MERCK

reThink
Western Blotting

What are your Western blot results telling you?

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

fisher scientific
part of Thermo Fisher Scientific

western blotting tools

EXPLORE OUR PRODUCTS DESIGNED TO IMPROVE KEY STEPS OF THE WESTERN BLOTTING WORKFLOW.

Western blotting (also known as immunoblotting) is one of the most commonly used techniques in the lab, yet difficulties persist in obtaining consistent, quality results. At Merck, we've been helping scientists perform their Western blots for decades, with continuous problem-solving and steadfast technical support.

With legacy products from Millipore and Sigma-Aldrich, our catalog includes thousands of trusted products for Western blotting on which protein detection experts rely, including Roche cOmplete protease inhibitor cocktails, Immobilon® PVDF and Amersham™ Protran nitrocellulose transfer membranes, and ECL detection substrates. We never stop working to find innovative solutions that improve immunoblot reliability, speed, sensitivity, and quantitative potential to get you to publishable results in time, every time.

Access to our Western blotting expertise is easy—flip to our troubleshooting section at the end of this brochure.

TABLE OF CONTENTS

Protein extraction & sample preparation	Quantitation	Electrophoresis	Protein Transfer	Blocking & antibody incubation	Detection
Gentle protein extraction kits	Protein assay kits	TruPAGE™ precast gels	Immobilon® high protein binding membranes	30-minute blot processing with the SNAP i.d.® 2.0 system	Immobilon® HRP substrates
Rapid protein isolation with PureProteome™ magnetic beads		Molecular weight markers	Protran™ nitrocellulose membranes	Immobilon® signal enhancer	Luminata™ premixed HRP substrates
		Acrylamides	Blotting papers	Protein-free Bløk® noice cancelling reagents	Amersham ECL substrates
Fast, effective concentration with Amicon® Ultra centrifugal filters		Buffers	Blotting buffers	~75,000 primary antibodies for Western blotting: **SigmaAldrich.com/antibodies**	Hyperfilm™
		Protein stains	Protein blot stains	Secondary antibody conjugates	Chromogenic substrates
					Substrates for AP detection

ReThink

protein extraction & sample preparation

Experts agree that 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. Our quality reagents unite superior performance with speed to reduce exposure of proteins to unfavorable conditions, leading to more stable, intact proteins for downstream analysis.

Best practices for protein extraction and preparation:

- Extract using a fractionation technique suitable for your cell type, tissue, or organism.
- Protect samples from degradation by endogenous proteases and phosphatases with relevant enzyme inhibitors.

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.

- Nonmechanical extraction using BugBuster® or CytoBuster™ kits and reagents provides a simple and rapid release without denaturing soluble proteins.
- Pair with SIGMA*FAST*™ or Roche cOmplete® inhibitor tablets to ensure maximum yields of stabilized and intact proteins.
- Add Benzonase® nuclease to degrade nucleic acids and reduce viscosity of cellular extracts.

Our complete range of extraction kits and reagents provide you with an array of options so that you can construct the perfect extraction protocol for your specific cells or tissue and protein of interest.

Affinity purification

Affinity purification is based on the specific interaction of a target molecule with an immobilized ligand. We offer a wide range of tools for protein purification, including affinity magnetic beads, affinity agarose resins, and protease cleavage enzymes. To ensure that samples are enriched for protein(s) of interest, our depletion reagents eliminate common irrelevant, abundant proteins that may confound protein analysis.

- PureProteome™ magnetic beads are ideal for small volume affinity purification assays, such as immunoprecipitation and serum depletion or enrichment.
- Affinity agarose formats are suited for larger volume applications, such as antibody or recombinant protein purification.
- Protease cleavage enzymes are available in restriction grade or in kits for cleaving fusion proteins.

Purification portfolio

Application	Magnetic	Agarose
IP and Antibody Purification	Protein A	Protein A
	Protein G	Protein G
	Kappa Ig Binder	Protein G/Protein A
	Lambda Ig Binder	
Recombinant Tag Purification	His•Tag® purification	His•Tag® purification
	HIS-Select® purification resins	GST•Tag™ purification
		S•Tag™ purification
		Strep•Tag® II purification
		T7•Tag® purification
		Flag®, 3XFlag® expression/ purification
Protease Cleavage	Thrombin Factor Xa Enterokinase HRV 3C Protease	
Biotinylated Molecule Purification	Streptavidin	Streptavidin
Custom Labeled	NHS FlexiBind	
	Carboxy FlexiBind	

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.

protein quantitation

QuantiPro™ BCA Assay Kit

Kit provides components to measure very dilute protein concentrations in small sample volumes.

- Accurately measures protein concentrations from 0.5 to 30 µg/mL in tube assays and 1 to 20 µg/mL in 96- or 384-well plate assays

Bradford Reagent

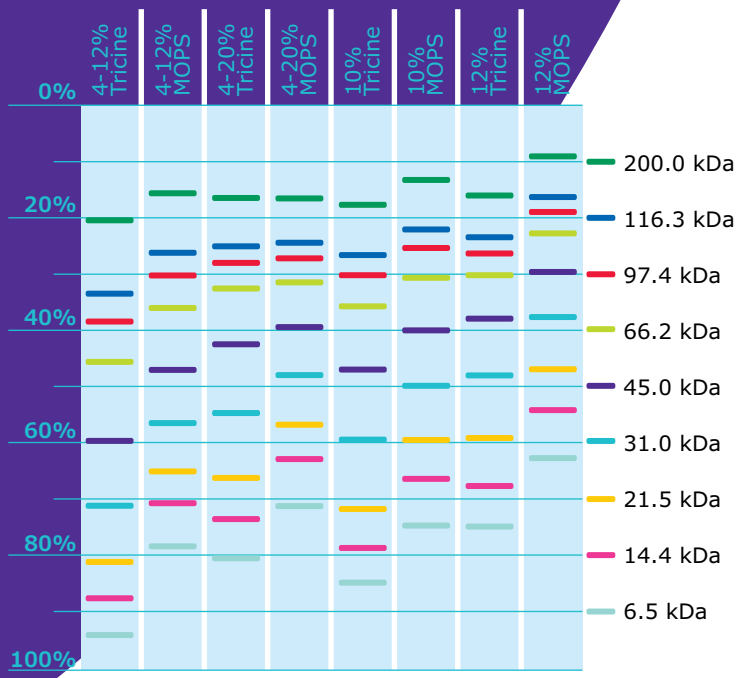
The Bradford assay is based on the complexing of proteins with Brilliant Blue G.

- The reagent is ready-to-use – no mixing or dilution required
- Color development is rapid. Incubate for five minutes; then read the sample at 595 nm
- Does not interfere with reducing sugars, reducing substances, or thiols
- Reagent is suitable for micro (1–10 µg/mL) and standard (50–1400 µg/mL) assays

electrophoresis

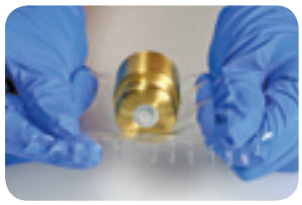
Electrophoresis is the technique most commonly used to separate extracted proteins for Western blot analysis. Applying an electrical current to a gel causes the proteins within the sample to migrate at different rates and separate within the medium according to their inherent properties. In the most common method, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), proteins separate according to their molecular weights. Attributes such as gel chemistry, running conditions, and acrylamide percentage can affect protein migration and should be considered in order to optimize results.

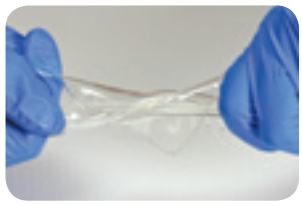
TruPAGE™ Migration Table



Best practices for electrophoresis

Select Gel & Buffer	Determine the gel electrophoresis system that is appropriate for your protein sample and targeted analyte, including acrylamide concentration and running buffer.
Prepare Sample	For SDS-PAGE, samples are treated with sodium dodecyl sulfate, which denatures the protein and equilibrates the charges across samples. Reducing agents, such as dithiothreitol (DTT) should be added to samples that contain proteins with disulfide bonds.
Load	Always load control samples (positive, negative, etc.) to ensure reliability of results. Consider published kDa of target when choosing a molecular weight marker to estimate protein size and monitor mobility progress and transfer efficiency.
Stain	Following electrophoresis, the gel can be stained in order to visualize and confirm successful migration of proteins.





TruPAGE™ Precast Gels & Buffers

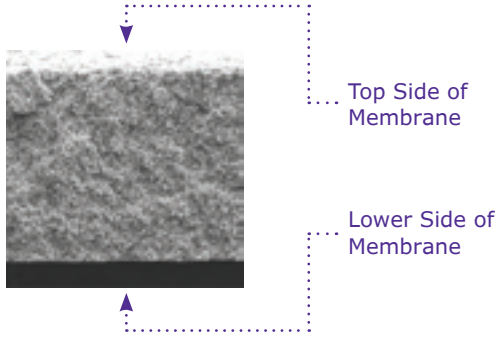
- Durable to resist tearing during experimental handling
- Compatible with popular electrophoresis equipment
- Deep wells with teeth that protrude above the backplate of the gel cassette, increasing well capacity and decreasing lane-to-lane overflow
- Extended shelf life means gels last up to 2 years from the date of manufacture when properly stored
- Fast – Run times as short as 30 minutes
- Affordable – Gels and buffers offer a cost-saving alternative without compromising performance and experimental integrity

protein transfer

Publications citing Immobilon® Membrane: >20,000
This family of trusted, quality transfer membranes includes Immobilon®-P Membrane, the first and most commonly used PVDF membrane for Western transfers.

How do Immobilon® membranes work?

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



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

Key Benefits

- Stronger protein signals due to high adsorption & retention of protein in the membrane
- Prolonged shelf life due to higher tensile strength
- Easier stripping & reprobing with PVDF membranes
- A variety of pore sizes provides optimal protein retention

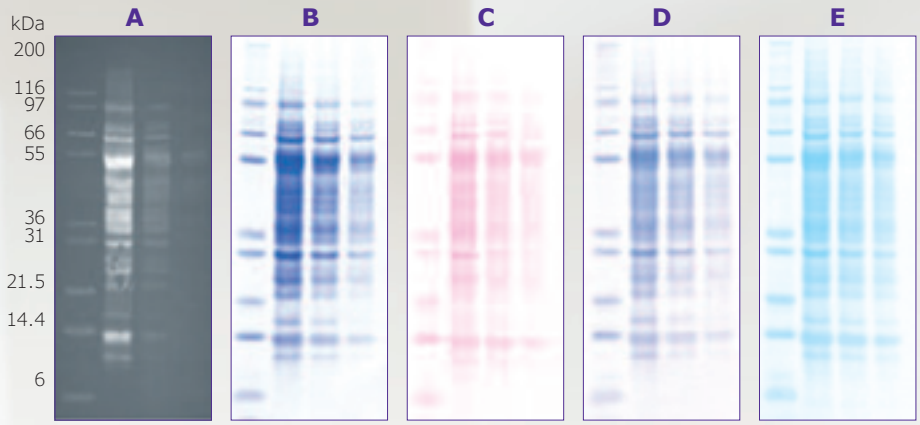
Membrane comparison table

	PVDF Membranes			Nitrocellulose Membranes			
Manufacturer	**Merck**			**GE Healthcare Life Sciences**			
Membrane Name	**Immobilon® P**	**Immobilon® FL**	**Immobilon® P^SQ**	**Amersham® Protran**	**Amersham® Protran premium**	**Amersham® Protran**	**Amersham® Protran premium**
Pore Size	0.45 µm	0.45 µm	0.2 µm	0.45 µm	0.45 µm	0.2 µm	0.2 µm
Detection methods	Chemiluminescence	Chemililuminescence, Fluorescence	Chemiluminescence, Fluorescence	Chemiluminescence	Chemiluminescence, Fluorescence	Chemiluminescence	Chemiluminescence, Fluorescence
Goat IgG	294 µg/cm²	300 µg/cm²	448 µg/cm²	115–125 µg	162–180 µg	150–176 µg	173–203 µg
BSA	215 µg/cm²	205 µg/cm²	340 µg/cm²	NA	NA	NA	NA
Insulin	160 µg/cm²	155 µg/cm²	262 µg/cm²	NA	NA	NA	NA
Pre-wet with ethanol, isopropanol or methanol	Yes			Not required			
Applications	Western blotting & other protein blotting methods. Durability of the membrane allows stripping and reprobing.			Protein and nucleic acid blotting.			
Western blotting selection criteria	• Target protein expressed over a broad dynamic range • Wide range of molecular weights		• Target protein <20 kDa • Very low abundance target protein • Low affinity antibody	• High-abundance target protein • Wide range of molecular weights		• High-abundance target proteins • Target protein <20 kDa	

Binding capacity

Immobilon®-P membrane transillumination for stain-free protein visualization, comparison to other stains

After transfer, proteins may be visualized by a variety of methods. If Immobilon® membranes are used, proteins may be visualized directly via the transillumination method (Reig and Klein, 1988). Detection sensitivity is comparable to Coomassie Brilliant Blue R stain when this method used in conjunction with an imaging system.



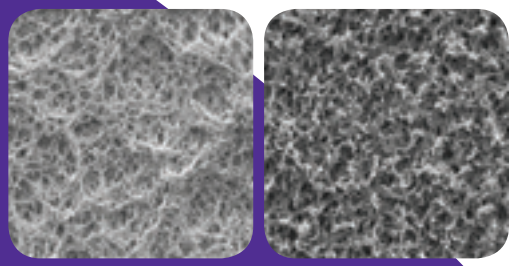
Calf liver proteins are visualized after electroblotting to Immobilon®-P membranes: (A) Transillumination, (B) Coomassie Brilliant Blue, (C) Ponceau-S red, (D) Amido black and (E) CPTS total protein stains. Left to right, molecular weight standards and 12.2 µg, 6.1 µg, 3.1 µg of the lysate per lane. For a detailed protocol, please refer to our Protein Blotting Handbook.

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

Publications citing Immobilon®-P^{SQ} Membrane: ~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} membranes have higher protein binding capacity, improving retention of small proteins.



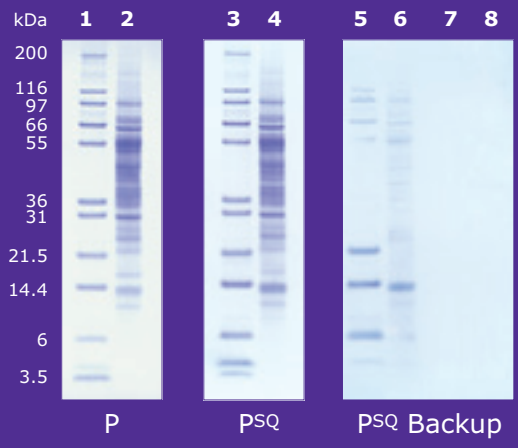
Scanning electron microscopy images (3000x magnification) showing the smaller & more uniform pores in the Immobilon®-P^{SQ} membrane (right) relative to Immobilon®-P membrane (left).

Key Benefits

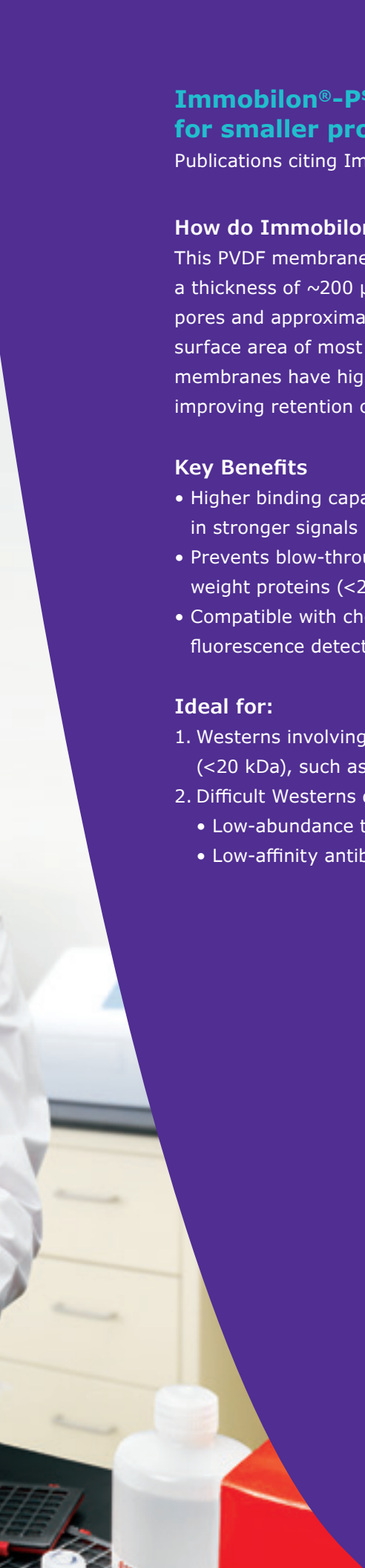
- Higher binding capacity and retention resulting in stronger signals
- Prevents blow-through of low molecular weight proteins (<20 kDa)
- Compatible with chemiluminescent and fluorescence detection techniques

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



Immobilon®-P^{SQ} membrane prevents low MW 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).



Immobilon®-FL transfer membrane

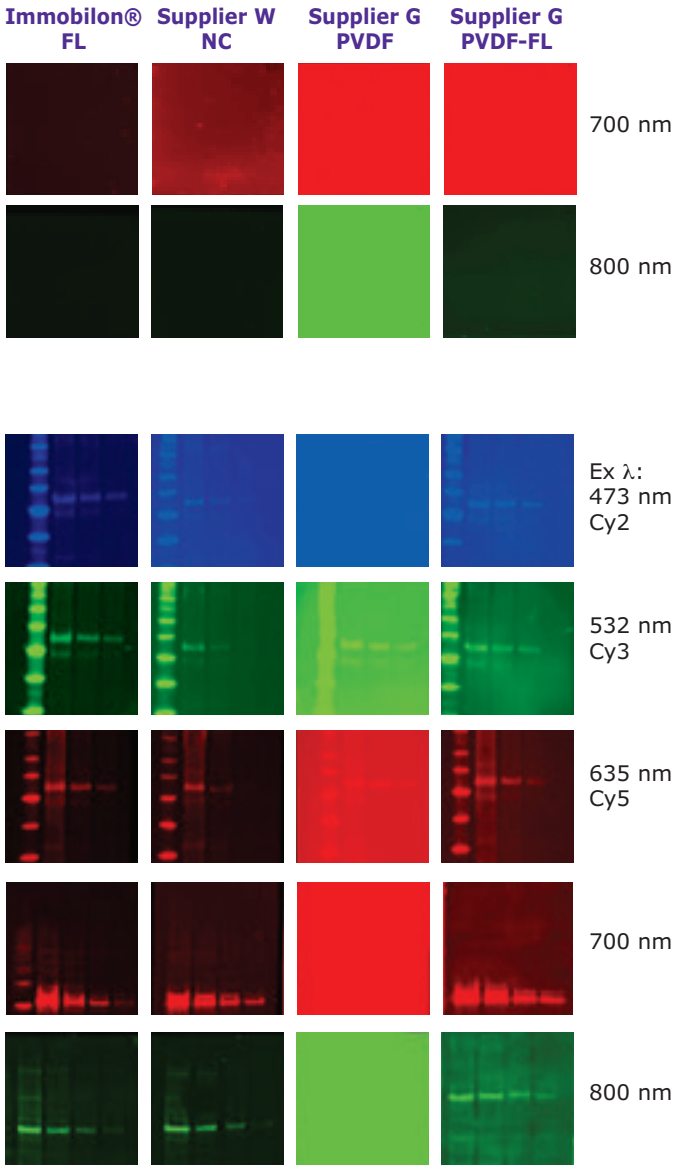
Publications citing Immobilon®-FL Membrane: ~9,000

Why choose Immobilon®-FL Membrane over other membranes for fluorescent Westerns?

This 0.45 µm-pore 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 best membrane for near-infrared wavelengths (700-800 nm)
- Strong signals due to higher protein adsorption and retention on the membrane
- Low background to detect even faint bands
- High tensile strength for multiple stripping and reprobing cycles

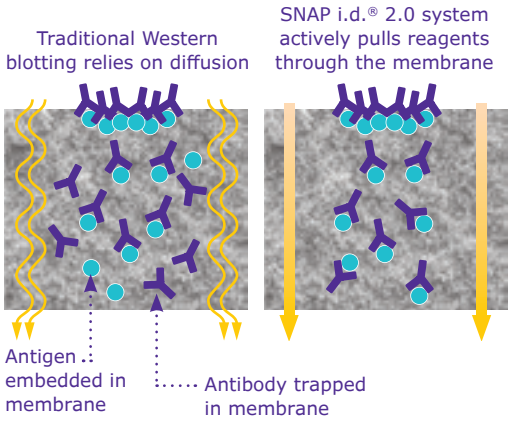


blocking & antibody incubation

SNAP i.d.® 2.0 protein detection system for Western blotting.

SNAP i.d.® 2.0 Protein Detection System

Unlike conventional Western blotting, where diffusion is the primary means of reagent transport, the SNAP i.d.® 2.0 system applies a vacuum to actively drive reagents through the membrane. This innovative technology promotes antigen binding and thorough washing, enabling you to better optimize your Western blotting conditions.



How does the SNAP i.d.® 2.0 system reduce 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 working solutions through the membrane for maximum interaction of antibodies with targeted antigens and high efficiency in blocking and washing that reduces nonspecific binding.

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

The vacuum-driven SNAP i.d.® 2.0 system takes full advantage of three-dimensional reagent distribution and reduces 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, reducing nonspecific binding that leads to background signal.

Key Benefits

- Faster results
- Reduces antibody optimization time
- Increases daily Western blot throughput

Key Features

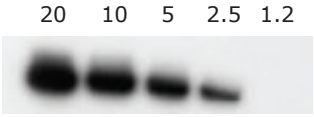
- Fastest immunodetection on the market
- Increased antibody-antigen binding
- Superior washes for lower background
- Save antibody by collecting and reusing your solutions

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

Immunodetection in as little as 30 minutes!

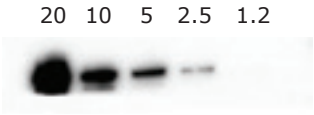
	Blocking	1st Antibody Addition & Incubation	Washing	2nd Antibody Addition & Incubation	Washing	
Conventional Immunodetection	~1 hr	>1 hr–overnight	~15 min	>1 hr	~15 min	4–20 Hrs
						vs.
SNAP i.d.® 2.0	20 sec	10 min	3 min	10 min	3 min	≤30 min

SNAP i.d.® Analysis



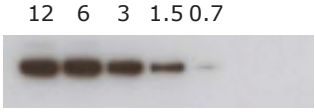
Anti-Huntingtin Protein (Cat. No. MAB2166)
1:400 dilution of this antibody detected Huntingtin protein in rat brain lysate (20–1.2 µg).

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.



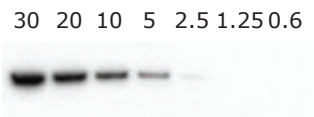
Anti-Metabotropic Glutamate Receptor 5 (Cat. No. AB5675)
1:200 dilution of this antibody detected Metabotropic Glutamate Receptor 5 in rat brain lysate (20–1.2 µg).

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) (Cat. 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 (Cat. 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.

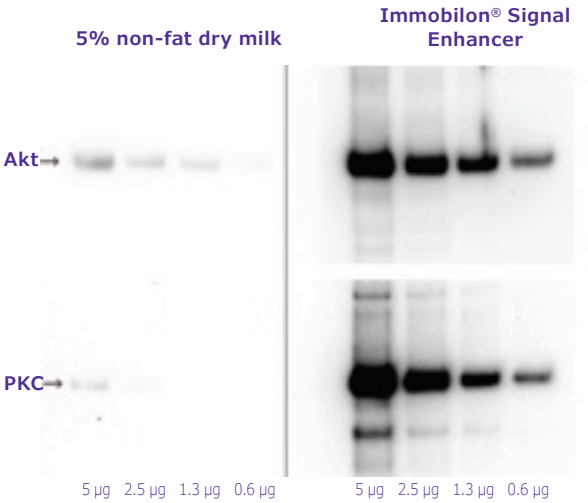
Immobilon® Signal Enhancer

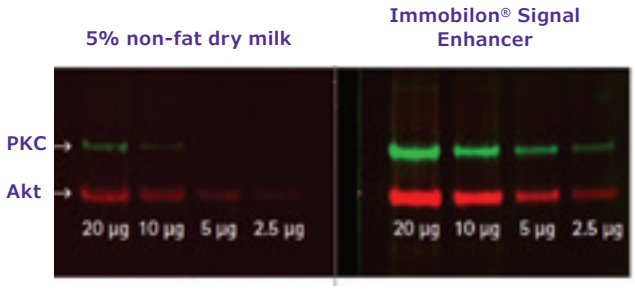
New Immobilon® Signal Enhancer combines signal amplification and blocking in one ready-to-use reagent.

Boost signal: Amplify low-intensity signal in Western blots—without amplifying noise

Save primary antibody: Use Immobilon® Signal Enhancer to reduce the amount of valuable primary antibody required

More signal. Less antibody. Blot A: 5% non-fat dry milk in TBST was used for blocking and antibody dilutions. Anti-Akt antibody was diluted 1:5,000. Blot B: Immobilon® Signal Enhancer was used for blocking and antibody dilutions. The amount of primary antibody was reduced ten-fold, allowing it to be used at a dilution of 1:50,000.





Two-fold dilution series of EGF-stimulated A431 cell lysate were resolved by SDS-PAGE and transferred onto Immobilon®-P or Immobilon®-FL membrane. Blots were blocked with either 5% non-fat dry milk or Immobilon® Signal Enhancer. Primary antibodies (rabbit anti-Akt, Cat. No. 05-796 and mouse anti-PKC antibody, Cat. No. 05-983) and the labeled secondary antibodies were diluted in the respective reagents at identical dilution ratios. All blots were compared under the same conditions.

Bløk® Noise cancelling reagents

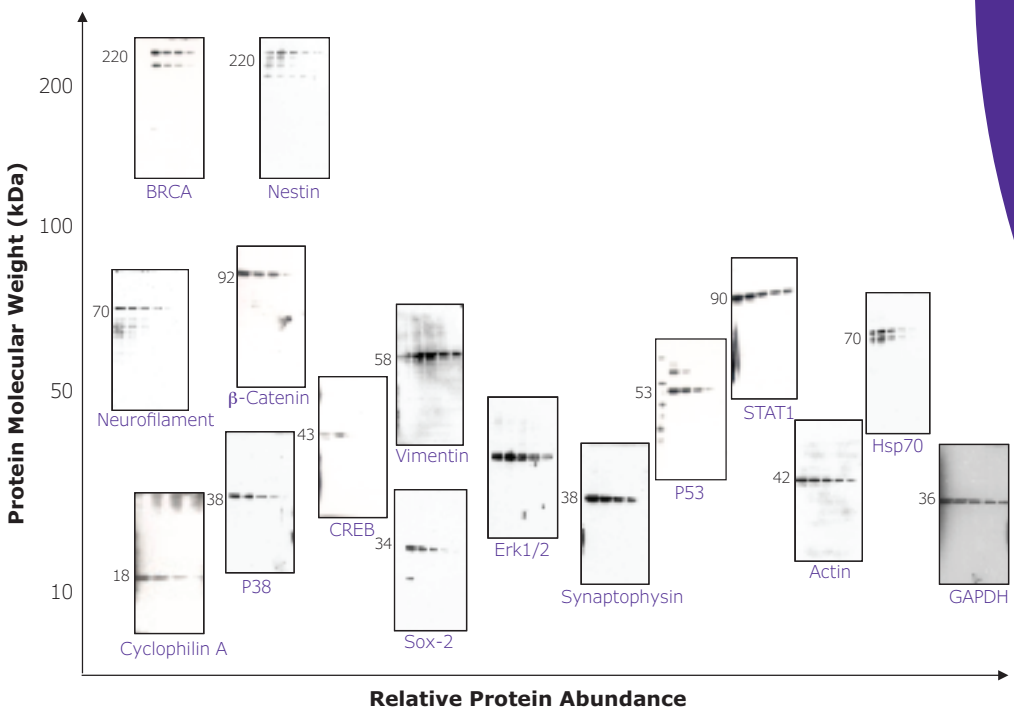
In Western blotting, blocking of unbound membrane sites is necessary to prevent the non-specific binding by antibodies that leads to high backgrounds. **Traditional milk/protein-blockers** can leave a thick layer of sticky proteins that:

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

Bløk® Reagent Key Benefits

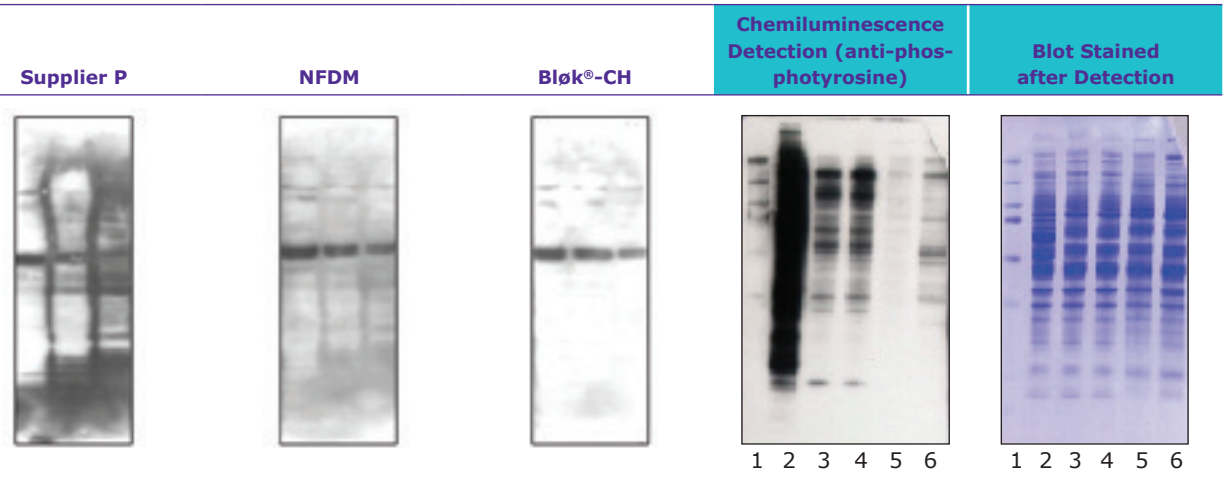
- Reduced background for better protein detection
- No need to run a second gel for Coomassie staining
- Stable at room temperature for 2 years
- Ready to use, no mixing required

Bløk® reagents excel with diverse antibodies and lysates



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

Bløk®-CH noise cancelling reagent



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 indicated were used during the blocking and antibody incubation steps.
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 reagent 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 immediately after immunodetection ruled out the possibilities of loading and transfer errors.

Technique Spotlight

Bløk®-PO noise cancelling reagent

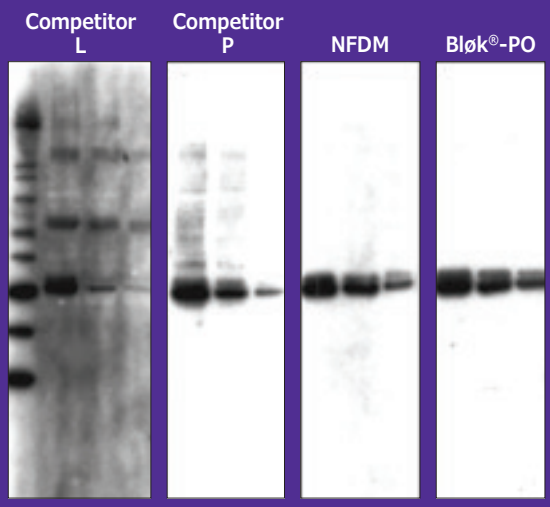
Blocking of non-specific protein binding sites on a blot is essential to decreasing the background and obtaining meaningful results. Although milk is the most commonly used blocker, the presence of phosphorylated mammalian proteins in milk can results in a very high background. For that reason, non-protein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

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



Chemiluminescence detection of pERK in EGF-stimulated A431 lysate (serial dilution ranging from 10–2.5 µg/lane, Cat. No. 12-110). Blots were blocked in the specified reagent (above) then probed with anti-pERK antibody (1:10,000, Cat. No. 05-797R) diluted in the respective blocking buffer. Bands were detected using Luminata™ Forte Western HRP substrate (Cat. No. WBLUF0500). NFDM= Non-fat dry milk.

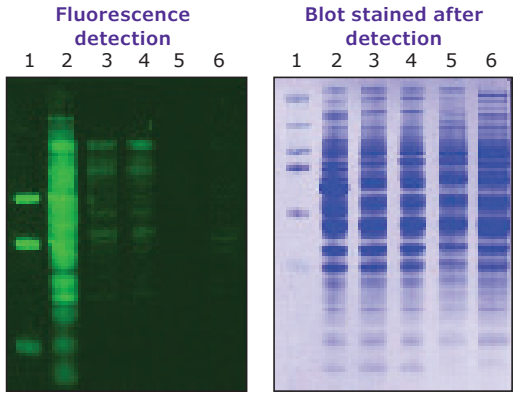
Bløk®-FL noise cancelling reagent

Key Benefits

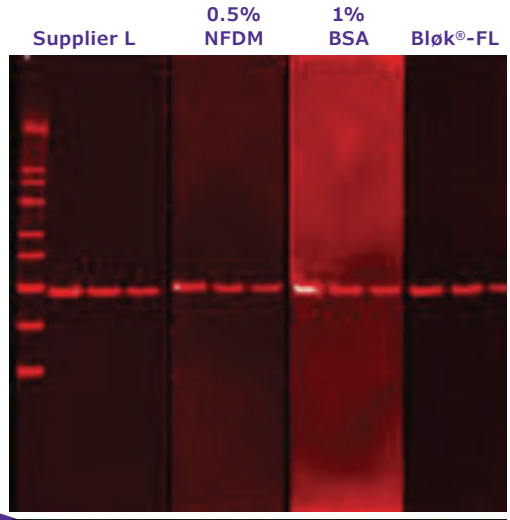
- Specially formulated for reduced background on fluorescent westerns
- Ready to use straight from the bottle
- Stable at room temperature for 2 years
- Enables colorimetric staining of blots after immunodetection

Avoid running a gel just for Coomassie staining

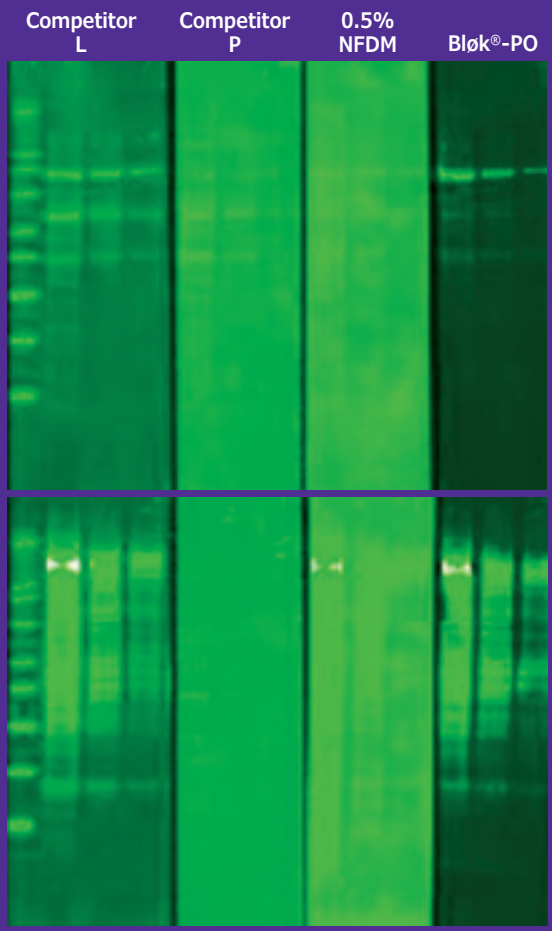
The combination of Bløk® Noise Cancelling Reagents and Immobilon®-PVDF membranes enable membrane staining after immunodetection.



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 reagent and probed with anti-phosphotyrosine, clone 4G10®, and detected by fluorescence. Lanes 5 and 6 showed significantly lower signal than lanes 3 and 4 with both detection methods. Staining with Coomassie blue immediately after immunodetection ruled out the possibilities of loading and transfer errors.



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



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

Technique Spotlight

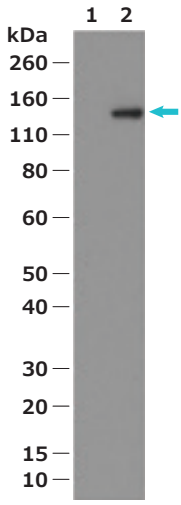
Phosphospecific antibodies

Merck's extensive portfolio of antibodies includes over 600 validated, phosphospecific antibodies. These antibodies are critical tools for the exploration of biological pathways and signals that involve phosphorylation.

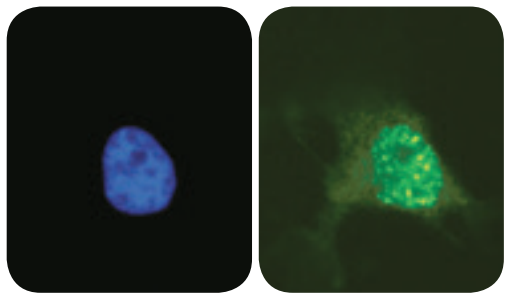
Anti-phospho-MYPT1 (Thr696) (Cat. No. ABS45)

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 Ca2+ 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.

Description	Qty.	Cat. 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



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).



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, Cat. No. 05-636), clone JBW301 (green, right panel); DNA stained with DAPI (left panel).

Loading control antibodies for Western blotting

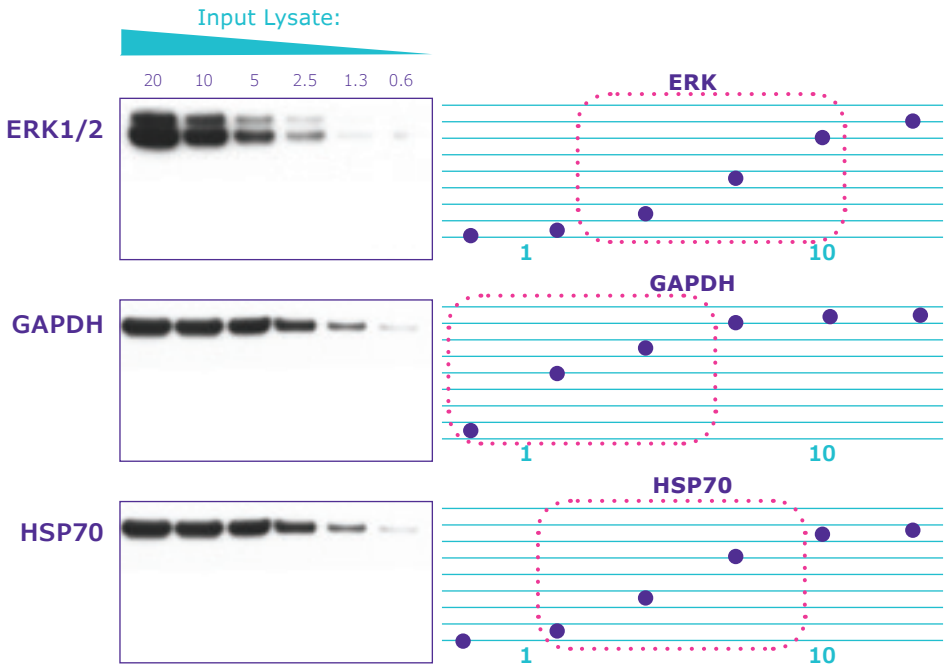
Proteins that exhibit abundant, constitutive levels in the Western blot sample can be used to control for technical variability. These so-called loading controls are essential for confirming that differences in observed signal in Western blot experiments are caused due to changes in protein expression, and not to artifacts introduced during sample loading, transfer and other workflow steps.

Although a few key so-called “housekeeping” proteins have sometimes been used as default loading controls, it is important to consider the characteristics of the target protein as well as experimental conditions of your Western blot assay.

Factors that should influence selection of an antibody against a loading control protein include:

- Constitutive expression of the loading control protein in the sample of interest
- A molecular weight dissimilar from that of the target, so that bands may be resolved
- Linear signal at the amount of lysate loaded in each gel lane
- Antibody validated for use in Western blot

Evaluation of signal linearity for prospective loading controls



Western blot of ERK1/2, GAPDH, and HSP70 shows the linear range of quantitation for each protein for the same sample. Cell line source for lysate, A431, Cat. No. 12-110. (x-GAPDH, Cat. No. MAB374; x-HSP70, Sigma SAB4200714; x-Erk1/2, Merck 06-182*) (ECL reagent, Luminata™ Classico Western HRP substrate, Cat. No. WBLUC0500).

Loading Control Antibodies

Loading control antibody	Species reactivity	Host	MW of antigen	Catalog No.
Anti-Vinculin, Clone V284	Hu, Ms, Rt, Rb, Ch	M	124 kDa	05-386
Anti-Vinculin, Clone hVIN-1	Hu, Ms, Rt, Ca, Ch, Bv, Tk, Fg	M	116 kDa	V9131
Anti-HSP90, Clone D7a	Hu, Ms, Rt, Rb, Bv, Ch, Po	M	90 kDa	05-594
Anti-HSP90, Clone 803CT9	Hu, Ms	M	85 kDa	SAB1305541
Anti-HSP70, Clone C92F3-5	A broad range of species	M	70 kDa	386032
Anti-HSP70, Clone BRM-22	A broad range of species	M	70 kDa	SAB4200714
Anti-Lamin B1, Clone 8F10.1	Hu, Rt	M	66 kDa	MABS492
Anti-Lamin B1, polyclonal	Hu	R	66 kDa	SAB1306342
Anti-HDAC1, Clone 2E10	Hu, Ms	Ms	55 kDa	05-100-I
Anti-HDAC1, polyclonal	Hu, Ms	Rb	65 kDa	H3284
Anti-GAPDH	H, M, Rt, B, Gp	Rb	36 kDa	ABS16
anti-GAPDH, polyclonal	Rb, Sh, Gp, Rt, Ms, Ca, Eq, Gt, Bv	Rb	36 kDa	SAB2108668
Anti-Actin, Smooth Muscle, Clone ASM-1/1A4	Hu, Ms, Rt, Ch, Bv	Ms	45 kDa	CBL171-I
Anti-Actin, Clone JLA20	Hu, Ch	Ms	42 kDa	MABT219
Anti-Actin, polyclonal	A wide range of organisms	Rb	42 kDa	A2066
Anti-Actin, polyclonal	plant	Rb	42 kDa	SAB4301137
Anti-Tubulin, Beta III, Clone 2G10	Hu, Ms, Rt, Bv	Ms	50 kDa	05-559
Anti-Cyclophilin B, polyclonal	Hu, Ms, Rt, Mk	Rb	20 kDa	SAB4200201
Anti-Cofilin, polyclonal	Hu, Rt	Rb	21 kDa	07-300
Anti-COX IV, polyclonal	Hu	Rb	17 kDa	AB10526
Anti-Cox IV-2 (COX42), polyclonal	Hu, Rt, Ms	Rb	20 kDa	SAB4503384
Anti-Histone H3, Clone 6.6.2	A wide range of organisms	Ms	17 kDa	05-499
Anti-Histone H3, polyclonal	Hu, Rt, Ms, Ch, Dr, Xe, plant	Rb	17 kDa	H0164
Anti-VDAC1, Clone N152B/23	Hu, Rt, Ms, Bv, Eq, RhM, Chp, Po	Rb	33 kDa	AB10527
Anti-VDAC1	Rt, Ms, Eq, Bv, Rb, Sh, Ca, Gp, Zbf	Rb	31 kDa	SAB2108496
Anti-PCNA, polyclonal	Hu, Ms, Rt, Chp	Rb	35 kDa	07-2162

detection

Chemiluminescent substrates for Western blotting

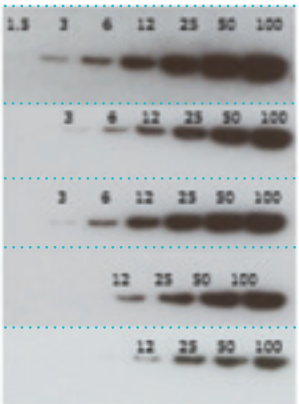
We offer a broad selection of ECL (enhanced chemiluminescence) substrates for every application.

Luminata™ Western HRP Substrates are a family of three premixed reagents for horseradish peroxidase-based detection that offer significant advantages over other ECL reagents:

- Single component, ready-to-use formulations simplify detection for enhanced reproducibility and convenience
- A range of sensitivities to provide optimal signal-to-background ratio across a spectrum of target protein concentrations

Immobilon© HRP substrates deliver exceptional sensitivity and long signal life in standard 2-component formats. These formulations permit the use of more dilute primary antibody solutions for immunoblot detection. Our newest product, Immobilon© ECL Ultra substrate, provides sensitivity at the low femtogram range with longer signal duration than other substrates in its class.

Relative Sensitivity of Chemiluminescent Substrates



Immobilon® ECL Ultra HRP Substrate

Immobilon® HRP Substrate

2-component reagents

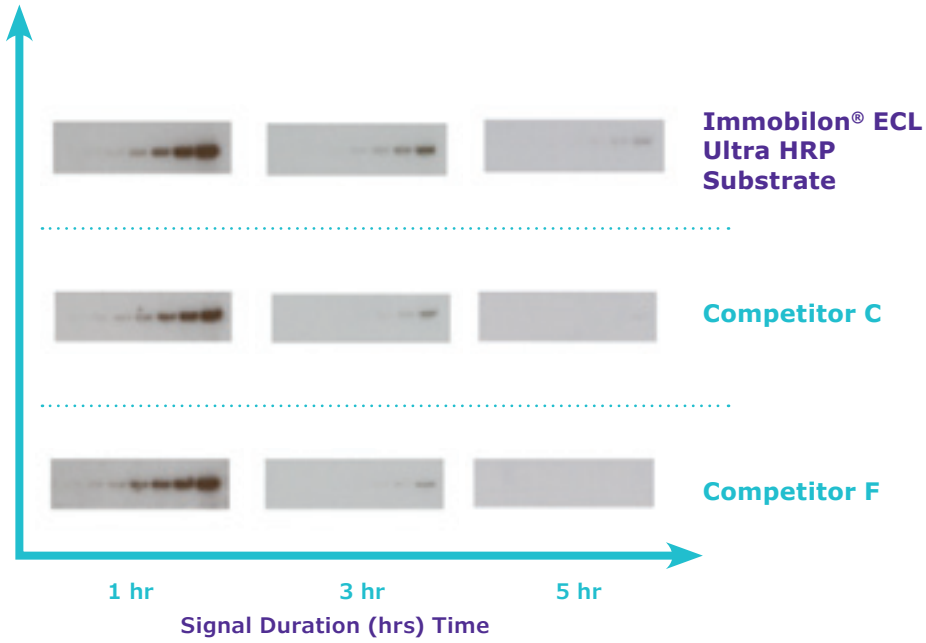
Luminata™ Forte

Luminata™ Crescendo

Luminata™ Classico

1-Component (pre-mixed, ready-to-use)

Comparison of signal duration and sensitivity between 3 different maximum sensitivity detection reagents:



	Luminata™ Classico	Luminata™ Crescendo	Luminata™ Forte	Immobilon® HRP Substrate	Immobilon® ECL Ultra HRP Substrate
Approximate Detection Limit*	**~ 6 pg**	**~ 1 – 3 pg**	**~ 400 fg**	**~ 400 fg**	**low fg**
Format	1-component	1-component	1-component	2-component	2-component
Signal Duration	1 hr	3 hr	3 hr	3 hr	5 hr
Stock Solution Stability	1 yr at 4 °C	1 yr at 4 °C	1 yr at room temperature	1 yr at 4 °C	1 yr at 4 °C
Working Solution Stability	1 yr at 4 °C	1 yr at 4 °C	1 yr at room temperature	7 days at 4 °C	30 days at 4 °C
2-component ECL reagents with similar sensitivity:					
Thermo	Pierce ECL	SuperSignal® Pico	SuperSignal® Dura	SuperSignal® Dura	SuperSignal® Femto
GE Healthcare	ECL	---	---	---	ECL Select
Bio-Rad	---	---	Clarity	Clarity	Clarity Max

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 with detecting with Luminata™ substrates alone. Try it, and you may detect bands you were not able to visualize previously.

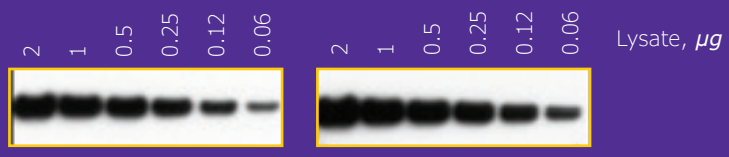
Visualized with specified HRP substrate

Blots were washed
2 x 5 min. with TBST

Luminata™ Classico was added

Revisualized with Luminata™ Classico

Reprobing blots with weak signal resulting from other HRP substrates with more sensitive reagents like Luminata™ Classico can improve signal intensity



Detection of GAPDH. Three Western blots containing a 2-fold dilution series of A431 extract (ranging from 2 µg–0.06 µg) were probed with 1:1000 dilution of anti-GAPDH (Cat. No. MAB374) and 1:1000 dilution of anti-mouse HRP-conjugated secondary antibody (Cat. No. AP124P) using a SNAP i.d.® system. Blots were first visualized with the indicated HRP substrate, then blots 2 and 3 were washed and re-visualized with Luminata™ Classico substrate. All blots were exposed to X-ray film for 1 minute.

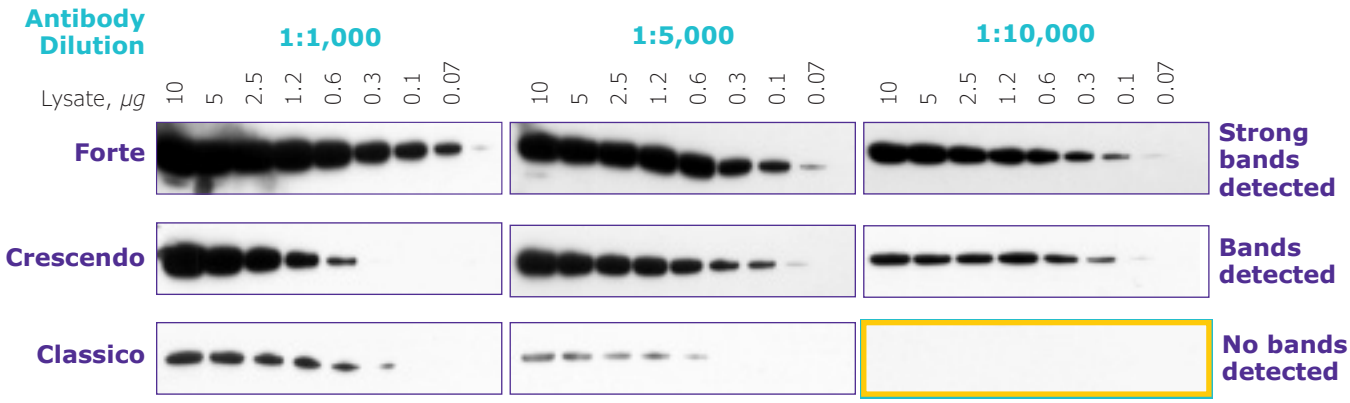
Obtain the best Western blots possible using Luminata™ Western HRP substrates

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

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

Using higher sensitivity HRP substrates produced the best results and was beneficial in three ways:

1. Better results: The increased-sensitivity detection reagent 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 the blot and apply a new substrate compared with 2.5 hours required to repeat antibody incubations.
3. Reduced cost: Appropriate selection of HRP substrates costs less than increasing the concentration of antibody.



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

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.

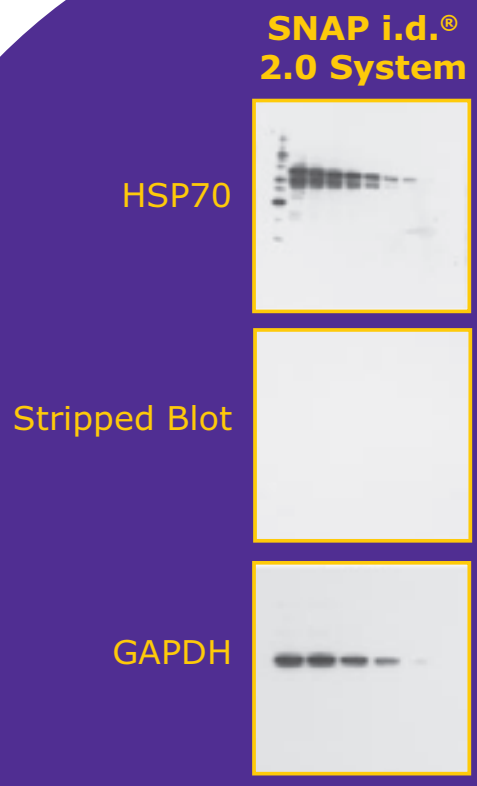
What is ReBlot™ Plus?

ReBlot™ Plus reagents efficiently strip probed blots of bound antibodies. ReBlot™ Plus reagents are available in “Mild” and “Strong” formulations.

- 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 odors
- Room temperature stripping in just 15 minutes
- Fast reuse of blots for multiple antibody probings
- Non-acidic, for reduced risk of protein degradation (such as in Edman degradation)



ReBlot™ efficiently strips blots to allow for fast reprobing with different antibodies.

Two-fold dilutions of A431 lysate were resolved by SDS-PAGE then transferred onto Immobilon®-P. The blot was initially probed with HSP70 (1:8,000, Cat. No. H5147) (top) using the SNAP i.d.® system. Following stripping using ReBlot™ Plus Strong for 15 minutes (middle), the blot was reprobed with anti-GAPDH (1:8,000, Cat. No. MAB374) (bottom).

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

Troubleshooting Western blots

Symptom	Possible Cause	Remedy
Immunodetection		
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 detergent (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.

Troubleshooting Western blots

Symptom	Possible Cause	Remedy
Immunodetection (continued)		
Persistent background	Non-specific binding	Use High Salt Wash. (PBS or TBS supplemented with 0.5% NaCl and 0.2% SDS)
High background (rapid immunodetection)	Membrane wets out during rapid immunodetection	Reduce the Tween®-20 detergent (<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 detec- tion 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		
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 manufacturer's instructions for blot imaging.

Timeless Recipes and Solutions for Blotting Success

Standard recipes for Western blotting

TBS 10x (concentrated Tris-buffered saline)
200 mM Tris
1500 mM NaCl

For 1 L:
24 g Tris base (formula weight: 121.1 g)
88 g NaCl (formula weight: 58.4 g)
Dissolve in 900 mL Milli-Q® water
pH to 7.6 with 12 N HCl
Add Milli-Q® water to a final volume of 1 L

1X solution: mix 1 part of the 10X solution with 9 parts Milli-Q® water and adjust pH to 7.6.

TBST (Tris-buffered saline, 0.1% Tween® 20)
For 1 L:
100 mL of TBS 10X
900 mL of Milli-Q® water
1 mL Tween®-20 detergent

4X SDS-PAGE sample loading buffer
1.5 mL of 1 M Tris-HCl pH 6.8
3 mL of 1 M DTT (dithiothreitol)
0.6 g of SDS (sodium dodecyl sulfate)
0.03 g of bromophenol blue
2.4 mL of glycerol
Bring final volume to 7.5 mL with Milli-Q® water
If solution is orange/yellow in color, add 1 drop of
5 M NaOH to adjust pH
Make 500 µL aliquots and store at -20°C

SDS-PAGE gel making buffer 1.5 M Tris-HCl (for separating gel)
118.2 g of Tris-HCl,
Add 450 mL Milli-Q® water and adjust pH to 8.8
Add Milli-Q® water to final volume 500 mL
Filter and degas

SDS-PAGE gel making buffer 1 M Tris-HCl (for stacking gel)
78.8 g of Tris-HCl
Add 450 mL Milli-Q® water and a djust pH to 6.8
Add Milli-Q® water to final volume 500 mL
Filter and degas

SDS-PAGE 10X gel running buffer
60 g Tris base (248 mM)
288 g glycine (1.92 M)
20 g SDS (1% w/v)
Milli-Q® water to final volume 2 L
No need to pH, filter, or degas
Dilute to 1X for running SDS-PAGE gels

10X PBS (1 L)
81.8 g NaCl (1.4 M)
20.1 g KCl (270 mM)
14.2 g Na_2HPO_4 (100 mM)
2.45 g KH_2PO_4 (18 mM)
pH 7.4
Add Milli-Q® water to final volume of 1 L

Transfer buffer 10X
15.2 g Tris base
72.1 g glycine
5.0 g SDS
Milli-Q® water to final volume 500 mL

Transfer buffer 1X (make fresh each time)
50 mL 10X transfer buffer
100 mL methanol
Milli-Q® water to final volume 500 mL

SDS-PAGE Coomassie staining solution
1.25 g Coomassie blue R-250
225 mL methanol
225 mL Milli-Q® water
50 mL glacial acetic acid

SDS-PAGE destaining solution
300 mL methanol (30%)
100 mL acetic acid (10%)
600 mL Milli-Q® water

Recipes for TruPAGE™ Precast Gel System

20X TruPAGE™ TEA-Tricine SDS Running Buffer
179.0 g triethanolamine
143.3 g tricine
20 g SDS
Milli-Q® water to final volume 1.0 L
The pH should be between 8.2–8.3 at 25 °C.

20X TruPAGE™ Tris-MOPS SDS Express Running Buffer
145.2 g Tris base
143.3 g MOPS
20 g SDS
Milli-Q® water to final volume 1.0 L
The pH should be between 8.2–8.3 at 25 °C.
NOTE: Antioxidant (5 mM sodium bisulfite) is required in the inner chamber if using this buffer.

4X TruPAGE™ LDS Sample Buffer
4.0 g glycerol
0.40 g lithium dodecyl sulfate
1.2 g triethanolamine
0.40 g Ficoll® 400
2.5 mg Phenol Red
2.5 mg Brilliant Blue G250
7.0 mg EDTA
Milli-Q® water to final volume 10 mL
The pH should be between 7.7-7.8 at 25 °C.
Dissolve triethanolamine (0.8 M) and pH to 7.6 before adding other reagents.

800X TruPAGE™ Running Antioxidant
4.2 g Sodium Bisulfite
Milli-Q® water to final volume 10 mL

20X TruPAGE™ Transfer Buffer
30.3 g Tris base
144.1 g glycine
Milli-Q® water to final volume 1.0 L.

10X Dithiothreitol sample reducer
38.6 mg DTT
Milli-Q® water to final volume 1.0 mL


SIGMA-ALDRICH

Sample Prep and Quantitation

Extraction & Purification

Description	Cat. No.
Lysis & Extraction Kits	
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
PhosphoSafe™ Extraction Reagent	71296
CelLytic™ B Plus Kit	CB0500
CelLytic™ Express	C1990
CelLytic™ IB Inclusion Body Solubilization Reagent	C5236
CelLytic™ MT Cell Lysis Reagent, For mammalian tissues	C3228
CelLytic™ PN Isolation/Extraction Kit, For plant leaves	CELLYTPN1
CelLytic™ Y Plus Kit, For enzymatic yeast cell lysis	CYP1
CHAPS hydrate, BioReagent, ≥98% (TLC)	C9426
Laemmli Lysis-buffer, non smelling	38733
Mammalian Protein Extraction Buffer, volume 500 mL	GE28-9412-79
RIPA Buffer	R0278
Sample Grinding Kit, GE Healthcare, 80-6483-37, sufficient for 50 preparations	GE80-6483-37
Protease Inhibitors	
Calbiochem® Protease Inhibitor Cocktail Set III, EDTA-Free	539134-1SET
Pepstatin A, 100 mg	516481
Chymostatin, 100 mg	EI6
Leupeptin, 100 mg	EI8
EDTA-Free, Roche cOmplete™	COEDTAF-RO
Mini, EDTA-Free Roche cOmplete™	11836170001
Mini Roche cOmplete™	11836153001
Protease Inhibitor Cocktail, for general use, lyophilized powder	P2714
SIGMA*FAST*™ Protease Inhibitor Cocktail Tablets, EDTA-Free, for use in purification of Histidine-tagged proteins	S8830
SIGMA*FAST*™ Protease Inhibitor Tablets, For General Use	S8820
Standard Roche cOmplete™	CO-RO
ULTRA-Roche cOmplete™	COUL-RO
ULTRA, EDTA-Free Roche cOmplete™	COUEDTAF-RO
PhosSTOP™	4906845001
Affinity Purification	
EZview™ Red Protein A Affinity Gel	EP6486
EZview™ Red Protein G Affinity Gel	E3403
PureProteome™ Protein A Magnetic Beads, 10 mL	LSKMAGA10
PureProteome™ Protein G Magnetic Beads, 10 mL	LSKMAGG10
PureProteome™ Protein A/G Mix Magnetic Beads, 10 mL	LSKMAGAG10
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

Sample Prep and Quantitation

Extraction & Purification

Description	Cat. No.
Buffer exchange and concentration	
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
Protein Quantitation Reagents	
Bradford Reagent, for 1-1,400 µg/ml protein	B6916
Copper(II) sulfate solution, 4 % (w/v) (prepared from copper (II) sulfate pentahydrate)	C2284
2-D Quant Kit, GE Healthcare, 80-6483-56	GE80-6483-56
QuantiPro™ BCA Assay Kit, for 0.5-30 µg/ml protein	QPBCA
FluoroProfile® Protein Quantification Kit	FP0010-1KT

For complete ordering information on all of our Amicon® Ultra filters, please visit:
www.merckmillipore.com/oneamicon

Electrophoresis

Description	Cat. No.
TruPAGE™ precast gels and bufffers	
TruPAGE™ Precast Gels, 10%, 10 x 10cm, 12-well	PCG2001
TruPAGE™ Precast Gels, 10%, 10 x 10cm, 17-well	PCG2005
TruPAGE™ Precast Gels, 10%, 10 x 8cm, 12-well	PCG2009
TruPAGE™ Precast Gels, 10%, 10 x 8cm, 17-well	PCG2013
TruPAGE™ Precast Gels, 12%, 10 x 10cm, 12-well	PCG2002
TruPAGE™ Precast Gels, 12%, 10 x 10cm, 17-well	PCG2006
TruPAGE™ Precast Gels, 12%, 10 x 8cm, 12-well	PCG2010
TruPAGE™ Precast Gels, 12%, 10 x 8cm, 17-well	PCG2014
TruPAGE™ Precast Gels, 4–12%, 10 x 10cm, 12-well	PCG2003
TruPAGE™ Precast Gels, 4–12%, 10 x 10cm, 17-well	PCG2007
TruPAGE™ Precast Gels, 4–12%, 10 x 8cm, 12-well	PCG2011
TruPAGE™ Precast Gels, 4–12%, 10 x 8cm, 17-well	PCG2015
TruPAGE™ Precast Gels, 4–20%, 10 x 10cm, 12-well	PCG2004
TruPAGE™ Precast Gels, 4–20%, 10 x 10cm, 17-well	PCG2008
TruPAGE™ Precast Gels, 4–20%, 10 x 8cm, 12-well	PCG2012
TruPAGE™ Precast Gels, 4–20%, 10 x 8cm, 17-well	PCG2016
TruPAGE™ LDS Sample Buffer, 4x	PCG3009
TruPAGE™ TEA-Tricine SDS Running Buffer, 20x	PCG3001
TruPAGE™ Tris-MOPS SDS Express Running Buffer, 20x	PCG3003
TruPAGE™ DTT Sample Reducer, 10x	PCG3005
TruPAGE™ Running Antioxidant, 800x	PCG3007

Electrophoresis

Description	Cat. No.
MW Markers	
Biotinylated Molecular Weight Marker, mol wt 6,500–180,000 Da	B2787
Bovine Serum Albumin, dimer mol wt ~132 kDa	A8654
Bovine Serum Albumin, For use as a marker in SDS-PAGE	A7517
Bromophenol Blue sodium salt, for molecular biology, for electrophoresis	B8026
Color Marker Ultra-low Range (M.W. 1,060–26,600)	C6210
ColorBurst™ Electrophoresis Marker, mol wt 8,000–220,000 Da	C1992
ECL™ DualVue Western Markers, GE Healthcare, RPN810, sufficient for 25 blots	GERPN810
ECL Plex™ Fluorescent Rainbow™ Markers, GE Healthcare, RPN850E, pack of 120 µL	GERPN850E
Full Range Molecular Weight Marker Rainbow™, GE Healthcare, RPN800E	GERPN800E
High Range Molecular Weight Marker Rainbow™, GE Healthcare, RPN756E	GERPN756E
Low Range Molecular Weight Marker Rainbow™, GE Healthcare, RPN755E	GERPN755E
Prestained Molecular Weight Marker, mol wt 26,600–180,000 Da	SDS7B2
Protein Gel Stains	
Brilliant Blue G solution, Concentrate	B8522
Brilliant Blue R, pure	B7920
EZBlue™ Gel Staining Reagent	G1041
Fast Green FCF, Dye content ≥85 %	F7252
Instant*Blue*™, Ultrafast Protein Stain	ISB1L
SYPRO® Orange Protein Gel Stain	S5692-500UL
ProteoSilver™ Silver Stain Kit	PROTSIL1-1KT
ProteoSilver™ Plus Silver Stain Kit	PROTSIL2-1KT
Acrylamides	
Acrylamide, for electrophoresis, ≥99% (HPLC), powder	A3553
Acrylamide, suitable for electrophoresis, ≥99%	A8887
Acrylamide solution, 40%, for electrophoresis, sterile-filtered	A4058
Acrylamide/Bis-acrylamide, 30% solution, BioReagent, suitable for electrophoresis, 29:1	A3574
Acrylamide/Bis-acrylamide, 30% solution, BioReagent, suitable for electrophoresis, 37.5:1	A3699
Acrylamide/bis-acrylamide, 40% solution, BioReagent, suitable for electrophoresis, 19:1	A9926
Acrylamide/bis-acrylamide, 40% solution, BioReagent, suitable for electrophoresis, 29:1	A7802
Acrylamide/bis-acrylamide, 40% solution, BioReagent, suitable for electrophoresis, 37.5:1	A7168
Buffers	
BICINE, BioXtra, ≥99% (titration)	B8660
Ficoll® 400, BioXtra, for molecular biology, lyophilized powder	F2637
Glycine, for electrophoresis, ≥99%	G8898
Laemmli 2x Concentrate Sample Buffer	S3401
MES solution, BioPerformance Certified, 1 M, suitable for cell culture	M1317
Phenol Red, powder, BioReagent, suitable for cell culture	P3532
Sodium bisulfite, ACS reagent, mixture of $NaHSO_3$ and $Na_2S_2O_5$	243973
Trichloroacetic acid, for electrophoresis, suitable for fixing solution (for IEF and PAGE gels), ≥99%	T8657
Tricine, BioPerformance Certified, cell culture tested, ≥99% (titration)	T5816
Triethanolamine, ≥99.0% (GC)	90279
Tris-Glycine Buffer, 10x Concentrate	T4904
Tris-Glycine-SDS Buffer, 10x Concentrate	T7777
Tris-Tricine-SDS Buffer, 10x Concentrate	T1165
Trizma® hydrochloride solution, BioPerformance Certified, pH 7.5, 1 M, suitable for cell culture	T2319
Trizma® base, anhydrous, free-flowing, Redi-Dri™, ≥99.9%	RDD008
Detergents	
3-(Benzyldimethylammonio)propanesulfonate, BioXtra, ≥99.0% (HPCE)	17236
IGEPAL® CA-630, CMC 0.083 mM	56741
Octyl β-D-glucopyranoside solution, ≥98% (HPLC), 50 % (w/v) in H_2O	O3757

Electrophoresis

Description	Cat. No.
Gel Casting Reagents	
Ammonium persulfate, for molecular biology, for electrophoresis, ≥98%	A3678
1,4-Bis(acryloyl)piperazine, BioXtra, suitable for electrophoresis, ≥99.0% (TLC)	14470
(-)-Riboflavin, BioReagent, suitable for cell culture, suitable for insect cell culture, ≥98%	R9504
N,N,N',N'-Tetramethylethylenediamine, BioReagent, suitable for electrophoresis, ~99%	T9281
SDS Gel Preparation Kit	8091
Gel Cross-linkers	
N,N'-Bis(acryloyl)cystamine, BioReagent, suitable for electrophoresis	A4929
N,N'-Methylenebisacrylamide, powder, for molecular biology, for electrophoresis, ≥99.5%	M7279
N,N'-Methylenebisacrylamide solution, for electrophoresis, 2% in H_2O	M1533
Piperazine diacrylamide, For acrylamide gel electrophoresis	D1538
Reducing Agents	
DL-Dithiothreitol, for electrophoresis, ≥99%	D9163
Lithium dodecyl sulfate, BioReagent, for molecular biology, suitable for electrophoresis	L9781
Sodium dodecyl sulfate, BioReagent, suitable for electrophoresis, for molecular biology, ≥98.5% (GC)	L3771
5-Sulfosalicylic acid dihydrate, for electrophoresis, ≥99%	S3147
Tris(2-carboxyethyl)phosphine hydrochloride, BioUltra, for electrophoresis, SDS-PAGE tested	68957

Transfer

Description	Cat. No.
Blotting Papers	
Immobilon® Blotting Filter Paper, 7 x 8.4 cm sheet	IBFP0785C
Immobilon® Blotting Filter Paper, 8.5 x 13.5 cm sheet	IBFP0813C
Hybond blotting paper, L x W 20 cm x 20 cm, sheets, pack of 100 ea	GERPN6101M
Whatman® gel blotting paper, Grade GB003, GB003 blotting sheets, 15 x 20 cm, 100/pk	WHA10427812
Whatman® gel blotting paper, Grade GB003, GB003 blotting sheets, 20 x 20 cm, 100/pk	WHA10427818
Whatman® membrane marking pen	WHA10499001
Blotting Papers	
CAPS, BioXtra, pH 3.0-7.0 (20 C, 0.5 M in H_2O), ≥98.0% NaOH basis (titration)	C6070
Methanol, Laboratory Reagent, ≥99.6%	179957
TruPAGE™ Transfer Buffer, 20x	PCG3011
Immobilon®-P PVDF Transfer Membrane, 0.45 µm	
26.5 cm x 3.75 m 1 roll	IPVH00010
26.5 cm x 1.875 m 1 roll	IPVH00005
7 x 8.4 cm 50/pk	IPVH07850
8.5 x 13.5 cm 10/pk	IPVH08130
20 x 20 cm 10/pk	IPVH20200
Blotting Sandwich Immobilon®-P membrane interleaved with blotting paper 7 x 8.4 cm	IPSN07852
Blotting Sandwich Immobilon®-P membrane interleaved with blotting paper 8.5 x 13.5 cm	IPSN08132
Immobilon®-P^{SQ} PVDF Transfer Membrane, 0.2 µm	
26.5 cm x 3.75 m 1 roll	ISEQ00010
26.5 cm x 1.875 m 1 roll	ISEQ00005
7 x 8.4 cm 50/pk	ISEQ07850
8.5 x 13.5 cm 10/pk	ISEQ08130
20 x 20 cm 10/pk	ISEQ20200
Immobilon®-FL Membrane, 0.45 µm	
26.5 cm x 3.75 m 1 roll	IPFL00010
26.5 cm x 1.875 m 1 roll	IPFL00005
7 x 8.4 cm 10/pk	IPFL07810
10 x 10 cm 10/pk	IPFL10100

Transfer

Description	Cat. No.
Protran® Nitrocellulose Membranes	
Amersham™ Protran® Western blotting membranes, nitrocellulose pore size 0.2 µm, roll W × L 300 mm × 4 m, pkg of 1 ea	GE10600001
Amersham™ Protran® Western blotting membranes, nitrocellulose pore size 0.2 µm, sheet W × L 200 mm × 200 mm, pkg of 25 ea	GE10600043
Amersham™ Protran® Western blotting membranes, nitrocellulose pore size 0.2 µm, sheet W × L 200 mm × 200 mm, pkg of 10 ea	GE10600044
Amersham™ Protran® Western blotting membranes, nitrocellulose pore size 0.2 µm, roll W × L 200 mm × 4 m, pkg of 1 ea	GE10600006
Amersham™ Protran® Premium Western blotting membranes, nitrocellulose pore size 0.2 µm, roll W × L 300 mm × 4 m, pkg of 1 ea	GE10600004
Amersham™ Protran® Supported Western blotting membranes, nitrocellulose pore size 0.2 µm, roll W × L 300 mm × 4 m, pkg of 1 ea	GE10600015
Amersham™ Protran® Western blotting membranes, nitrocellulose pore size 0.45 µm, sheet W × L 200 mm × 200 mm, pkg of 25 ea	GE10600041
Amersham™ Protran® Western blotting membranes, nitrocellulose pore size 0.45 µm, roll W × L 300 mm × 4 m, pkg of 1 ea	GE10600002
Amersham™ Protran® Western blotting membranes, nitrocellulose pore size 0.45 µm, roll W × L 200 mm × 4 m, pkg of 1 ea	GE10600007
Amersham™ Protran® Western blotting membranes, nitrocellulose pore size 0.45 µm, roll W × L 150 mm × 4 m, pkg of 1 ea	GE10600012
Amersham™ Protran® Premium Western blotting membranes, nitrocellulose pore size 0.45 µm, sheet W × L 80 mm × 90 mm, pkg of 25 ea	GE10600096
Amersham™ Protran® Premium Western blotting membranes, nitrocellulose pore size 0.45 µm, sheet W × L 200 mm × 200 mm, pkg of 10 ea	GE10600048
Amersham™ Protran® Premium Western blotting membranes, nitrocellulose pore size 0.45 µm, roll W × L 300 mm × 4 m, pkg of 1 ea	GE10600003
Amersham™ Protran® Premium Western blotting membranes, nitrocellulose pore size 0.45 µm, roll W × L 200 mm × 4 m, pkg of 1 ea	GE10600008
Amersham™ Protran® Supported Western blotting membranes, nitrocellulose pore size 0.45 µm, roll W × L 300 mm × 4 m, pkg of 1 ea	GE10600016
Amersham™ Protran® Supported Western blotting membranes, nitrocellulose pore size 0.45 µm, roll W × L 200 mm × 4 m, pkg of 1 ea	GE10600018
Protein Blot Stains	
Alcian Blue 8GX, certified by the Biological Stain Commission	A3157
Amido Black Staining Solution 2x, electrophoresis reagent	A8181
Glycoprotein Detection Kit	GLYCOPRO
Gold solution, colloidal, 0.0065% gold basis (AAS)	50755
Ponceau S, BioReagent, suitable for electrophoresis	P3504
Ponceau S solution, BioReagent, suitable for electrophoresis, 0.1 % (w/v) in 5% acetic acid	P7170
Reversible Protein Detection Kit for Membranes and Polyacrylamide Gels	RPROB

Blocking & Antibody Addition

Description	Cat. No.
SNAP i.d.® 2.0 systems	
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	
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	
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
Signal enhancer reagents	
Immobilon® Signal Enhancer for Immunodetection	WBSH0500

Blocking & Antibody Addition

Description	Cat. No.
Blocking buffers and reagents	
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
Western Blocker™ Solution, for HRP detection systems	W0138
Bovine Serum Albumin, heat shock fraction, protease free, fatty acid free, essentially globulin free, pH 7, ≥98%	A7030
Bovine Serum Albumin, heat shock fraction, pH 7, ≥98%	A7906
Gelatin blocking buffer, powder blend	G7663
Phosphate buffered saline, 10x concentrate, BioPerformance Certified, suitable for cell culture	P5493
Phosphate buffered saline, dry powder, pH 7.4, contains 3% nonfat milk	P2194
Phosphate buffered saline, pH 7.4, contains BSA, powder	P3688
Phosphate buffered saline, powder, pH 7.4	P3813
Tris Buffered Saline, with BSA, pH 8.0, powder	T6789
Wash buffers	
Phosphate buffered saline, BioUltra, pH 7.4 (in solution), contains TWEEN 20, tablets	08057
Phosphate buffered saline, pH 7.4, contains TWEEN® 20, dry powder	P3563
Tris Buffered Saline, pH 8.0, powder	T6664
Tris buffered saline with TWEEN® 20, BioUltra, tablets (for 500 mL), pH 7.6	91414
Tris Buffered Saline, with TWEEN® 20, pH 8.0, powder	T9039
Peroxidase conjugates	
Anti-Mouse IgG (Fab specific)-Peroxidase antibody produced in goat, affinity isolated antibody buffered aqueous solution	A3682
Anti-Mouse IgG (Fab specific)-Peroxidase antibody produced in goat, affinity isolated antibody buffered aqueous solution	A9917
Anti-Mouse IgG (Fc specific)-Peroxidase antibody produced in goat, affinity isolated antibody	A0168
Anti-Mouse IgG (Fc specific)-Peroxidase antibody produced in goat, affinity isolated antibody buffered aqueous solution	A2554
Anti-Mouse IgG (whole molecule)-Peroxidase antibody produced in rabbit, IgG fraction of antiserum buffered aqueous solution	A9044
Anti-Rabbit IgG (whole molecule)-Peroxidase antibody produced in goat, affinity isolated antibody	A0545
Peroxidase conjugates (continued)	
Avidin-Peroxidase, lyophilized powder	A3151
Anti-Goat IgG (whole molecule)-Peroxidase antibody produced in rabbit, affinity isolated antibody, buffered aqueous solution	A5420
Anti-Mouse IgG (whole molecule)-Peroxidase antibody produced in goat, affinity isolated antibody buffered aqueous solution	A4416
Anti-Rabbit IgG (whole molecule)-Peroxidase antibody produced in goat, affinity isolated antibody buffered aqueous solution	A6154
Anti-Goat IgG Antibody, HRP conjugate; produced in rabbit; Purified rabbit IgG conjugated to horseradish peroxidase in buffer containing 0.01 M Sodium Phosphate, 0.15 M NaCl, pH 7.1 with 15 mg/mL BSA and 0.01% thimerosal. Lyophilized.	AP106P
Anti-Rabbit IgG Antibody, HRP-conjugate; produced in rabbit; Goat polyclonal conjugated with horseradish peroxidase IgG in buffer containing 50% storage buffer 0.02 M potassium phosphate, 0.15 M NaCl, pH 7.2, 10 mg/mL BSA, 0.01% (w/v) gentamicin sulfate and 50% glycerol. Lyophilized.	12-348
Anti-Mouse IgG Antibody, HRP conjugate; produced in goat; Purified goat polyclonal IgG in buffer containing conjugated to horseradish peroxidase lyophilized from 0.02 M Potassium Phosphate, 0.15 M NaCl, pH 7.2, 10 mg/mL BSA, and 0.01% gentamicin sulfate.	12-349
Anti-Mouse IgG Antibody, (H+L) HRP conjugate; produced in goat; Purified Goat IgG in buffer containing liquid in PBS, pH 7.1 with 15 mg/mL BSA and 0.01% thimerosal.	AP308P
Anti-Rabbit light chain, HRP conjugate Antibody; produced in mouse; Purified from antiserum by chromatographic procedures. Lyophilized in 0.01M Sodium Phosphate, 0.25M NaCl, pH 7.6. 15 mg/mL BSA as stabilizer.	MAB201P
Anti-Mouse light chain Antibody, HRP conjugate; produced in goat; Lyophilized in 0.01M Sodium Phosphate, 0.25M NaCl, pH 7.6. 15 mg/mL BSA as stabilizer. Purified by immunoaffinity chromatography.	AP200P
Flurophore conjugates	
ECL Plex™ G-A-M IgG, Cy®3, GE Healthcare, PA43009, pack of 150 µg	GEPA43009
ECL Plex™ G-A-M IgG, Cy®5, GE Healthcare, PA45010, pack of 600 µg	GEPA45010
ECL Plex™ Gar IgG Cy®3, GE Healthcare, 28-9011-06, pack of 150 µg	GE28-9011-06
ECL Plex™ G-A-R IgG, Cy®5, GE Healthcare, PA45011, pack of 150 µg	GEPA45011

Blot Stripping Reagents

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

Detection

Description	Cat. No.
Immobilon® Western Chemiluminescent HRP Substrate	
2 x 25 mL	WBKLS0050
2 x 50 mL	WBKLS0100
2 x 250 mL	WBKLS0500
Immobilon® ECL Ultra Western HRP Substrate	
2 x 50 mL	WBULS0100
2 x 250 mL	WBULS0500
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
Amersham ECL Western Blotting Detection Reagent	
Amersham™ ECL™ Western Blotting Reagent Pack GE Healthcare, RPN2124, pack of 1 kit	GERPN2124
ECL™ Western Blotting Analysis System GE Healthcare, RPN2108	GERPN2108
ECL™ Western Blotting Detection Reagents GE Healthcare, RPN2209	GERPN2209
ECL™ Western Blotting Reagents GE Healthcare, RPN2106	GERPN2106
ECL™ Western Blotting Reagents GE Healthcare, RPN2134	GERPN2134
ECL™ Western Blotting Reagents GE Healthcare, RPN2109	GERPN2109
ECL™ Start Western Blotting Reagent GE Healthcare, RPN2109	GERPN3243
ECL™ Start Western Blotting Reagent GE Healthcare, RPN2109	GERPN3244
Amersham ECL Prime Western Blotting Detection Reagent	
ECL™ Prime Western Blotting System GE Healthcare, RPN2232	GERPN2232
ECL™ Prime Western Blotting Detection Reagent GE Healthcare, RPN2236	GERPN2236
Amersham ECL Select Western Blotting Detection Reagent	
ECL™ Select Western Blotting Detection Reagent GE Healthcare, RPN2235	GERPN2235
Detection Films	
Hyperfilm™ ECL™, W x L 18 cm x 24 cm, pack of 100 ea	GE28-9068-37
Hyperfilm™ MP, W x L 8 in. x 10 in., pack of 50 ea	GE28-9068-45
Chromogenic Substrates	
3,3',5,5'-Tetramethylbenzidine (TMB) Liquid Substrate System for Membranes, ready-to-use solution	T0565
3,3'-Diaminobenzidine (DAB) Enhanced Liquid Substrate System tetrahydrochloride, for Membrane Applications	D6815

Accessories

Description	Cat. No.
Scienceware® gel incubating tray W × L 1.6 cm (0.63 in.) × 11.5 cm (4.5 in.)	BAF451000000-5EA
Scienceware® gel incubating tray W × L 9.0 cm (3.5 in.) × 9.0 cm (3.5 in.)	BAF451000001-5EA
Scienceware® gel incubating tray W × L 16.5 cm (6.5 in.) × 16.5 cm (6.5 in.)	BAF451000002-5EA
Scienceware® electrophoresis plate rack, polypropylene	E1028-1EA
Gel Handler™ gel support	Z376957-1PAK

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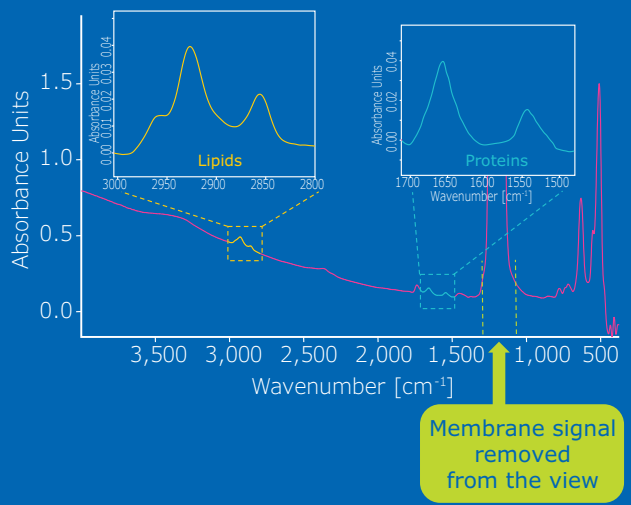

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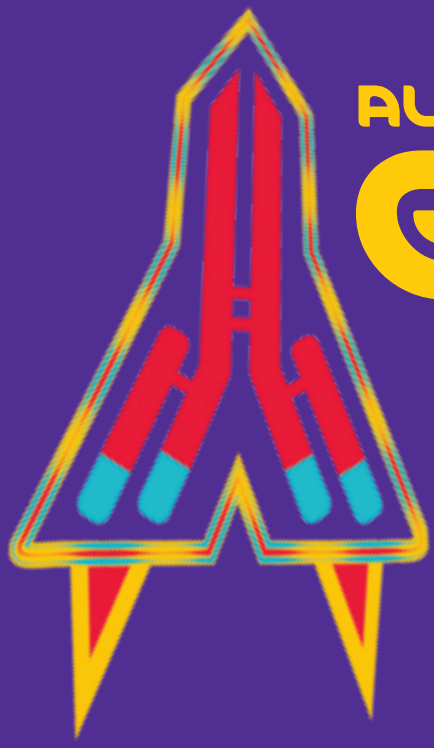

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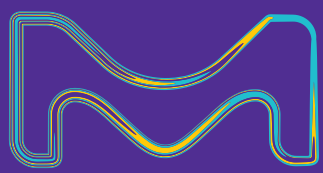

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