Rethink Western Blotting

MilliporeSigma

What are your Western blot results telling you?

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

Millipore®

Preparation, Separation, Filtration & Monitoring Products

western blotting tools

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

MilliporeSigma has brought together the world's leading Life Science brands, so regardless of your needs, our experts are ready with our unparalleled products and services to help you solve your toughest problems.

Millipore®

The Millipore® portfolio of MilliporeSigma offers an ecosystem of industry-leading products and services, spanning preparation, separation, filtration and monitoring – all of which are deeply rooted in quality, reliability and timetested processes. Our proven products, regulatory and application expertise are a strong foundation you can rely on to consistently perform at the highest level.

Sigma-Aldrich®

The Sigma-Aldrich® portfolio of MilliporeSigma offers a strong and ever-expanding offering of lab and production materials. Through our technical support and scientific partnerships, we help connect our customers with a whole world of progress.

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 MilliporeSigma, we've been helping scientists perform Western blots for decades, with continuous problem-solving and steadfast technical support.

With a combined portfolio of legacy Millipore® products and Sigma-Aldrich® products, our catalog includes thousands of products for Western blotting, including Immobilon® transfer membranes, Amersham Protran® nitrocellulose membranes, ECL® detection substrates, and Roche cOmplete™ Protease inhibitor cocktails. 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, or learn more at:

SigmaAldrich.com/westernblot

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Protein Extraction & Sample Preparation	Quantitation	Electrophoresis	Protein Transfer	Blocking & Antibody Incubation	Detection
Gentle protein extraction kits	Protein assay kits	mPAGE™ precast gels	Immobilon® high protein binding membranes	30-minute blot processing with the SNAP i.d.® 2.0 system	Immobilon® Western HRP substrates
Rapid protein isolation with PureProteome™ magnetic beads		Molecular weight markers	Protran® nitrocellulose membranes	Immobilon® signal enhancer	Immobilon® premixed HRP substrates
Fast, effective concentration with Amicon® Ultra centrifugal filters		Reagents for hand-casting polyacrylamide gels	Blotting papers	Immobilon® GO for walk-away immunodetection	Amersham ECL® substrates
		Buffers	Blotting buffers	~75,000 primary antibodies for Western blotting: SigmaAldrich.com/antibodies	
		Protein gel stains	Protein blot stains	Secondary antibody conjugates	Chromogenic substrates
					Substrates for AP detection

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 reagents unite superior performance with speed to reduce protein exposure 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 SIGMAFAST™ 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.
- Use Stabilyser™ reagent and protect both nucleic acids and functional proteins in one uniform lysate mixture.

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
	GST•Tag™ purification	S•Tag™ purification
	FLAG® Tag purification	Strep•Tag® II purification
		T7•Tag® purification
		FLAG®, 3X FLAG® 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
- Reducing agents including dithiothreitol and thiols do not interfere with the assay
- Reagent is suitable for micro (1–10 µg/mL) and standard (50–1400 µg/mL) assays

Sigma-Aldrich®
Lab & Production Materials

electrophoresis

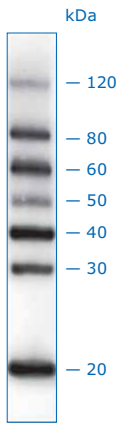
Electrophoresis is the technique most commonly used to separate extracted proteins for Western blot analysis. Applying an electrical current to a gel causes the proteins within the sample to migrate at different rates and separate within the medium according to their inherent properties. In the most 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 when optimizing results.

Best practices for electrophoresis

Select Gel & Buffer	Determine the appropriate gel electrophoresis system 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, to denature the protein and equilibrate 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 (e.g., positive, negative) 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 to visualize and confirm successful protein migration.

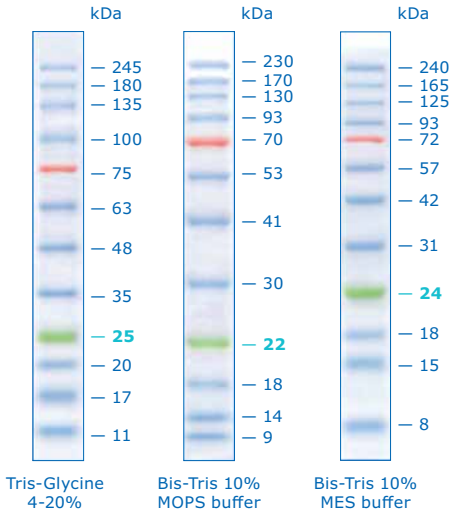
mPAGE™ Western Protein Standard

The mPAGE™ Western blotting protein standard consists of 7 recombinant proteins that incorporate an IgG binding site. The addition of this binding site allows for the visualization of the protein marker and target protein, without requiring additional reagents.



BLUEye Prestained Protein Ladder

Provided in a gel loading buffer, the ready-to-use BLUeye prestained protein ladder has 12 sharp bands and reference bands to enable easier identification.

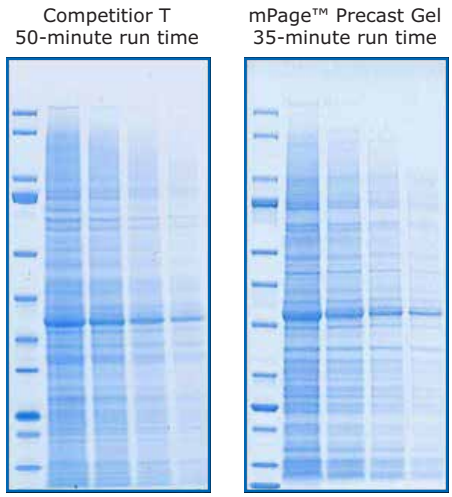


mPAGE™ Bis-Tris Precast Gels for Protein Electrophoresis

Without compromising the high resolution you need, mPAGE™ gels require shorter run times and are compatible with large sample volumes, making mPAGE™ precast gels a valuable tool for your protein research at a budget-friendly price.

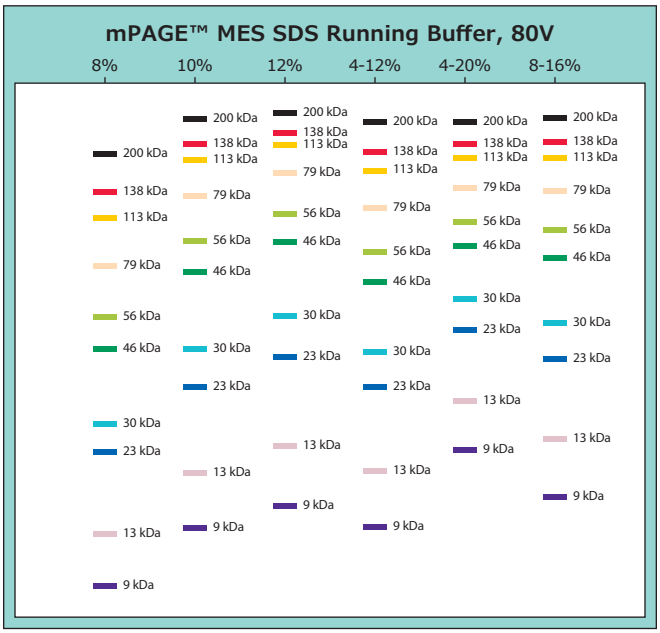
Benefits of mPAGE™ Precast Gels:

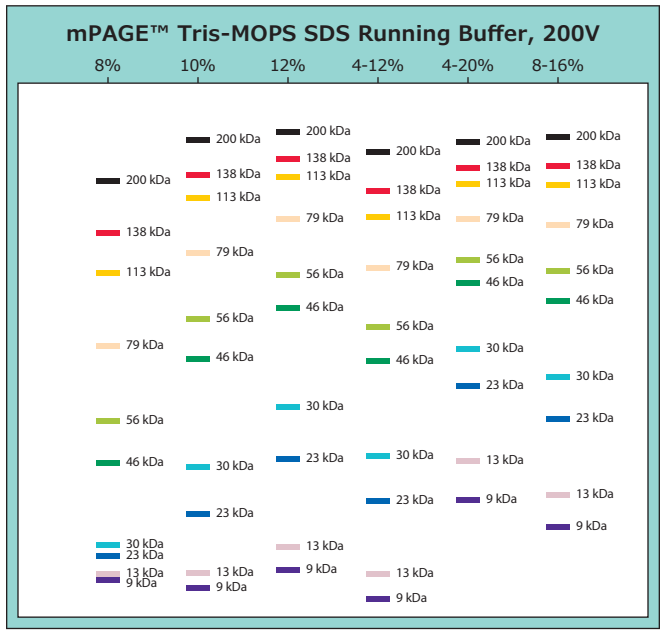
- Publication-ready resolution at a fraction of the cost
- Compatible with popular electrophoresis tanks*
- Up to 80 µL sample per well
- Efficient wet and semi-dry Western blot transfer
- Up to 15-minute shorter run time
- Neutral pH prevents protein modification



Protein separation using MES running buffer comparing mPAGE™ Bis-Tris Precast Gel (4-12%) to a competitor precast bis-tris gel. Samples: unstained protein marker and serial dilution of E.coli extract.

mPAGE™ migration tables





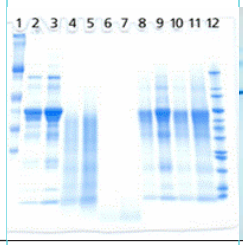
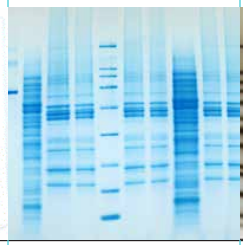
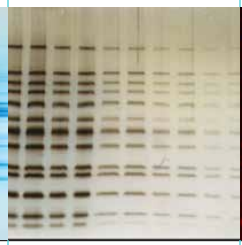
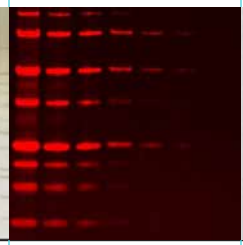
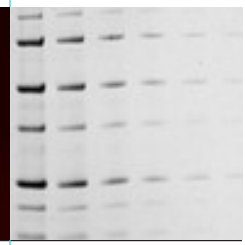
mPAGE™ precast gels are compatible with:

- Bio-Rad Mini-PROTEAN® II tanks
- Bio-Rad Mini-PROTEAN® Tetra System
- Sigma-Aldrich® Dual Run and Blot System
- Thermo Fisher Scientific® XCell I, II, and Surelock™ mini-cell tanks
- LONZA PAGEr® minigel chamber tanks

While mPAGE™ precast gels are compatible with the tanks listed previously, different tank configurations or adapters may be required to prevent buffer leaking. Please verify the correct procedure in the mPAGE™ user guide prior to use.

Protein gel stains

After separation by electrophoresis, protein bands are commonly visualized with gel stains. Utilizing either a dye-binding or color-producing chemical reaction, protein gel stains react selectively with proteins to yield a stained gel. Protein gel stains are typically selected based on the initial sample size, desired detection method, and compatibility requirements for downstream analysis.

	Colormetric			Flourescent	
	EZBlue™ Gel Stain	InstantBlue™ Stain	ProteoSilver™ Stain	EZFluor™ Stain	EZFluor™ UV Stain
					
Sensitivity	≥5 ng	≥5 ng	≥0.1 ng	1-10 ng	1-10 ng
Format	Premixed solution	Premixed solution	Premixed solutions	Premixed solution	Premixed solution
Reaction time	60 minutes	<15 minutes	3 - 12 minutes	5 - 60 minutes	5 - 60 minutes
Destaining required	Optional	Optional	No	Optional	Optional
Benefits	• No solvent waste	• Rapid reaction time • Non-toxic	• MALDI compatible • Stable at room temperature	• Image with a UV or blue light transilluminator, or a laser gel scanner • Compatible with mass spectrometry • Aqueous-based, without methanol or acetic acid	• Image with UV transilluminator and ethidium bromide filter. • Compatible with mass spectrometry • Aqueous based, without methanol or acetic acid

protein transfer

Over 20,000 publications cite Immobilon® membranes

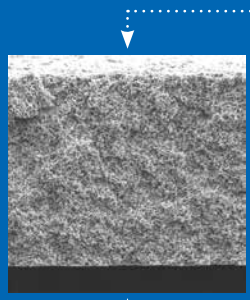
This family of trusted, quality transfer membranes includes Immobilon®-P Membrane, the first and most commonly used PVDF membrane for Western transfers.

How do Immobilon® membranes work?

Polyvinylidene fluoride (PVDF) membranes bind biomolecules through hydrophobic interactions. Membrane pores increase the surface binding area while restricting sizes of bound proteins.

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
- A variety of pore sizes provides optimal protein retention

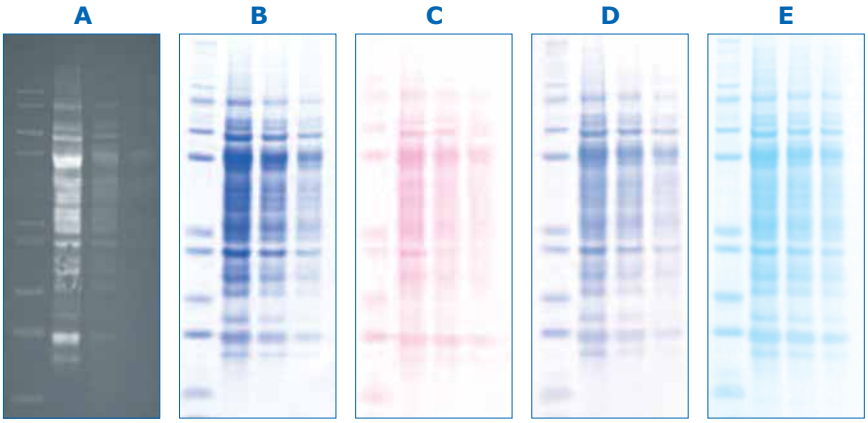


Membranes are 3-dimensional structures full of microscopic pores (Scanning electron microscope image of a cross-section of Immobilon®-P Membrane, Magnification: 500x).

		PVDF Membranes				Nitrocellulose Membranes			
Manufacturer		Millipore® Brand				GE ® Healthcare Life Sciences			
Membrane Name		Immobilon®-E	Immobilon®-P	Immobilon®-FL	Immobilon®-PSQ	Amersham Protran®	Amersham Protran® premium	Amersham Protran®	Amersham Protran® premium
Chemistry		PVDF	PVDF	PVDF	PVDF	Nitrocellulose	Nitrocellulose	Nitrocellulose	Nitrocellulose
Pore Size		0.45 µm	0.45 µm	0.45 µm	0.2 µm	0.45 µm	0.45 µm	0.2 µm	0.2 µm
Detection methods		Chemiluminescence, Colorimetric	Chemiluminescence, Colorimetric	Chemiluminescence, Colorimetric, Fluorescence (including near-infrared)	Chemiluminescence, Colorimetric	Chemiluminescence, Colorimetric	Chemiluminescence, Colorimetric, Fluorescence	Chemiluminescence, Colorimetric	Chemiluminescence, Colorimetric, Fluorescence
Binding capacity	Goat IgG	225-293 µg/cm²	294 µg/cm²	300 µg/cm²	448 µg/cm²	115-125 µg/cm²	162-180 µg/cm²	150-176 µg/cm²	173-203 µg/cm²
	BSA	N/A	215 µg/cm²	205 µg/cm²	340 µg/cm²	N/A	N/A	N/A	N/A
	Insulin	N/A	160 µg/cm²	155 µg/cm²	262 µg/cm²	N/A	N/A	N/A	N/A
Applications		Western blots	Western blots, dot blots			Western, Southern and Northern blots, dot blots			
		The durability and high protein retention of Immobilon® PVDF membranes make them the preferred option for sequential detection of multiple targets via antibody stripping and reprobing.							
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	

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® NOW Transfer Membrane rolls for Western blotting

The convenience of pre-cut sheets with the flexibility of rolls

With standard rolls of transfer membrane, plan for extra bench time with ruler and scissors to cut the size needed for your experiment. Over time, this handling leads to waste of your valuable membrane. Pre-cut sheets offer convenience, but are costly and lack flexibility if a different size is needed.

Immobilon® NOW rolls offer the convenience of pre-cut sheets with the flexibility of rolls in the formats you depend on: Immobilon®-E, Immobilon®-P, Immobilon®-FL and Immobilon®-PSQ membranes.

- One-cut convenience
- Flexibility for mini or midi blots
- Measurement marks on the lid. No ruler needed!
- No waste
- Smaller package saves space in your lab

The optional Immobilon® NOW Dispenser adds even more convenience. It offers a complete solution to measure, cut and store the transfer membrane. Simply retract the unused membrane into the container and easily see when to refill.

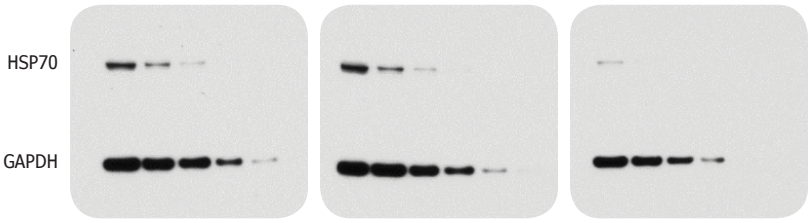
Immobilon®-E Transfer Membrane

No alcohol pre-wet step required

Unlike other PVDF transfer membranes, Immobilon®-E membrane does not require an alcohol pre-wet step prior to Western blotting. It wets out easily with standard transfer buffers or even water, and provides experimental results similar to Immobilon®-P membrane.

- Shorter workflow than other PVDF membranes
- Less organic waste
- Immobilon®-P membrane performance

PVDF performance with the workflow of nitrocellulose

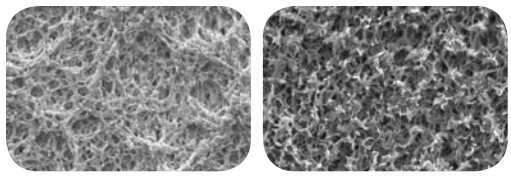


Serial dilutions of EGF-stimulated A431 cell lysate (12-110) were separated via SDS-PAGE. Proteins were transferred from a single polyacrylamide gel to strips of Immobilon®-E, Immobilon®-P and nitrocellulose membranes. All three membranes were processed identically for immunodetection using a single tray. Membranes were blocked with 3% non-fat dry milk and then probed with antibodies to HSP70 (SAB4200714) and GAPDH (MAB374). Target proteins were visualized using anti-mouse HRP (AP124P) at 1:40,000 dilution followed by development with Immobilon® Classico Western HRP Substrate (WBLUC0500).

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

How do Immobilon®-PSQ membranes work?

This PVDF membrane has a thickness of ~200 µm and approximately 3 times the internal surface area of most membranes. These properties give Immobilon®-P^{SQ} higher protein binding capacity, improving retention of small proteins.



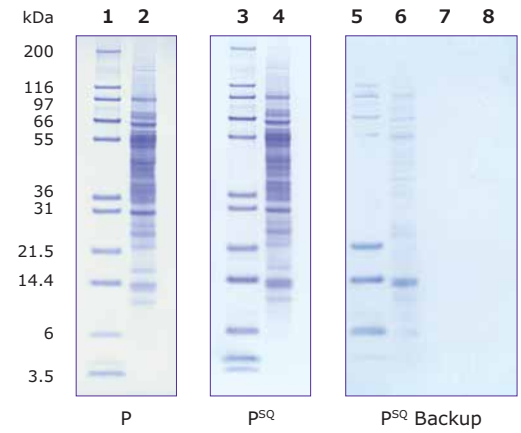
Scanning electron microscopy images (3000x magnification) show differences in the pore structure of Immobilon®-P^{SQ} membrane (right) relative to Immobilon®-P membrane (left).

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 chromogenic 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).

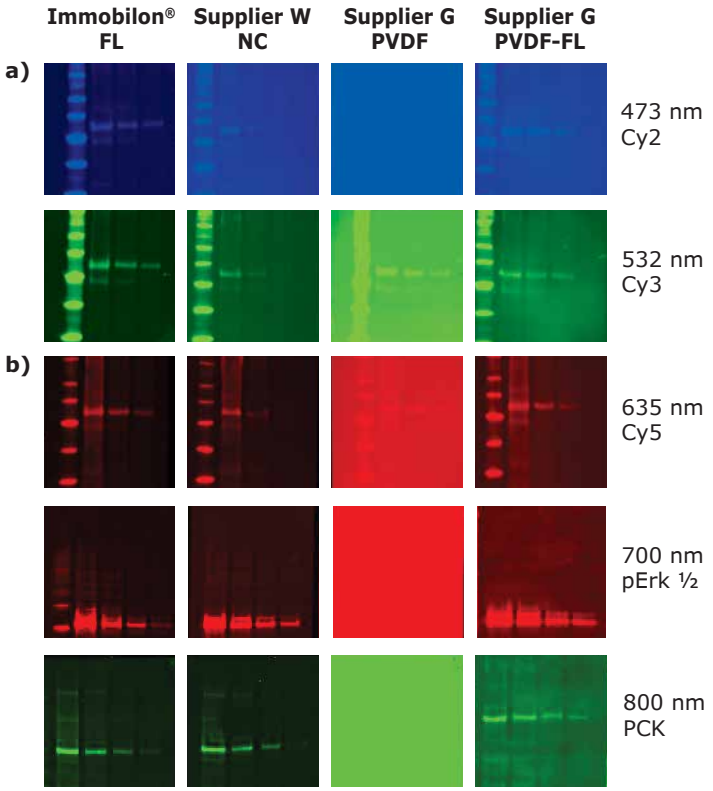
Immobilon®-FL transfer membrane

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.

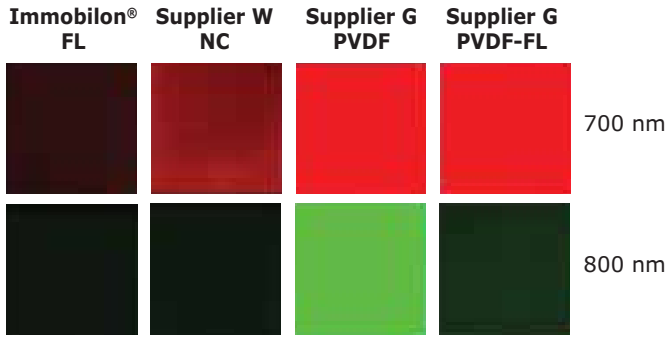
Key Benefits

- 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



a) Two-fold dilution series of (10-2.5 µg/lane) of human brain lysate (CL302-250UG, MilliporeSigma) were resolved by SDS-PAGE and transferred onto either an Immobilon®-FL membrane, Protran® BA85 membrane, Hybond®-P membrane, or Hybond®-LFP membrane. The blots were probed with Anti-GSK3-β antibody (MAB8687, MilliporeSigma) followed by anti-rabbit IgG antibody either Cy2, Cy3, or Cy5 conjugated (AP132J, AP187C, AP187S, MilliporeSigma). The blot was scanned on Fuji FLA-5100 after drying the blot for 1 hr under vacuum.

b) Two-fold dilution series of (10-2.5 µg/lane) of human brain lysate (CL302-250UG, MilliporeSigma) were resolved by SDS-PAGE and transferred onto either an Immobilon®-FL membrane, Protran® BA85 membrane, Hybond®-P membrane, or Hybond®-LFP membrane. The blots were probed using the SNAP i.d. system with Anti-GSK3-β antibody (MAB8687, MilliporeSigma) followed by pErk ½ or PCK. The blot was scanned on the Odyssey® Infrared Imaging System (LI-COR) after drying the blot for 1 hr under vacuum.



Comparison of background fluorescence observed in PVDF membranes from different manufacturers. Only Immobilon®-FL membrane offers low background fluorescence at both 700 and 800 nm, increasing its ability to used in multiple excitation and emission wavelengths.

blocking & antibody incubation

Immobilon® GO device for walk-away immunodetection.

Immobilon® GO Immunodetection Device

for walk-away Western blot immunodetection

Immunodetection in Western blotting usually takes four hours or more and requires frequent manual intervention. The Immobilon® GO immunodetection device applies the principles of lateral flow immunoassays to eliminate those tedious, manual steps. Simply load the Immobilon® GO device with your blot, wash buffer, and primary and secondary antibody solutions. Once setup is complete, no manual intervention is required - saving time and improving productivity in your lab.

Walk-away Western blot immunodetection

- Load your blot with blocking, wash and antibody solutions, and move on to other tasks
- Return in 3 hours (or the next morning) to briefly wash the blot, apply detection substrate, image, and analyze your results.

Open system

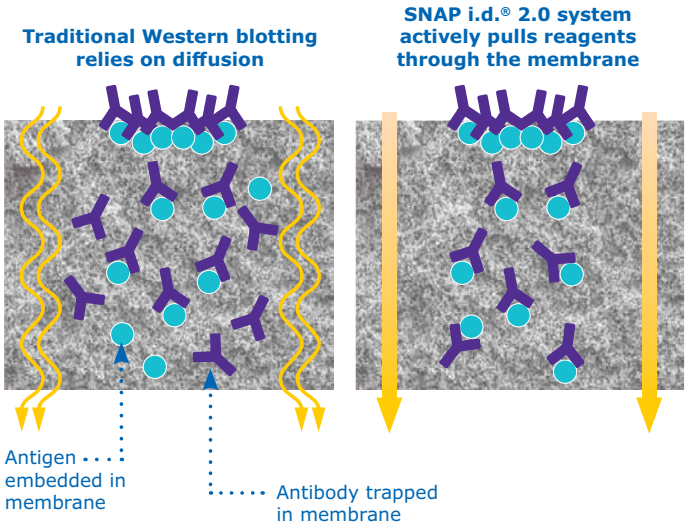
- Use standard buffers and your preferred primary and secondary antibodies
- Compatible with chemiluminescent or fluorescent detection

No hardware to buy

- Simple, consumable device

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

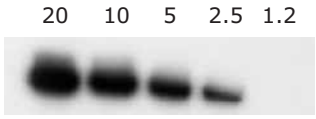
- Fastest immunodetection on the market
- Increased antibody-antigen binding
- Superior washes for lower background
- Save antibody by collecting and reusing your solutions

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	Total
Conventional Immunodetection	~1 hr	>1 hr–overnight	~15 min	>1 hr	~15 min	4–20 Hrs
SNAP i.d.® 2.0	**20 sec**	**10 min**	**3 min**	**10 min**	**3 min**	**≤30 min**

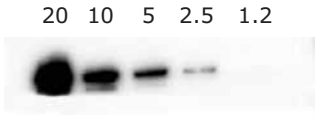
SNAP i.d.® Analysis



Anti-Huntingtin Protein (Cat. No. MAB2166)

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

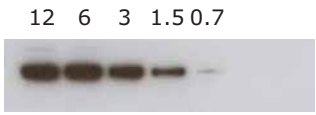
Proteins were detected using Immobilon® Forte HRP detection reagent.
Exposure of the blots to X-ray film time varies from 20 sec. to 30 min.



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).

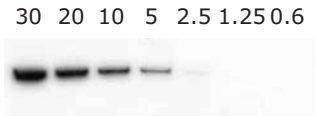
Proteins were detected using Immobilon® Forte HRP detection reagent.
Exposure of the blots to X-ray film time varies from 20 sec. to 30 min.



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 Immobilon® Forte HRP detection reagent.
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).

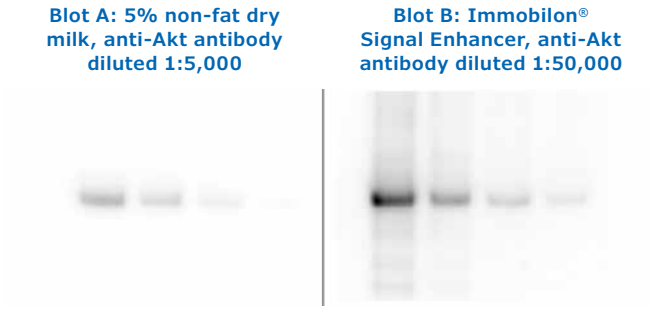
Proteins were detected using Immobilon® Forte HRP detection reagent.
Exposure of the blots to X-ray film varies from 20 sec. to 30 min.

Immobilon® Signal Enhancer

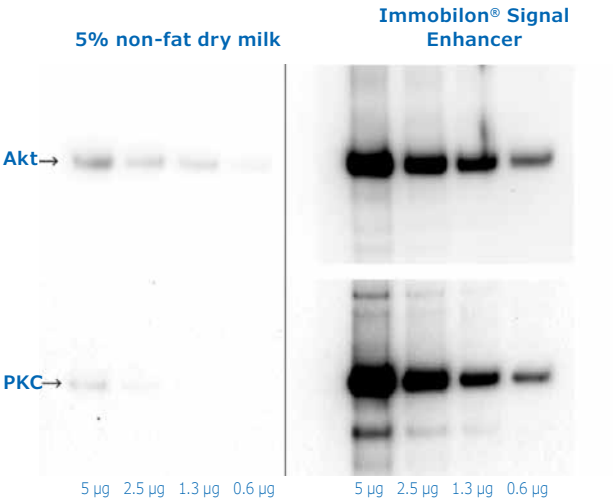
New Immobilon® Signal Enhancer combines signal amplification and blocking in one ready-to-use reagent.

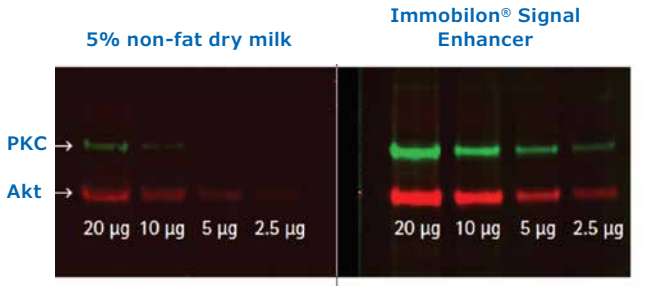
Boost signal: Amplify low-intensity signal in Western blots—without amplifying noise

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.

Immobilon® Block noise cancelling reagents

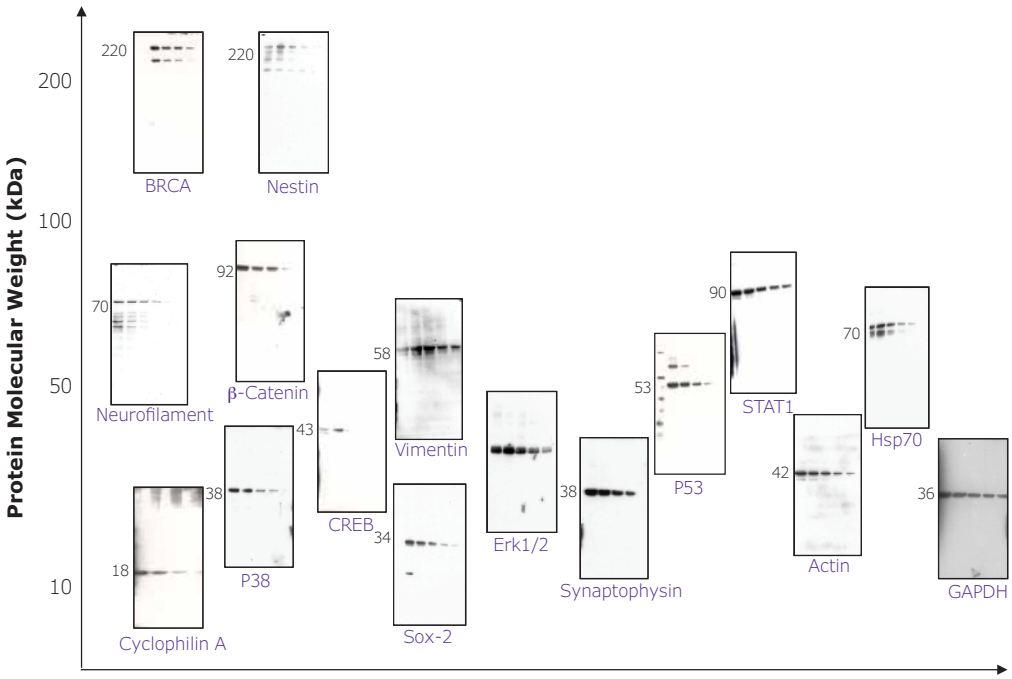
In Western blotting, blocking of unbound membrane sites is necessary to prevent the non-specific binding by antibodies that leads to high backgrounds. **Traditional milk or other protein-blockers** can leave a thick layer of sticky proteins that:

- 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.

Immobilon® Block reagent offers:

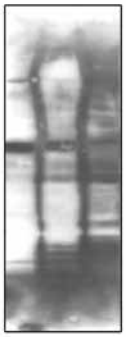
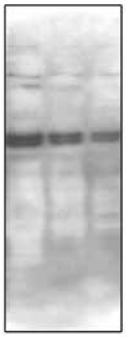
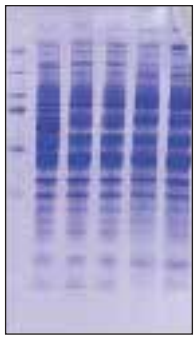
- Reduced background for better protein detection
- No need to run a second gel for Coomassie® staining
- Stable at room temperature for two years
- Ready-to-use, no mixing required

Immobilon® Block reagents excel with diverse antibodies and lysates



All primary and secondary antibodies were diluted in Immobilon® Block-CH

Immobilon® Block-CH noise cancelling reagent

Supplier P	Non-fat dry milk (NFDM)	Immobilon® Block-CH	Chemiluminescence Detection (anti-phosphotyrosine)	Blot Stained after Immunodetection (Highlight)
			 1 2 3 4 5 6	 1 2 3 4 5 6

Immobilon® Block 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.

Immobilon® Block 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 Immobilon® Block-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

Immobilon® Block-PO noise cancelling reagent

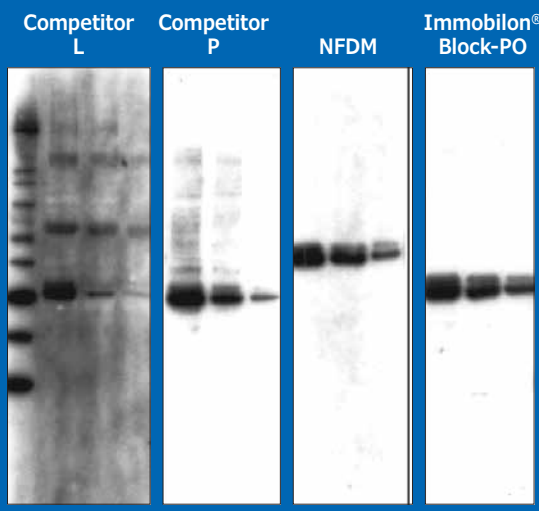
Blocking of non-specific protein binding sites on a blot is essential to decreasing the background and obtaining meaningful results. Although milk is the most commonly used blocker, the presence of phosphorylated mammalian proteins in milk can result in a very high background. For that reason, non-protein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

How does Immobilon® Block-PO reagent improve results?

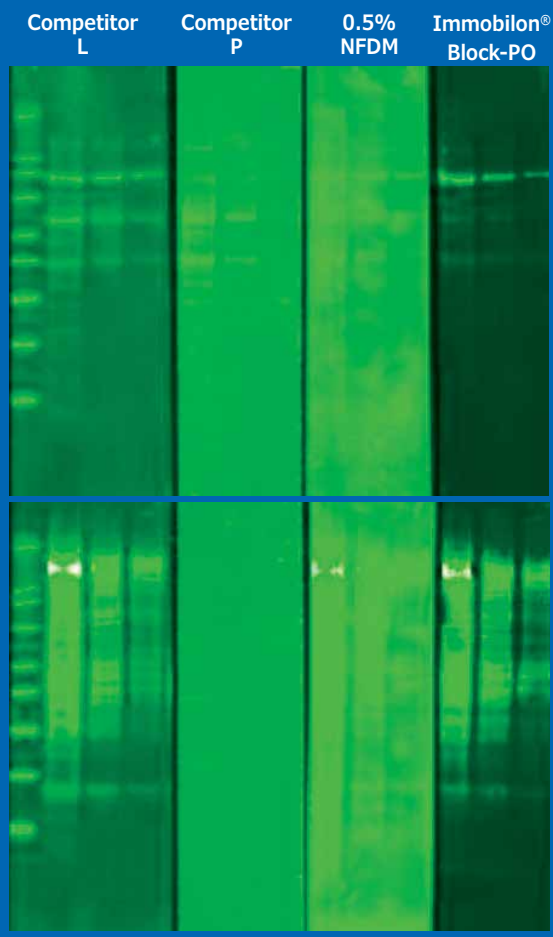
This chemical-based blocker contains phosphatase inhibitors to preserve the phosphorylation state of the blotted proteins.

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 one 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 Immobilon® Forte Western HRP substrate (Cat. No. WBLUF0500). NFDM= Non-fat dry milk.



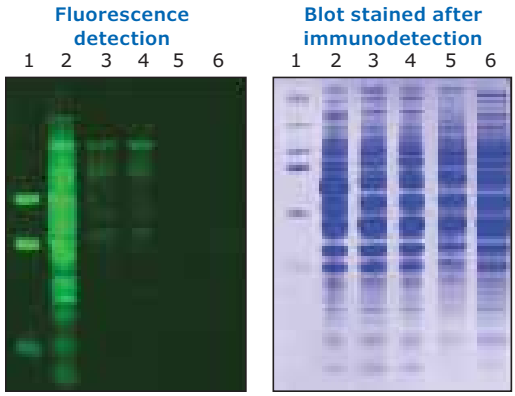
Immobilon® Block-PO reagent works best for detection of phosphoproteins.

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.

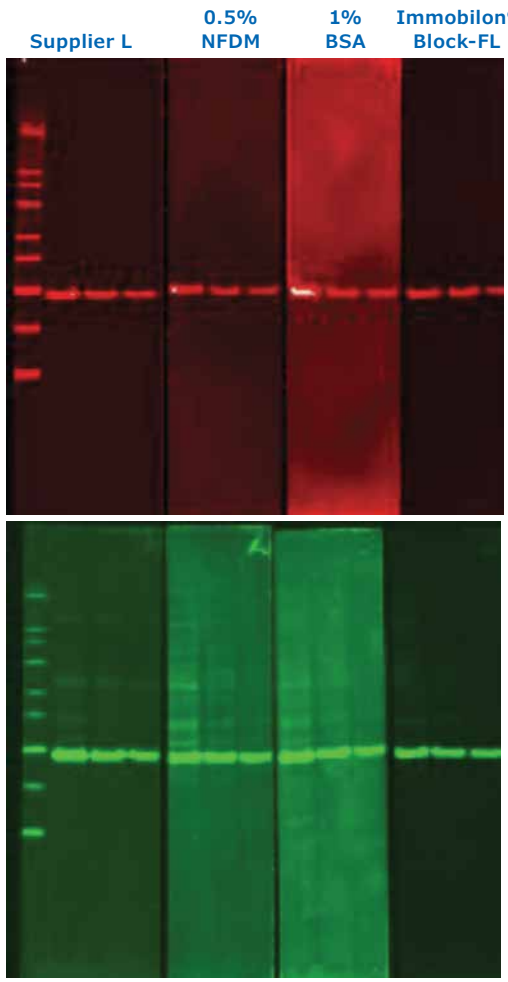
Immobilon® Block-FL noise cancelling reagent

Key Benefits

- Specially formulated for reduced background on fluorescent westerns
- Ready-to-use straight from the bottle
- Stable at room temperature for two years
- Enables colorimetric staining of blots 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 Immobilon® Block-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.



Immobilon® Block-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.

Avoid running a gel just for Coomassie® staining

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

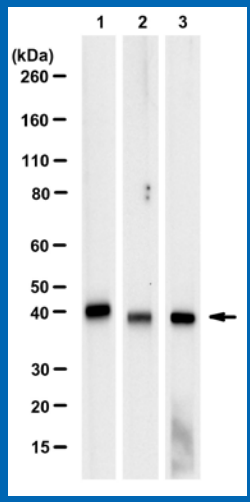
Technique Spotlight

ZooMAb® Antibodies

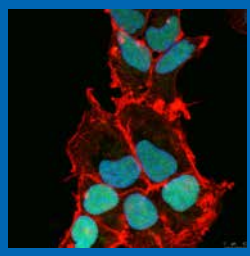
ZooMAb® antibodies represent an entirely new generation of recombinant monoclonal antibodies. They are specifically engineered to combine state- of-the-art consistency and applications performance with the most user-friendly formulation, handling, and storage features available today. With a long history of delivering highly cited antibodies for research applications, we are excited to provide you with the next revolution in recombinant monoclonal antibody technology.

Unlike previous conventional technologies, ZooMab® antibodies are developed from a proprietary B-cell transfection and recombinant expression platform using tissue culture-based methods. This technology opens the door to a much wider “Zoological” range of species to produce recombinant monoclonal antibodies. Our first group of ZooMAb® antibodies are rabbit- derived, which are well recognized today for producing monoclonal antibodies of the highest affinity and specificity, but future iterations will be from a variety of species.

Learn all about our new ZooMAb® antibodies at **SigmaAldrich.com/ZooMAb**



Lysates from NIH3T3 (A) and HepG2 (B) cells were probed with Anti-SOX-9, clone 2B10, ZooMAb® Rabbit Monoclonal (Cat. No. ZRB5535). Proteins were visualized using a Donkey Anti-Rabbit IgG secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates SOX-9 (~65 kDa).



Immunofluorescent analysis with Anti-Sox-2, clone 1A2, ZooMAb® Rabbit Monoclonal (Cat. No. ZRB5603).

These ZooMAb® antibodies are validated for Western blot and at least two other key applications at similar recommended dilutions as traditional antibodies.

Loading control antibodies for Western blotting

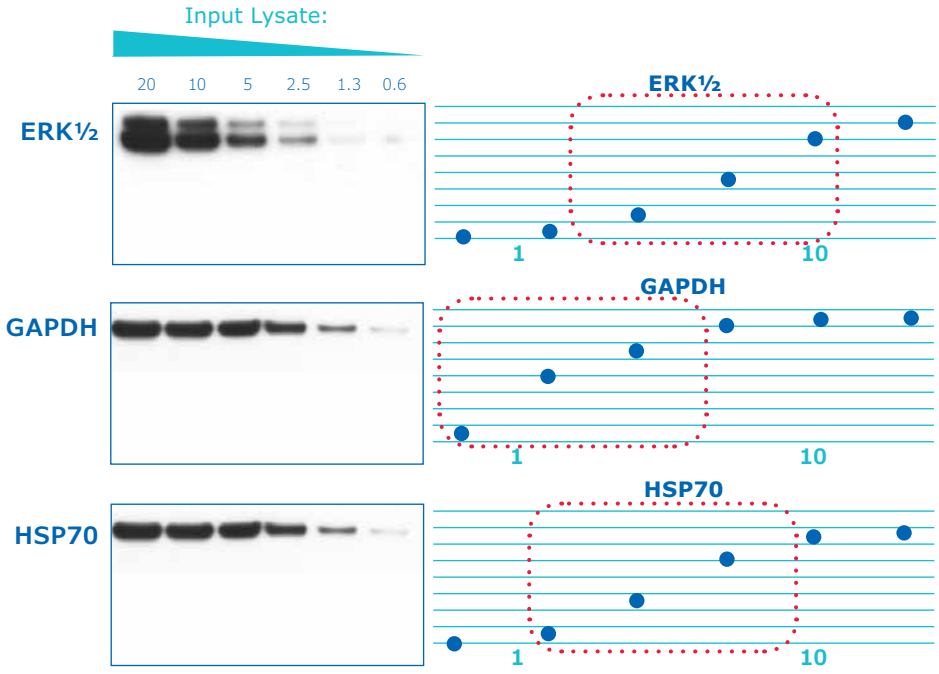
Proteins exhibiting abundant, constitutive levels in Western blot samples can be used to control for technical variability. These so-called loading controls are essential for confirming that differences in observed signal in Western blot experiments are caused due to changes in protein expression, and 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 ERK½, 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, SAB4200714; x-Erk½, 06-182) (ECL reagent, Immobilon® 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	Ms	124 kDa	05-386
Anti-Vinculin, Clone hVIN-1	Hu, Ms, Rt, Ca, Ch, Bv, Tk, Fg	Ms	116 kDa	V9131
Anti-HSP90, Clone D7a	Hu, Ms, Rt, Rb, Bv, Ch, Po	Ms	90 kDa	05-594
Anti-HSP90, Clone 803CT9	Hu, Ms	Ms	85 kDa	SAB1305541
Anti-HSP70, Clone C92F3-5	A broad range of species	Ms	70 kDa	386032
Anti-HSP70, Clone BRM-22	A broad range of species	Ms	70 kDa	SAB4200714
Anti-Lamin B1, Clone 8F10.1	Hu, Rt	Ms	66 kDa	MABS492
Anti-Lamin B1, polyclonal	Hu	Rb	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

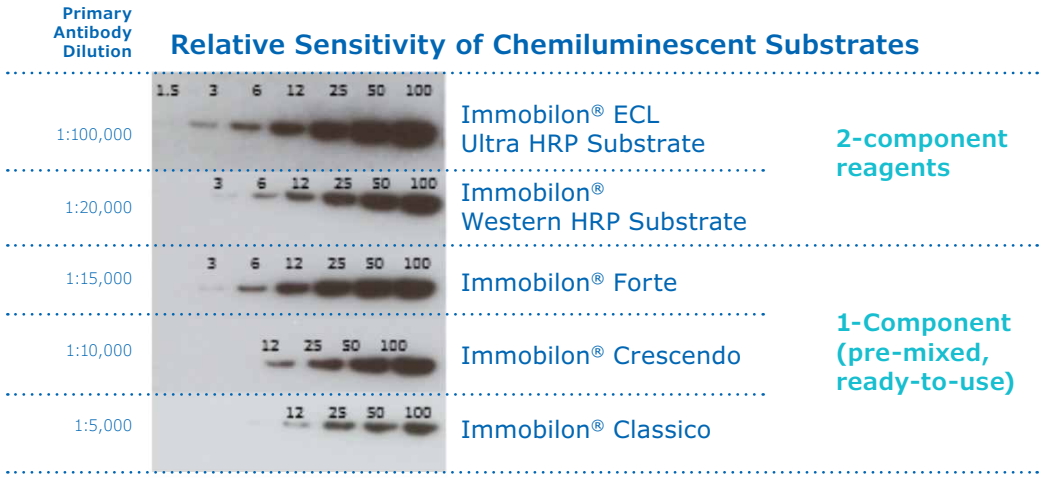
We offer a broad selection of ECL (enhanced chemiluminescence) substrates for every application.

Immobilon® Classico, Crescendo and Forte substrates are a family of premixed reagents for peroxidase-based detection that offer significant advantages over other ECL reagents:

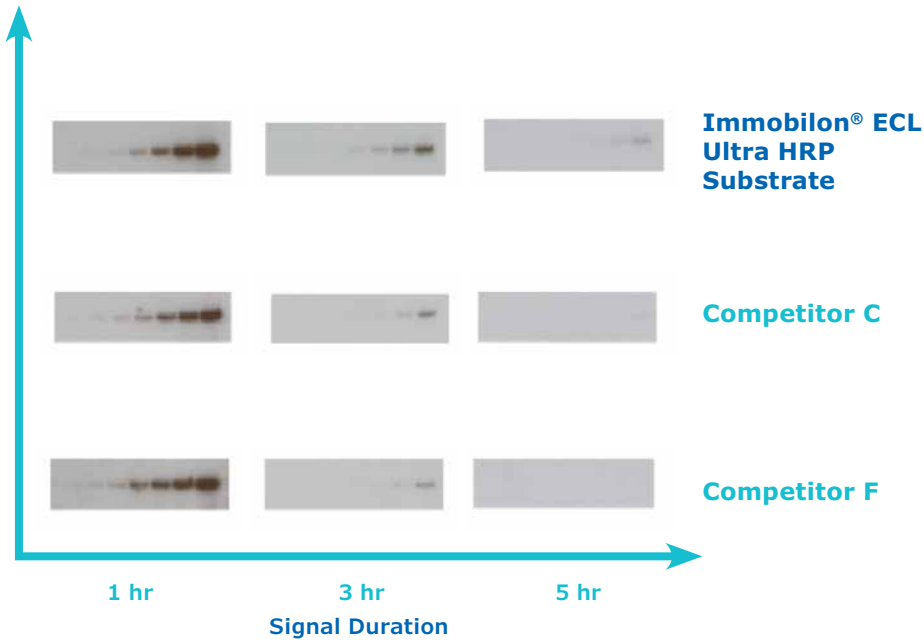
- Single component, ready-to-use formulations simplify detection for enhanced reproducibility and convenience
- A range of sensitivities to provide optimal signal-to-background ratio across a spectrum of target protein concentrations

Immobilon® ECL Ultra and Western HRP substrates 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



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

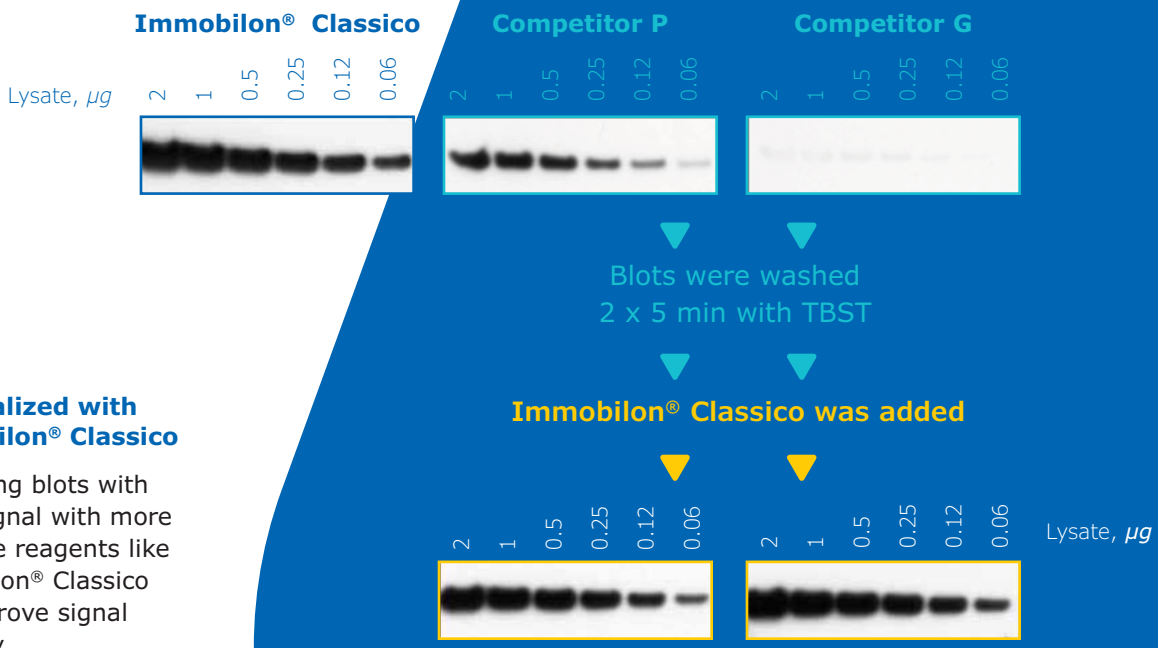


	Immobilon® Classico	Immobilon® Crescendo	Immobilon® Forte	Immobilon® Western HRP Substrate	Immobilon® ECL Ultra HRP Substrate
Approximate Detection Limit	**~ 6 pg**	**~ 1 – 3 pg**	**~ 400 fg**	**~ 400 fg**	**low fg**
Format	1-component	1-component	1-component	2-component	2-component
Signal Duration	1 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
Two-component ECL reagents with similar sensitivity					
ThermoFisher Scientific	Pierce™ ECL	SuperSignal® Pico	SuperSignal® Dura	SuperSignal® Dura	SuperSignal® Femto
GE® Healthcare	Amersham ECL® reagent	---	Amersham ECL® Prime	Amersham ECL® Prime	Amersham ECL Select®
Bio-Rad	---	---	Clarity™ reagent	Clarity™ reagent	Clarity Max™ reagent

Test Immobilon® substrates after your regular HRP substrate

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

Visualized with specified HRP substrate



Revisualized with Immobilon® Classico

Reprobing blots with weak signal with more sensitive reagents like Immobilon® 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 Immobilon® Classico substrate. All blots were exposed to X-ray film for 1 minute.

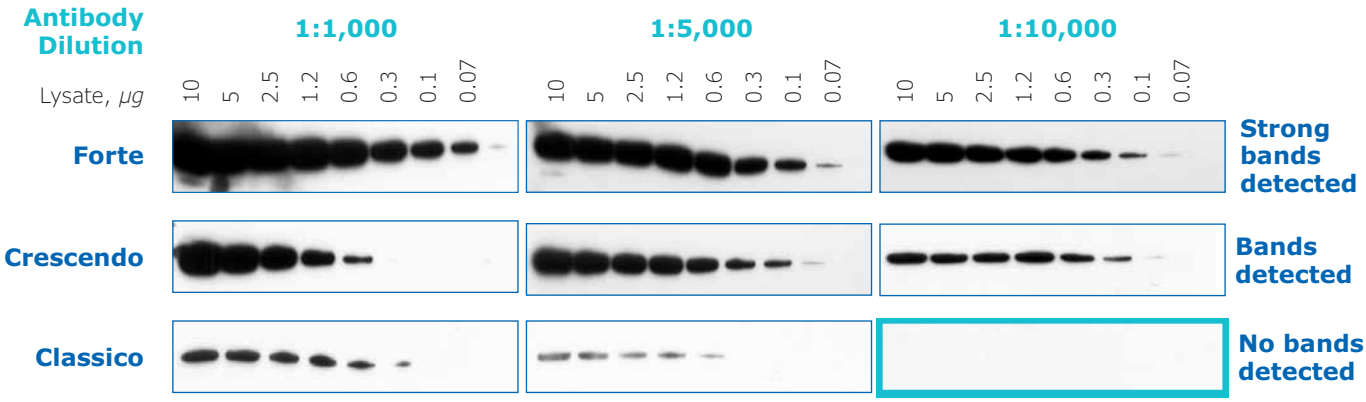
Optimizing Western blots with Immobilon® Western HRP substrates

If no bands are detected with Immobilon® Classico Western HRP substrate (boxed blot), two potential options can be considered:

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

Using higher sensitivity HRP substrates produces better results while saving time and reducing cost:

1. **Better results:** The increased-sensitivity detection reagent produced stronger bands for a more quantitative blot (compare the increase in band intensities for Immobilon® 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 Immobilon® HRP substrate and exposed to x-ray film for 5 minutes.

ReBlot™ Plus Western blot recycling kit

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.

- **Mild:** Provides good results on both nitrocellulose and PVDF membranes.
- **Strong:** Use when stripping high-signal membranes or 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 (e.g., Edman degradation)

ReBlot™ reagent 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 membrane. 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).

Efficient blot stripping with ReBlot™ reagent led to a clean GAPDH blot, even though both primary antibodies share the same anti-mouse secondary antibody.

Accessories

Millipore® Electrophoresis Power Supplies

Our power supplies offer the reproducibility your research requires with the quality you've come to expect. Both models provide the versatility needed for electrophoresis and transfer, ensuring the power is always in your hands.

mA700 Essential Power Supply

- Higher maximum output provides increased capacity
- Wet (tank) & semi-dry transfer
- Stores 30 programs, each with up to 6 steps
- Color display shows all parameters during operation
- Compact, stackable design

mA400 Basic Power Supply

- Wet (tank) transfer for up to 2 gels
- Timer control and 2-step programs
- Compact design

Specifications

	mA400 Basic Power Supply	mA700 Essential Power Supply
Output range		
Volts	10-300 V, adjustable in 1 V steps	5-300 V, adjustable in 1 V steps
Current	10-400 mA, adjustable in 1 mA steps	1-700 mA, adjustable in 1 mA steps
Output type	Constant voltage or current	Constant voltage, current, or power (with automatic crossover)
Maximum output power	60W	150W
Programmable	1 method up to 2 steps	Stores up to 30 programs, each with up to 6 steps
Display	3-digit LED	2.4-inch color TFT LCD
Timer	Up to 999 min	Constant: up to 9,999 min Program: up to 999 min
Output terminals	2 sets	4 sets
Dimensions (W x L x H)	14.0 x 19.1 x 8.4 cm	21.5 x 33.5 x 10.4 cm
Weight	1 kg	2.1 kg

Ordering Information

Model	Plug Type	Country†	Catalog Number
mA700 Essential Power Supply	NEMA 5-15P (YP12)	US, Canada	MA700-US
	CEE 7/7 (YP22)	France, Germany	MA700-EU
	Type G (YP61)	UK, Ireland	MA700-UK
	YP18	Japan	MA700-NI
	Type I (YP03)	China	MA700-ZH
mA400 Basic Power Supply	NEMA 5-15P (YP12)	US, Canada	MA400-US
	CEE 7/7 (YP22)	France, Germany	MA400-EU
	Type G (YP61)	UK, Ireland	MA400-UK
	YP18	Japan	MA400-NI
	Type I (YP03)	China	MA400-ZH

†Countries listed based on standard plug type. Other locations may use the same plug type and are not listed. Please verify the plug type in your lab prior to purchase.

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 a 0.2 µm or 0.45 µm Millex® syringe filter.
	Aggregates in HRP-conjugated secondary antibody	Filter secondary antibody solution through a 0.2 µm or 0.45 µm Millex® syringe filter.
High background	Insufficient washes	Increase washing volumes and times. Pre-filter all of your solutions including the transfer buffer, using Millex® syringe filters 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 0.5 M 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 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		
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 (conc. tris-buffered saline)
[Tris]: 200 mM
[NaCl]: 1500 mM
For 1 L:
24 g Tris base (Product 93362 – FW: 121.1 g/mol)
88 g NaCl (Product 71376)
Dissolve in 900 mL Milli-Q® water. Adjust pH to 7.6 with 12 N HCl (Product H1758). 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)
[Tris]: 20 mM
[NaCl]: 150 mM
[Tween® 20]: 0.1% (w/v)
For 1 L:
100 mL TBS 10X
900 mL Milli-Q® water
1 mL Tween®-20 detergent (Product P9416)

4X SDS-PAGE sample loading buffer
[Tris-HCl]: 0.2 M
[DTT]: 0.4 M
[SDS]: 277 mM, 8.0% (w/v)
[Bromophenol blue]: 6 mM
[Glycerol]: 4.3 M
For 7.5 mL:
1.5 mL 1 M Tris-HCl pH 6.5 (Product 20-160)
3 mL 1 M dithiothreitol (DTT, Product 43816)
0.6 g sodium dodecyl sulfate (SDS, Product L3771)
30 mg bromophenol blue (Product B0126)
2.4 mL glycerol (Product G5516)
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 (Product S8263) to adjust pH
Make 500 µL aliquots and store at -20°C

SDS-PAGE separating gel casting buffer
[Tris-HCl]: 1.5 M
For 500 mL:
118.2 g Tris-HCl (Product 93363 - FW:157.60 g/mol)
Add 450 mL Milli-Q® water and adjust pH to 8.8
Add Milli-Q® water to final volume of 500 mL
Filter and degas

SDS-PAGE stacking gel casting buffer
[Tris-HCl]: 1 M
For 500 mL:
78.8 g Tris-HCl (Product 93363 - FW:157.60 g/mol)
Add 450 mL Milli-Q® water and adjust pH to 6.8
Add Milli-Q® water to final volume 500 mL
Filter and degas

SDS-PAGE 10X gel running buffer
[Tris]: 248 mM
[Glycine]: 1.92 M
[SDS]: 35 mM, 1% (w/v)
For 2 L:
60 g Tris base (Product 93362 – FW: 121.1 g/mol)
288 g glycine (Product 50046)
20 g sodium dodecyl sulfate (SDS, Product L3771)
Add Milli-Q® water to yield solution with final volume of 2 L
No need to pH, filter, or degas
Dilute to 1X for running SDS-PAGE gels

10X PBS
[NaCl]: 1.37 M
[KCl]: 27 mM
[Na_2HPO_4]: 100 mM
[KH_2PO_4]: 18 mM
For 1 L:
80 g NaCl (Product 71376)
2 g KCl (Product P9541)
17.8 g $Na_2HPO_4 \cdot 2H_2O$ (Product 71643 – FW: 177.99 g/mol)
2.45 g KH_2PO_4 (Product P9791 – FW: 136.09 g/mol)
Add to 900 mL of Milli-Q® water and adjust pH to 7.4.
Add Milli-Q® water to final volume of 1 L.

Transfer buffer 10X
[Tris]: 250 mM
[Glycine]: 1.9 M
[SDS]: 17 mM, 0.5% (w/v)
For 500 mL:
15.2 g Tris base (Product 93362 – FW: 121.1 g/mol)
72.1 g glycine (Product 50046)
2.5 g sodium dodecyl sulfate (SDS, Product L3771)
Add Milli-Q® water to final volume of 500 mL

Transfer buffer 1X
Make fresh each time
For 500 mL:
50 mL 10X transfer buffer
100 mL methanol (Product 494437)
Add Milli-Q® water to final volume of 500 mL

SDS-PAGE Coomassie® staining solution
[Coomassie® Blue R-250]: 3.0 mM
[Methanol]: 50% (v/v)
[Acetic acid]: 1.74 M, 10% (v/v)
For 500 mL:
1.25 g Coomassie® Blue R-250 (Product B7920)
225 mL methanol (Product 494437)
225 mL Milli-Q® water
50 mL glacial acetic acid (Product 45726)

SDS-PAGE destaining solution
[Methanol]: 30% (v/v)
[Acetic acid]: 1.74 M, 10% (v/v)
For 1 L:
300 mL methanol (Product 494437)
100 mL glacial acetic acid (Product 45726)
600 mL Milli-Q® water

mPAGE™ MES SDS Running Buffer
[Tris]: 50 mM
[MES]: 50 mM
[SDS]: 0.1%, 3.5 mM
[EDTA]: 1 mM
For 1000 mL:
6.06 g Tris base (Product 93362 – FW: 121.1 g/mol)
9.76 g 2-(N-Morpholino)ethanesulfonic acid (MES)
1.0 g sodium dodecyl sulfate (SDS, Product L3771)
0.3 g Ethylenediaminetetraacetic acid (EDTA – Product 03609)
Dissolve in 900 mL Milli-Q® water and mix well. Add Milli-Q® water to a final volume of 1 L. Do not adjust pH.

mPAGE™ MOPS SDS Running Buffer
[Tris]: 50 mM
[MOPS]: 50 mM
[SDS]: 0.1%, 3.5 mM
[EDTA]: 1 mM
For 1000 mL:
6.06 g Tris base (Product 93362 – FW: 121.1 g/mol)
1.046 g 3-(N-morpholino)propanesulfonic acid (MOPS, Product 69947)
1.0 g sodium dodecyl sulfate (SDS, Product L3771)
0.3 g Ethylenediaminetetraacetic acid (EDTA – Product 03609)
Dissolve in 900 mL Milli-Q® water and mix well. Add Milli-Q® water to a final volume of 1 L. Do not adjust pH.

mPAGE™ 1X Transfer Buffer, pH 8.2, for Wet Transfer
[Tris]: 25 mM
[Bicine]: 25 mM
[Methanol]: 10%
For 1000 mL:
3.0 g Tris base (Product 93362 – FW: 121.1 g/mol)
4.08 g N,N-Bis(2-hydroxyethyl)glycine (Bicine, Product B8660)
100 mL methanol (Product 494437)
Combine and mix well. Add Milli-Q® water to final volume 1000 mL. Do not adjust pH.

mPAGE™ 4X LDS Sample Buffer
[Tris-HCl]: 424 mM
[Tris]: 564 mM
[LDS]: 8%, 294 mM
[EDTA]: 2.07 mM
[Glycerol]: 40%, 4.34 M
[Coomassie® Blue G250]: 0.88 mM
[Phenol Red]: 0.7 mM
For 10 mL:
0.666 g Tris-HCl (Product 93363 - FW:157.60 g/mol)
0.682 g Tris base (Product 93362 – FW: 121.1 g/mol)
0.800 g Lithium dodecyl sulfate (LDS, Product L9781)
0.006 g Ethylenediaminetetraacetic acid (EDTA, Product 03609)
3.2 mL Glycerol (Product G5516)
0.75 mL 1% Coomassie® Blue G250 solution
0.25 mL 1% Phenol Red solution
Combine and add Milli-Q® water to final volume 10 mL. Store at 2-8 °C. The pH of the 1X solution is 8.5. Do not adjust pH with acid or base

mPAGE™ Transfer Buffer (with Methanol), pH 8.2, for Semi-Dry Transfer
[Tris]: 50 mM
[Bicine]: 50 mM
[Methanol]: 10%
For 500 mL:
3.0 g Tris base (Product 93362 – FW: 121.1 g/mol)
4.08 g N,N-Bis(2-hydroxyethyl)glycine (Bicine, Product B8660)
50 mL methanol (Product 494437)
Combine and mix well. Add Milli-Q® water to final volume 500 mL. Do not adjust pH.

mPAGE™ Gel Equilibration Buffer, pH 8.2, for Semi-Dry Transfer
[Tris]: 50 mM
[Bicine]: 50 mM
For 500 mL:
3.0 g Tris base (Product 93362 – FW: 121.1 g/mol)
4.08 g N,N-Bis(2-hydroxyethyl)glycine (Bicine, Product B8660)
Dissolve in 450 mL of Milli-Q® water and mix well. Add Milli-Q® water to final volume 500 mL. Do not adjust pH.

Prepare

Description	Cat. No.
Lysis & Extraction Kits	
BugBuster® Master Mix	**71456**
BugBuster® Plus Benzonase® Nuclease	**70750**
BugBuster® Protein Extraction Reagent (for bacterial lysis)	**70584**
CelLytic™ B Cell Lysis Reagent, For bacterial cell lysis, 10x concentrate	**C8740**
CelLytic™ Express	**C1990**
CelLytic™ IB Inclusion Body Solubilization Reagent	**C5236**
CelLytic™ M, Cell Lysis Reagent, Suitable for Mammalian cell lysis and protein solubilization.	**C2978**
CelLytic™ MEM Protein Extraction Kit	**CE0050**
CelLytic™ MT Cell Lysis Reagent, For mammalian tissues	**C3228**
CelLytic™ NuCLEAR™ Extraction Kit, For mammalian tissue or cultured cells	**NXTRACT**
CelLytic™ PN Isolation/Extraction Kit, For plant leaves	**CELLYTPN1**
CelLytic™ Y Cell Lysis Reagent, For yeast cells	**C4482**
CelLytic™ Y Plus Kit, For enzymatic yeast cell lysis	**CYP1**
CHAPS hydrate, BioReagent, ≥98% (TLC)	**C9426**
CytoBuster® Protein Extraction Reagent (for mammalian cell lysis)	**71009**
Laemmli Lysis-buffer, non smelling	**38733**
Mammalian Protein Extraction Buffer, volume 500 mL	**GE28-9412-79**
Nuclear Extraction Kit	**2900**
ProteoExtract® Complete Mammalian Proteome Extraction Kit	**539779**
ProteoExtract® Native Membrane Protein Extraction Kit	**444810**
ProteoExtract® Subcellular Proteome Extraction Kit	**539790**
ProteoExtract® Transmembrane Protein Extraction Kit	**71772**
RIPA Buffer	**R0278**
RIPA Lysis Buffer, 10X, 100 mL	**20-188**
Sample Grinding Kit, GE Healthcare, 80-6483-37, sufficient for 50 preparations	**GE80-6483-37**
YeastBuster® Protein Extraction Reagent (for yeast cell lysis)	**71186**
Affinity Purification	
Protein A Agarose, fast flow, 10 mL	**16-156**
Protein A/G Mix, 10 mL	**IP10-10ML**
Protein G Agarose, fast flow, 10 mL	**16-266**
PureProteome™ Protein A Magnetic Beads, 10 mL	**LSKMAGA10**
PureProteome™ Protein G Magnetic Beads, 10 mL	**LSKMAGG10**
Protease Inhibitors	
Calbiochem® Protease Inhibitor Cocktail Set III, EDTA-Free	**539134-1SET**
Chymostatin, 100 mg	**EI6**
Leupeptin, 100 mg	**EI8**
Mini Roche cOmplete™	**11836153001**
Mini, EDTA-Free Roche cOmplete™	**11836170001**
Pepstatin A, 100 mg	**516481**
Protease Inhibitor Cocktail, for general use, lyophilized powder	**P2714**
Roche cOmplete™, EDTA-Free	**COEDTAF-RO**
SIGMAFAST™ Protease Inhibitor Cocktail Tablets, EDTA-Free, for use in purification of Histidine-tagged proteins	**S8830**
SIGMAFAST™ Protease Inhibitor Tablets, For General Use	**S8820**
Standard Roche cOmplete™	**CO-RO**
ULTRA, EDTA-Free Roche cOmplete™	**COUEDTAF-RO**
ULTRA-Roche cOmplete™	**COUL-RO**

Prepare (continued)

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**

Quantify

Description	Cat. No.
Protein Quantitation Kits	
Bicinchoninic Acid Kit for Protein Determination	**BCA1-1KT**
QuantiPro™ BCA Assay Kit, for 0.5-30 µg/ml protein	**QPBCA**
Total Protein Kit, Micro	**TP0100-1KT**
Total Protein Kit, Micro Lowry, Peterson's Modification	**TP0300-1KT**
Total Protein Kit, Micro-Lowry, Onishi & Barr Modification	**TP0200-1KT**
2-D Quant Kit, GE Healthcare	**GE80-6483-56**
Protein Quantitation Reagents	
Bradford Reagent, for 1-1,400 µg/ml protein	**B6916**
Bradford Reagent for 0.1-1.4 mg/ml protein	**B6916-500ML**
Bicinchoninic Acid solution	**B9643-1L**
Biuret reagent	**B3934-110ML**
Lowry Reagent	**L3540-25VL**

Electrophoresis: mPAGE™ Precast Gels

mPAGE™ Bis-Tris Precast Gels	10-well	12-well	15-well
10 gels per box	**80 µL/well**	**60 µL/well**	**40 µL/well**
4-12%	MP41G10	MP41G12	MP41G15
4-20%	MP42G10	MP42G12	MP42G15
8-16%	MP81G10	MP81G12	MP81G15
8%	MP8W10	MP8W12	MP8W15
10%	MP10W10	MP10W12	MP10W15
12%	MP12W10	MP12W12	MP12W15

Description	Qty	Cat. No.
mPAGE™ Buffers		
mPAGE™ 4X LDS Sample Buffer	10 mL	**MPSB-10ML**
	250 mL	**MPSB-250ML**
mPAGE™ MES SDS Running Buffer Powder (each packet makes 1 L)	5 packets	**MPMES**
mPAGE™ MOPS SDS Running Buffer Powder (each packet makes 1 L)	5 packets	**MPM0PS**
mPAGE™ Transfer Buffer Powder (each packet makes 1 L)	10 packets	**MPTRB**
mPAGE™ Trial Kits		
Trial kits contain 2 gels, 1 x 1 L Running Buffer Powder, Adapter Plates, Cassette Opener		
mPAGE™ Trial Kit, 4-12%, 12-well, MOPS		**MP41G12TR1**
mPAGE™ Trial Kit, 4-20%, 12-well, MOPS		**MP42G12TR1**
mPAGE™ Trial Kit, 10%, 12-well, MOPS		**MP10W10TR1**
mPAGE™ Trial Kit, 4-12%, 12-well, MES		**MP41G12TR2**
mPAGE™ Trial Kit, 10%, 12-well, MES		**MP10W12TR2**
mPAGE™ Trial Kit, 12%, 12-well, MES		**MP12W12TR2**
mPAGE™ Accessories		
mPAGE™ Adapter Plates	2	**MPTA**
mPAGE™ Gel Cassette Opener	1	**MPC0**
mPAGE™ Buffer Dam	1	**MPBD**

Protein Molecular Weight Markers (Ladders)

Description	Cat. No.
Prestained Protein Standards	
BLUEye Prestained Protein Ladder	**94964-500UL**
Color Marker Ultra-Low Range (M.W. 1,060-26,600)	**C6210**
ColorBurst™ Electrophoresis Marker, mol wt 8,000-220,000 Da	**C1992**
Prestained Molecular Weight Marker, mol wt 26,600-180,000 Da	**SDS7B2**
Amersham ECL® Rainbow Marker - Full range	**GERPN800E**
Amersham ECL® Rainbow Marker - High range	**GERPN756E**
Amersham ECL® Rainbow Marker - Low range	**GERPN755E**
Unstained Protein Standards	
mPAGE™ Western Protein Standard	**MPSTD2**
Biotinylated Molecular Weight Marker, mol wt 6,500-180,000 Da	**B2787**
SigmaMarker™ low range, mol wt 6,500-66,000 Da	**M3913**
SigmaMarker™ wide range, mol wt 6,500-200,000 Da	**S8445**
β-Galactosidase from Escherichia coli	**G8511**

Electrophoresis Reagents & Buffers

Description	Cat. No.
Acrylamide/Bis-Acrylamide Solutions	
Acrylamide/Bis-acrylamide, 30% solution, BioReagent, 29:1	**A3574**
Acrylamide/Bis-acrylamide, 30% solution, BioReagent, 37.5:1	**A3699**
Acrylamide/bis-acrylamide, 40% solution, BioReagent, 19:1	**A9926**
Acrylamide/bis-acrylamide, 40% solution, BioReagent, 29:1	**A7802**
Acrylamide/bis-acrylamide, 40% solution, BioReagent, 37.5:1	**A7168**
Acrylamide/Bis-Acrylamide Powders	
Acrylamide/Bis-acrylamide, BioReagent, 19:1	**A2917**
Acrylamide/Bis-acrylamide, BioReagent, 29:1	**A2792**
Acrylamide/Bis-acrylamide, BioReagent, 37:1	**A6050**
Acrylamide/Bis-acrylamide, BioReagent, 41:1	**A0924**
Acrylamide, ≥99% (HPLC), powder	**A3553**
Acrylamide, ≥99%	**A8887**
N,*N*′-Methylenebis(acrylamide), 99%	**146072**
Electrophoresis Running and Loading Buffers	
Glycine, ≥99%	**G8898**
Laemmli 2x Concentrate Sample Buffer	**S3401**
Tricine, cell culture tested, ≥99%	**T5816**
Tris-Glycine Buffer, 10x Concentrate	**T4904**
Tris-Glycine-SDS Buffer, 10x Concentrate	**T7777**
Tris-Tricine-SDS Buffer, 10x Concentrate	**T1165**
TG-SDS Buffer, 10X Powder Pack, ULTROL® Grade	**585207**
Trizma® hydrochloride solution, pH 7.5, 1 M, suitable for cell culture	**T2319**
Bromophenol Blue sodium salt	**B8026**
Trizma® base, anhydrous, free-flowing, Redi-Dri™, ≥99.9%	**RDD008**
Detergents	
Sodium dodecyl sulfate ≥98.5%	**L3771**
Lithium dodecyl sulfate	**L9781**
Gel Casting Reagents	
Ammonium persulfate, for molecular biology, for electrophoresis, ≥98%	**A3678**
N,*N*,*N*',*N*'-Tetramethylethylenediamine, ~99%	**T9281**
Reducing & Denaturing Reagents	
2-Mercaptoethanol suitable for cell culture, 99%	**M3148**
DL-Dithiothreitol (DTT), ≥99%	**D9163**
DL-Dithiothreitol (DTT) solution, 1 M in H_2O	**43816**
Tris(2-carboxyethyl)phosphine hydrochloride	**68957**
Urea 8 M (after reconstitution)	**U4883-6X25ML**

Protein Gel Stains

Description	Cat. No.
Colorimetric	
EZBlue™ Gel Staining Reagent	**G1041**
InstantBlue™, Ultrafast Protein Stain	**ISB1L**
ProteoSilver™ Plus Silver Stain Kit	**PROTSIL2-1KT**
ProteoSilver™ Silver Stain Kit	**PROTSIL1-1KT**
Reversible Protein Detection Kit for Membranes and Polyacrylamide Gels	**RPROB-1KT**
Coomassie® Brilliant Blue G solution, Concentrate	**B8522**
Coomassie® Brilliant Blue R, pure	**B7920**
Fast Green FCF, Dye content ≥85 %	**F7252**
Fluorescent	
EZFluor™ 1-step Fluorescent Protein Gel Stain	**SCT145**
EZFluor™ UV 1-step Fluorescent Protein Gel Stain	**SCT147**
SYPRO® Orange Protein Gel Stain	**S5692**
SYPRO® Ruby Protein Gel Stain	**S4942**
Fixing solution	**F7264**

Transfer Membrane & Blotting Papers

Description	Format	Cat. No.
PVDF Transfer Membrane		
Immobilon®-E PVDF Transfer Membrane, 0.45 µm (no alcohol pre-wet step required)	8.5 cm x 10 m roll	**IEVH85R**
	26.5 cm x 1.875 m roll	**IEVH00005**
	7 cm x 8.4 cm sheets, 50/pk	**IEVH07850**
	8 cm x 10 cm sheets, 10/pk	**IEVH08100**
	Blotting Sandwich*, 7 cm x 8.4 cm, 20/pk	**IESN07852**
	Blotting Sandwich*, 8.5 cm x 13.5 cm, 20/pk	**IESN08132**
	7 cm x 8.4 cm sheets, 4/pk (trial size)	**IEVH07804**
Immobilon®-P PVDF Transfer Membrane, 0.45 µm	8.5 cm x 10 m roll	**IPVH85R**
	26.5 cm x 3.75 m roll	**IPVH00010**
	26.5 cm x 1.875 m roll	**IPVH00005**
	7 cm x 8.4 cm sheets, 50/pk	**IPVH07850**
	8.5 cm x 13.5 cm, 10/pk	**IPVH08130**
	Blotting Sandwich*, 7 cm x 8.4 cm, 20/pk	**IPSN07852**
	Blotting Sandwich*, 8.5 cm x 13.5 cm, 20/pk	**IPSN08132**
Immobilon®-FL PVDF Transfer Membrane, 0.45 µm	8.5 cm x 10 m roll	**IPFL85R**
	26.5 cm x 3.75 m roll	**IPFL00010**
	26.5 cm x 1.875 m roll	**IPFL00005**
	7 cm x 8.4 cm sheets, 10/pk	**IPFL07810**
	10 cm x 10 cm sheets, 10/pk	**IPFL10100**
Immobilon®-P^{SQ} PVDF Transfer Membrane, 0.2 µm	8.5 cm x 10 m roll	**ISEQ85R**
	26.5 cm x 3.75 m roll	**ISEQ00010**
	26.5 cm x 1.875 m roll	**ISEQ00005**
	7 cm x 8.4 cm sheets, 50/pk	**ISEQ07850**
	8.5 cm x 13.5 cm, 10/pk	**ISEQ08130**
	20 cm x 20 cm, 10/pk	**ISEQ20200**
Immobilon® NOW Dispenser for 8.5 cm x 10 cm rolls		**IMDISP**

*Blotting sandwiches are pre-assembled with membrane and 2 sheets of Immobilon® filter paper for standard tank transfer procedures. For more options, go to **SigmaAldrich.com/Immobilon**

Transfer Membrane & Blotting Papers (continued)

Description	Format	Cat. No.
Nitrocellulose Membranes		
Amersham Protran® Premium Western blotting membranes, nitrocellulose, pore size 0.45 µm	300 mm x 4 m roll	**GE10600003**
	80 mm x 90 mm sheets, 25/pk	**GE10600096**
	Blotting sandwich, 80 mm x 90 mm , 10/pk	**GE10600117**
Amersham Protran® Western blotting membranes, nitrocellulose, pore size 0.45 µm	300 mm x 4 m roll	**GE10600002**
	80 mm x 90 mm sheets, 25/pk	**GE10600093**
	Blotting sandwich, 80 mm x 90 mm , 10/pk	**GE10600115**
Amersham Protran® Western blotting membranes, nitrocellulose pore size 0.2 µm	300 mm x 4 m roll	**GE10600001**
	80 mm x 90 mm sheets, 25/pk	**GE10600094**
Amersham Protran® Premium Western blotting membranes, nitrocellulose pore size 0.2 µm	300 mm x 4 m roll	**GE10600004**
	80 mm x 90 mm sheets, 25/pk	**GE10600097**
Amersham Protran® Supported Western blotting membranes, nitrocellulose pore size 0.2 µm	300 mm x 4 m roll	**GE10600015**
	Blotting sandwich, 80 mm x 90 mm , 10/pk	**GE10600120**
Amersham Protran® Supported Western blotting membranes, nitrocellulose pore size 0.45 µm	300 mm x 4 m roll	**GE10600016**
	Blotting sandwich, 80 mm x 90 mm , 10/pk	**GE10600119**
Blotting Papers		
Immobilon® Blotting Filter Paper	7 cm x 8.4 cm sheets, 100/pk	**IBFP0785C**
	8.5 cm x 13.5 cm, 100/pk	**IBFP0813C**
Whatman® gel blotting paper, Grade GB003	15 cm x 20 cm, 100/pk	**WHA10427812**
	20 cm x 20 cm, 100/pk	**WHA10427818**

Protein Blotting Reagents & Buffers

Description	Cat. No.
Protein Blot Stains	
Amido Black Staining Solution 2X, electrophoresis reagent	**A8181**
ATX Ponceau S red staining solution	**09189-6X1L-F**
Gold solution, colloidal, 0.0065% gold basis (AAS)	**50755**
Ponceau S solution, 0.1 % (w/v) in 5% acetic acid	**P7170**
Reversible Protein Detection Kit for Membranes and Polyacrylamide Gels	**RPROB**
Transfer Buffers & Reagents	
CAPS, pH 3.0-7.0 (20 C, 0.5 M in H_2O), ≥98.0% NaOH basis (titration)	**C6070**
Methanol, ≥99.6%	**179957**
mPAGE™ Transfer Buffer Powder	**MPTRB**
Tris-Glycine Buffer, 10x Concentrate	**T4904**

Immunodetection Devices

Description	Format / Quantity	Cat. No.
Immobilon® GO For Simple Immunodetection		
Immobilon® GO Device	2/pk (trial size)	**IMGDV002**
	10/pk	**IMGDV010**
SNAP i.d.® 2.0 Protein Detection System for Western Blotting		
SNAP i.d.® 2.0 Systems	Mini x 2 (7.5 x 8.4 cm)	**SNAP2MINI**
	Midi x 2 (8.5 x 13.5 cm)	**SNAP2MIDI**
	MultiBlot x 2 (4.5 x 8.4 cm)	**SNAP2MB3**
	Mini and Midi (7.5 x 8.4 cm and 8.5 x 13.5 cm)	**SNAP2MM**
	Mini and MultiBlot (7.5 x 8.4 cm and 4.5 x 8.4 cm)	**SNAP2MB1**
	Midi and MultiBlot (8.5 x 13.5 cm and 4.5 x 8.4 cm)	**SNAP2MB2**
SNAP i.d.® 2.0 Consumables	Mini Blot Holders (7.5 x 8.4 cm) 100/pk	**SNAP2BHMN0100**
	Midi Blot Holders (8.5 x 13.5 cm) 100/pk	**SNAP2BHMD0100**
	MultiBlot Holders (4.5 x 8.4 cm) 50/pk	**SNAP2BHMB050**
SNAP i.d.® 2.0 Accessories	Antibody Collection Tray 20/pk	**SNAPABTR**
	Blot Roller 1/pk	**SNAP2RL**
	Mini Blot Holding Frames (double pack) 2/pk	**SNAP2FRMN02**
	Midi Blot Holding Frames (double pack) 2/pk	**SNAP2FRMD02**
	Mini Blot Holding Frame (single pack) 1/pk	**SNAP2FRMN01**
	Midi Blot Holding Frame (single pack) 1/pk	**SNAP2FRMD01**
	MultiBlot Frame (single pack) 1/pk	**SNAP2FRMB01**

Antibody Conjugates

Description	Cat. No.
Peroxidase Conjugates	
Goat Anti-Mouse IgG Antibody, Peroxidase Conjugated, H+L	**AP124P**
Goat Anti-Rabbit IgG Antibody, Peroxidase Conjugated	**AP132P**
Streptavidin–Peroxidase	**S5512**
Flurophore Conjugates	
ECL Plex™ G-A-M IgG, Cy®3, pack of 150 µg	**GEPA43009**
ECL Plex™ G-A-M IgG, Cy®5, pack of 600 µg	**GEPA45010**
ECL Plex™ Gar IgG Cy®3, pack of 150 µg	**GE28-9011-06**
ECL Plex™ G-A-R IgG, Cy®5, pack of 150 µg	**GEPA45011**
Anti-Rabbit IgG (H+L), F(ab')2 fragment, CF™680 antibody produced in goat	**SAB4600362**
Anti-Mouse IgG (H+L), F(ab')2 fragment, CF™680 antibody produced in goat	**SAB4600361**
Anti-Mouse IgG1 (γ1), CF™770 antibody produced in goat	**SAB4600380**
Anti-Goat IgG (H+L), highly cross-adsorbed, CF™770 antibody produced in donkey	**SAB4600374**

Immunodetection Buffers

Description	Quantity	Cat. No.
Signal enhancer reagents		
Immobilon® Signal Enhancer for Immunodetection	500 mL	**WBSH0500**
	100 mL (trial size)	**WBSH0500-100ML**
Blocking buffers		
Gelatin blocking buffer, powder blend	1L	**G7663-1L**
Immobilon® Block-CH Reagent, Chemiluminescence Detection	500 mL	**WBAVDCH01**
	100 mL (trial size)	**WBAVDCH01-100ML**
Immobilon® Block-FL Reagent, Fluorescence Detection	500 mL	**WBAVDFL01**
	100 mL (trial size)	**WBAVDFL01-100ML**
Immobilon® Block-PO Reagent, Phosphoprotein Detection	500 mL	**WBAVDP001**
	100 mL (trial size)	**WBAVDP001-100ML**
Phosphate-Buffered Saline, dry powder, pH 7.4, contains 3% non-fat milk		**P2194**
Phosphate buffered saline with 5% nonfat milk, powder, pH 7.3		**P4739**
Phosphate-Buffered Saline, powder, pH 7.4		**P3813**
Tris-Buffered Saline, with BSA, pH 8.0, powder		**T6789**
Western Blocker™ Solution, for HRP detection systems	400 mL	**W0138**
Bovine Serum Albumin, heat shock fraction, pH 7, ≥98%		**A7906**
Immunoblot Blocking Reagent	20 g	**20-200**
ChemiBLOCKER™ blocking agent	1 L	**2170**
5% Alkali-soluble Casein	225 mL	**70955**
Wash buffers and reagents		
Phosphate-Buffered Saline, pH 7.4 (in solution), contains Tween®20 detergent, tablets		**08057**
Phosphate-Buffered Saline, pH 7.4, contains Tween®20 detergent, dry powder		**P3563**
Tris-Buffered Saline, 10X solution		**T5912**
Tris-Buffered Saline with Tween®20 detergent, tablets pH 7.6		**91414**
Tris-Buffered Saline, pH 8.0, powder		**T6664**
Tris-Buffered Saline, with Tween®20 detergent, pH 8.0, powder		**T9039**
Tween®20 detergent		**P9416**

Detection Substrates

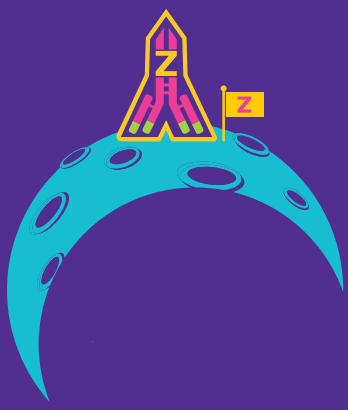
Description	Quantity	Cat. No.
Immobilon Chemiluminescence Peroxidase Substrates		
Immobilon® ECL Ultra Western HRP Substrate	2 x 50 mL	**WBULS0100**
	2 x 250 mL	**WBULS0500**
	2 x 10 mL (trial size)	**WBULS0100-20ML**
Immobilon® Western Chemiluminescent HRP Substrate	2 x 50 mL	**WBKLS0100**
	2 x 250 mL	**WBKLS0500**
	2 x 25 mL (trial size)	**WBKLS0050**
Immobilon® Forte Western HRP Substrate	100 mL	**WBLUF0100**
	500 mL	**WBLUF0500**
	20 mL (trial size)	**WBLUF0020**
Immobilon® Crescendo Western HRP Substrate	100 mL	**WBLUR0100**
	500 mL	**WBLUR0500**
	20 mL (trial size)	**WBLUR0020**
Immobilon® Classico Western HRP Substrate	100 mL	**WBLUC0100**
	500 mL	**WBLUC0500**
	20 mL (trial size)	**WBLUC0020**
Amersham ECL® Western Blotting Detection Reagents		
Amersham ECL® Western Blotting Detection Reagent	For 1000 cm^2 membrane	**GERPN2109**
	For 2000 cm^2 membrane	**GERPN2209**
	For 4000 cm^2 membrane	**GERPN2106**
	For 6000 cm^2 membrane	**GERPN2134**
Amersham ECL® Prime Western Blotting Detection Reagent	For 1000 cm^2 membrane	**GERPN2232**
	For 3000 cm^2 membrane	**GERPN2236**
Amersham ECL Select® Western Blotting Detection Reagent	For 1000 cm^2 membrane	**GERPN2235**
Chromogenic Substrates		
3,3′,5,5′-Tetramethylbenzidine (TMB), Enhanced HRP Membrane Substrate		**T9455**
3,3′,5,5′-Tetramethylbenzidine (TMB), Insoluble	100 mL	**613548-100ML**

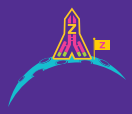
Blot stripping reagents

Description	Quantity	Cat. No.
ReBlot™ Plus Mild Antibody Stripping Solution	10 x 50 mL	**2502**
ReBlot™ Plus Strong Antibody Stripping Solution	10 x 50 mL	**2504**
Blot Restore Membrane Rejuvination Kit	1 kit	**2520-M**

Equipment and accessories

Description	Detail	Cat. No.
Power Supplies		
mA700 Essential Power Supply	mA700 Power Supply with cord for the US, Canada, and locations using NEMA 5-15P (YP12) plugs	**MA700-US**
	mA700 Power Supply with cord for France, Germany, and locations using CEE 7/7 (YP22) plugs	**MA700-EU**
	mA700 Power Supply with cord for the UK, Ireland and locations using type G (YP61) plugs	**MA700-UK**
	mA700 Power Supply with cord for Japan and locations using YP18 plugs	**MA700-NI**
	mA700 Power Supply with cord for China and locations using Type I (YP03) plugs	**MA700-ZH**
mA400 Basic Power Supply	mA400 Power Supply with cord for the US, Canada, and locations using NEMA 5-15P (YP12) plugs	**MA400-US**
	mA400 Power Supply with cord for France, Germany, and locations using CEE 7/7 (YP22) plugs	**MA400-EU**
	mA400 Power Supply with cord for the UK, Ireland and locations using type G (YP61) plugs	**MA400-UK**
	mA400 Power Supply with cord for Japan and locations using YP18 plugs	**MA400-NI**
	mA400 Power Supply with cord for China and locations using Type I (YP03) plugs	**MA400-ZH**
Accessories		
Blot Roller	1/pk	**SNAP2RL**
Filter Forceps	3/pk	**XX6200006P**
Sigma-Aldrich® Dual Run and Blot System	Gel tank and blotting module for 10 cm x 8 cm and 10 cm x 10 cm cassettes.	**Z741768-1EA**





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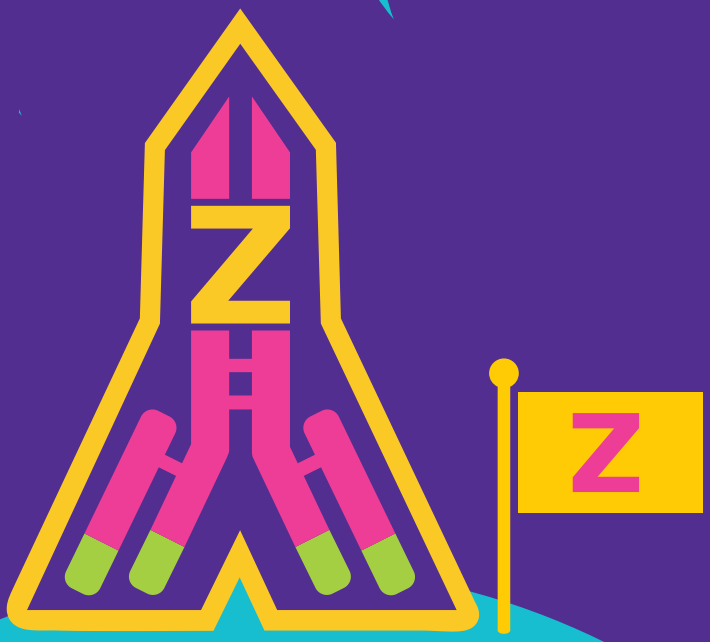

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Lit. No. MS_BR4306EN
2019-23943
10/2019