH should be 8.3, do not adjust

Ordering Information

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.® System

Description	Components	Qty	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	1/pk	WBAVDROLL

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

For technical assistance, contact Millipore:
1-800-MILLIPORE (1-800-645-5476)
E-mail: tech_service@millipore.com
www.emdmillipore.com

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To fax an order, use 1-800-926-1166.
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In Canada:
For customer service, call 1-800-234-7437.
To fax an order, use 1-800-463-2996.
To order online: www.fishersci.ca

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