# Rethink Western Blotting

Millipore SigMa

What are your Western blot results telling you?

MilliporeSigma is the U.S. and Canada Life Science business of Merck KGaA, Darmstadt, Germany. Millipore<sub>®</sub>

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<sub>®</sub>

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<sub>®</sub>

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® and Sigma-Aldrich® products, our catalog includes thousands of products for Western blotting, including Immobilon® PVDF and NC transfer membranes, Cytiva Amersham Protran® nitrocellulose membranes, Cytiva 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

#### **TABLE OF CONTENTS**

- 4 Protein Extraction and Sample Preparation
- **7** Protein Quantitation
- **8** Electrophoresis
- **16** Protein Transfer
- **21** Immunodetection
- **32** Detection
- **37** Accessories
- **39** Troubleshooting
- **42** Ordering Information

Protein Extraction & Sample Preparation	Protein Quantitation	Electrophoresis	Protein Transfer	Blocking & Antibody Incubation	Detection
Gentle protein extraction kits	Protein assay kits	mPAGE® Bis-Tris precast gels	Immobilon® PVDF and NC high protein binding membranes	30-minute blot processing with the SNAP i.d.® 2.0 system	Immobilon® Western HRP substrates
		mPAGE® TurboMix Bis-Tris Gel Solutions			
		mPAGE® Gel Caster and Mini Gel Tank			
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		Reagents for hand-casting polyacrylamide gels	Blotting papers	~75,000 primary antibodies for Western blotting:	Amersham ECL® substrates
centrifugal filters		Buffers	Blotting buffers	SigmaAldrich.com/ antibodies	Chromogenic
			mPAGE® Wet Transfer System		substrates
		Protein gel stains	Protein blot stains	Secondary antibody conjugates	Substrates for AP detection
		Auto2D® 2-D Electrophoresis Device			

# 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 you can construct the perfect extraction protocol for your specific cells or tissue and protein of interest.

## **Product Spotlight**

# skip the Thaw

The quality of your protein samples is critical for generating reliable results that meet your research demands. The ReadyShield® Inhibitor Cocktails' non-freezing formula means it's ready when you are. Optimized and suitable for various cell types and tissue extracts, including mammalian, plant, and microbial, our ReadyShield® Protease Inhibitor Cocktail contains inhibitors with broad specificity for serine, cysteine, acid proteases, and aminopeptidases.

With our ReadyShield® Phosphatase Inhibitor Cocktail or Combined Cocktail also available, you will be able to find the best fit for your protein sample needs.

ReadyShield® Protease Inhibitor Cocktail Features and Benefits:

- Does not freeze in storage for quick, convenient use
- Formulation tailored for mammalian cell and tissue extracts
- Tested for use with various sample types, including human placenta, bovine liver, and A431, CHO, and U937 cell extracts
- Provided in screw-top vials
- Can be stored in the cooler for approximately 1 month or room temperature for a week

To learn more, please visit ReadyShield® Phosphatase and Protease Inhibitor Cocktail FAQ

SigmaAldrich.com/readyshieldfaq





### **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<sup>™</sup> 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 <sup>™</sup> purification
	GST•Tag <sup>™</sup> 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
Customize your Beads	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.

# **Product Spotlight**

#### mPAGE® Gel Caster and Mini Gel Tank

Discover our new mPAGE® complete solution for casting mini gels, protein electrophoresis, and blotting.

The mPAGE® Gel Caster and Mini Gel System offer a leak-free solution for hand-casting mini protein gels and high-resolution protein separation on precast or hand-cast gels.

Compatible with mPAGE® TurboMix Bis-Tris Gel Casting Solutions.

#### mPAGE® Gel Caster



Gels cast are 10 cm x 8 cm with a gel thickness of 0.75 mm, 1.0 mm, or 1.5 mm, along with 10 or 15-well options for sample loading. The mPAGE® Gel Caster has been designed to simplify hand casting gels and to eliminate leakage during the casting process.

For maximum flexibility, the gel caster is available as a standalone device or comes as a kit with glass plates (0.75 mm, 1.0 mm, or 1.5 mm Mini Spacer Plates and Short Plates) and combs (10 wells and 15 wells).

#### **Ordering Information**

Description	Cat. No.
mPAGE® Caster, 2 pk Device for hand casting mini gels, does not include plates or combs	GCR2
mPAGE® Casting Kit, 0.75 mm For hand casting mini gels with 0.75 mm thickness, includes plates and combs	MGCK-75M
mPAGE® Gel Casting Kit, 1.0 mm For hand casting mini gels with 1.0 mm thickness, includes plates and combs	MGCK-10M
mPAGE® Gel Casting Kit, 1.5 mm For hand casting mini gels with 1.5 mm thickness, includes plates and combs	MGCK-15M

#### mPAGE® Mini Gel Tank



The mPAGE® Mini Gel Tank is available in 2 formats for running up to 2 or 4 mini gels and is designed to prevent incorrect assembly and leakage.

It is compatible with 10 cm x 8 cm gels including gels cast on the mPAGE® Gel Caster and mPAGE® Precast Gels.

Description	Cat. No.
mPAGE® Mini Gel Tank, 2-gel	MGT-2
mPAGE® Mini Gel Tank, 4-gel	MGT-4

#### mPAGE® TurboMix Bis-Tris Acrylamide Gel Casting Solutions

Faster gel casting. Outstanding resolution. Reduced run times.

#### mPAGE® TurboMix Gel Casting Benefits

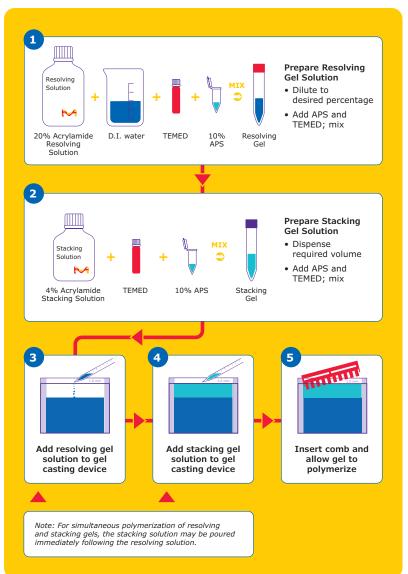
- Save time: premixed and readyto-pour acrylamide buffer solutions reduce hands-on prep time
- Rapid gel pouring protocol: Quick Cast protocol allows simultaneous polymerization of resolving and stacking gels
- Improved resolution: neutral pH running conditions result in reduced protein modification and sharper bands, compared to standard Tris-glycine gels
- Flexibility: easily dilute to any required percentage acrylamide gel between 8% and 15%, to separate proteins 6 kDa-400 kDa in size
- Longer cast gel shelf life:
   3-4 weeks, with proper storage
   neutral pH improves stability compared to tris-glycine gels



12% mPAGE® TurboMix Bis-Tris hand-cast gel loaded with E. coli lysate titrations (lanes 3–6), mPAGE® unstained protein standard (lanes 2 and 7), and mPAGE® color protein standard (lane 1). Gel was run with MOPS buffer for 35 min at 200 V and stained with ReadyBlue™ Protein Gel Stain.

#### mPAGE® TurboMix Quick Cast Workflow

The mPAGE® TurboMix Quick Cast method offers a rapid, 5-step process for casting polyacrylamide gels.



Description	Quantity	Cat. No.				
mPAGE® TurboMix Solutions						
mPAGE® TurboMix Bis-Tris Gel Casting Kit	~10 mini gels	TMKIT-10				
mPAGE® TurboMix Bis-Tris Gel Casting Kit	~60 mini gels	TMKIT-60				
mPAGE® TurboMix Resolving Solution	216 mL	TMRES-216ML				
mPAGE® TurboMix Stacking Solution	120 mL	TMSTK-120ML				
Reagents						
Ammonium persulfate (APS) for electrophoresis, ≥98%	25 g	A3678-25G				
N,N,N',N'-Tetramethylethylenediamine (TEMED) ReagentPlus®, 99%	5 mL	T22500-5ML				

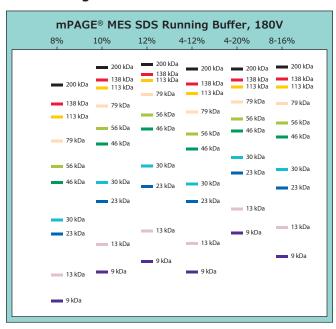
#### mPAGE® Bis-Tris Precast Gels for Protein Electrophoresis

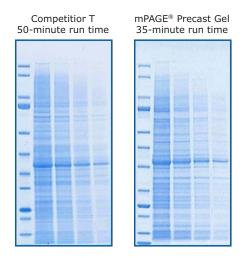
Without compromising the high resolution you need, mPAGE® gels require shorter run times and are compatible with large sample volumes, making mPAGE® precast gels a valuable tool for your protein research at a budget-friendly price.

#### Benefits of mPAGE® Precast Gels:

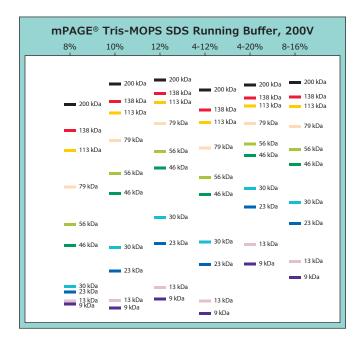
- Publication-ready resolution at a fraction of the cost
- · Compatible with popular electrophoresis tanks
- Up to 80 μL sample per well (10-well gels)
- · Efficient wet and semi-dry Western blot transfer
- Up to 15-minute shorter run time
- Neutral pH prevents protein modification

#### mPAGE® migration tables





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.



Use the migration charts above for MES (left) and MOPS running buffer (right) to identify the best mPAGE® gel and buffer for your electrophoresis application.

#### mPAGE® precast gels are compatible with:

- mPAGE® Mini Gel Tank
- 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.

mPAGE® Bis-Tris Precast Gels (10 gels per box)					
10-well (80 μL/well)	12-well (60 µL/well)	15-well (40 µL/well)			
MP41G10	MP41G12	MP41G15			
MP42G10	MP42G12	MP42G15			
MP81G10	MP81G12	MP81G15			
MP8W10	MP8W12	MP8W15			
MP10W10	MP10W12	MP10W15			
MP12W10	MP12W12	MP12W15			
	MP41G10 MP42G10 MP81G10 MP8W10 MP10W10	MP41G10 MP41G12 MP42G10 MP42G12 MP81G10 MP81G12 MP8W10 MP8W12 MP10W10 MP10W12			

#### mPAGE® Color Protein Standard

mPAGE® Color Protein Standard includes ten recombinant prestained proteins from 10 kDa to 203 kDa, covalently coupled with different chromophores. It is designed for observing protein separation during SDS-PAGE, verifying membrane transfer efficiency, and approximating the molecular weight of proteins.

- 10 sharp, recombinant bands over a broad MW range
- 2 orange reference bands at 23 kDa and 203 kDa
- 1 green reference band at 46 kDa
- Room-temperature stable for up to 2 weeks

#### mPAGE® Unstained Protein Standard

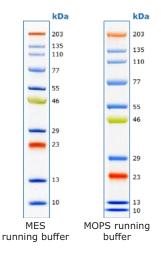
mPAGE® Unstained Protein Standard includes twelve recombinant unstained proteins ranging from 10 kDa to 200 kDa. The 85 kDa, 25 kDa, and 10 kDa bands have higher intensity to serve as reference points. mPAGE® Unstained Protein Standard is suitable for molecular weight determination after SDS-PAGE or Western blotting and serves as a standard to calibrate the mobility of prestained markers.

- 12 sharp, recombinant bands over a broad MW range
- Room-temperature stable for up to 2 weeks

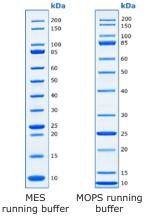
#### mPAGE® Western Protein Standard

mPAGE® Western Protein Standard consists of seven recombinant proteins at 20 kDa, 30 kDa, 40 kDa, 50 kDa, 60 kDa, 80 kDa and 120 kDa with an IgG binding site that binds to most antibodies. This enables visualization of both the protein marker and sample proteins on the same Western blot without additional reagents.

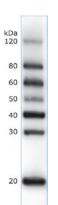
- 7 sharp, recombinant bands, 20-120 kDa
- Easily visualized on immunoblots without additional reagents



mPAGE® Color Protein Standard separated on mPAGE® 4-12% Bis-Tris gels.



mPAGE® Unstained Protein Standard separated on mPAGE® 4-12% Bis-Tris gels. Stained with ReadyBlue™ Protein Gel Stain.



mPAGE® Western Protein Standard separated on a 4-20% SDS-PAGE gel and transferred to a nitrocellulose membrane.

Description	Quantity	Cat. No.
mPAGE® Color Protein Standard	500 μL	MPSTD4
mPAGE® Unstained Protein Standard	500 μL	MPSTD3
mPAGE® Western Protein Standard	250 μL	MPSTD2

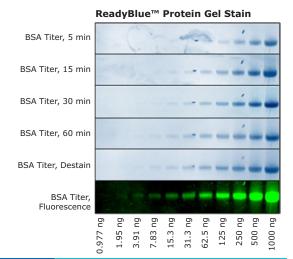
#### **Protein gel stains**

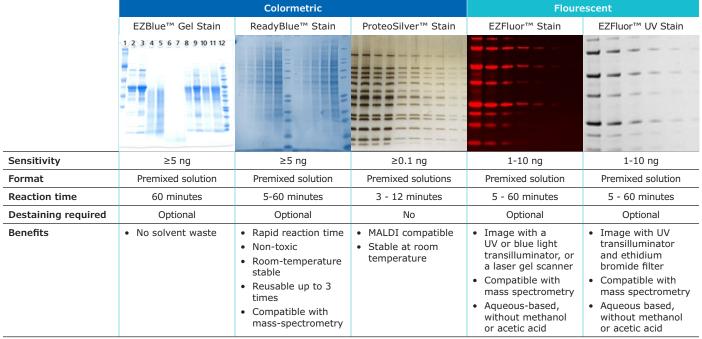
After separation by electrophoresis, protein bands are commonly visualized with gel stains. Utilizing either a dye-binding or color-producing chemical reaction, protein gel stains react selectively with proteins to yield a stained gel. Protein gel stains are typically selected based on the initial sample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

#### ReadyBlue™ Protein Gel Stain

ReadyBlue™ Protein Gel Stain simplifies your staining protocol for high sensitivity results in as fast as 5 minutes. No prior fixing step is required, and an optional microwave step can reduce time for staining even more.

- Low background and sharper bands for highly accurate quantitation of proteins
- Flexible colorimetric or IR fluorescence (700 or 800 nm) imaging
- Reusable up to 3 times
- No organic solvents or phosphoric acid
- · Compatible with mass spectrometry
- Room temperature storage—it's ready when you are





Description	Format	Cat. No.
EZBlue™ Gel Staining Reagent	500 mL	G1041-500ML
ReadyBlue™ Protein Gel Stai	1 L	RSB-1L
ProteoSilver™ Stain	1 Kit	PROTSIL1-1KT
EZFluor™ 1-step Fluorescent Protein Gel Stain	1 L	SCT145-1L
EZFluor™ UV 1-step Fluorescent Protein Gel Stain	1 L	SCT147-1L

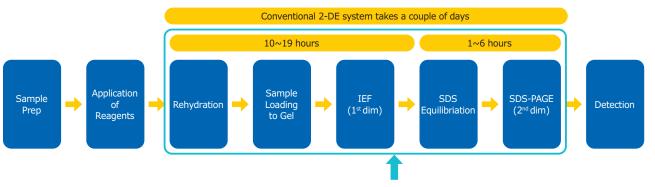
#### **2D Electrophoresis**

#### **Auto2D® 2-D Electrophoresis Device**

Our new Auto2D® 2-D Electrophoresis Device fully automates two-dimensional gel electrophoresis, simplifying protein analysis and providing more consistent, reproducible results that are user-independent. The efficient engineering of the Auto2D® system significantly reduces the amount of time spent during sample loading, isoelectric focusing, equilibration, and SDS-PAGE from 4-24 hours to only 1-2 hours. This makes the Auto2D® device unique compared to other semi-automated 2-DE systems on the market.



#### 2-DE Workflow and Where Auto2D® Device Automates



Auto2D<sup>®</sup> system automates this process in **~2 hours** 

#### Advantages of the Auto2D® 2-D Electrophoresis Device:

- Easy Operation: Eliminates the tedious handling steps of conventional 2-D electrophoresis
- High Reproducibility: Automation removes operator and day to day variability
- High Speed Separation: Achieves fast results in 60–120 min
- Good Sample Resolution: Gives deeper understanding of phosphorylation shifts in a shorter time

#### **Example applications**

The Auto2D® 2-D Electrophoresis Device has been proven in applications including:

- Differential protein expression analysis in cancer research and elucidation of disease mechanisms
- Separation of purified proteins for crystallization or post-translational modification analysis
- 2D Western blotting in research areas such as allergy research or cell signaling pathway analysis
- Contaminating host cell protein (HCP) analysis in therapeutic protein and antibody bioprocessing

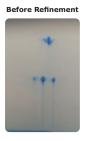
#### Differential protein expression analysis





#### **Protein separation**







Differential protein expression in cells and plasma samples. Left: Differential protein expression analysis in cultured cells for cancer biomarker research. Green represents more abundant proteins in normal cells. Red represents cancer-specific protein spots. Yellow represents the overlap in protein expression. Right: Differential protein expression in plasma samples.

Protein separation and analysis for downstream applications. Left: Assessing microheterogeneities in therapeutic antibody drug products. Red triangle indicates spots for target antibody; lower spots are antibody missing regions. Right: Protein analysis before and after purification. 2-DE can be used to check whether the sample is homogeneous for crystallization and X-ray structural analysis.

To learn more about the Auto2D® Automated 2-D Gel Electrophoresis Device, please visit **SigmaAldrich.com/Auto2D** 

Product Name	Quantity	Cat. No.
Auto2D® Base Unit		
Auto2D® 2-D Electrophoresis Device	1 unit	BM-100
Auto2D® Special Consumables		
Electrode Chip	1 chip	BM-1E
Solution Chip	10 chips	BM-1S
Auto2D® Plus Special Consumables		
Electrode Chip Plus	1 chip	BM-1EP
Solution Chip Plus	10 chips	BM-1SP
Auto2D®/Auto2D® Plus Common Consumables		
IEF Chip pH 3-10	10 chips	BM-113010
IEF Chip pH 3-10NL	10 chips	BM-113010NL
IEF Chip pH 4-7	10 chips	BM-114070
IEF Chip pH 4-5.5	10 chips	BM-114055
IEF Chip pH 5-6.5	10 chips	BM-115065
IEF Chip pH 6-10	10 chips	BM-116010
IEF Chip pH 7-10	10 chips	BM-117010
PAGE Chip 6.5%	10 chips	BM-12065
PAGE Chip 7.5%	10 chips	BM-12075
PAGE Chip 10.0%	10 chips	BM-12100
PAGE Chip 12.5%	10 chips	BM-12125
Tris-Glycine Reagent Kit	1 kit	BM-1RYSJ1
Tris-Tricine Reagent Kit	1 kit	BM-1RYTJ1

# 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.



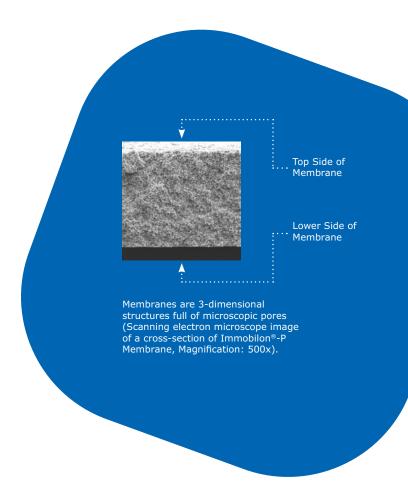
# How do Immobilon® membranes work?

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

Immobilon®-NC Transfer Membrane is our solution for customers who prefer nitrocellulose membranes. It is a mixed cellulose ester (MCE) membrane optimized for blotting proteins with molecular weights >20 kDa. MCE transfer membranes have improved mechanical strength over pure NC membranes.

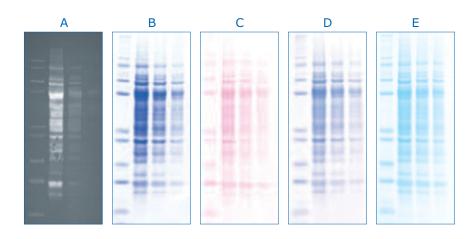


#### **Immobilon® Family of Transfer Membranes**

Membrane Name		Immobilon®-E	Immobilon®-P	Immobilon®-FL	Immobilon®-PSQ	Immobilon®-NC
Chemistry		PVDF	PVDF	PVDF	PVDF	MCE
Pore Size		0.45 μm	0.45 μm	0.45 μm	0.2 μm	0.45 μm
Detection methods		Chemiluminescence, Colorimetric	Chemiluminescence, Colorimetric	Chemiluminescence, Colorimetric, Fluorescence (including near-infrared)	Chemiluminescence, Colorimetric	Chemiluminescence, Colorimetric
t d	Goat IgG	225-293 μg/cm²	294 μg/cm²	300 μg/cm²	448 μg/cm²	237 μg/cm²
Binding capacity	BSA	N/A	215 μg/cm²	205 μg/cm²	340 μg/cm²	N/A
Bin	Insulin	N/A	160 μg/cm²	155 μg/cm²	262 μg/cm²	N/A
Applic	ations	Western blots	Western blots, dot blots			Western, Southern and
			igh protein retention of Immobilon® PVDF membranes make them the quential detection of multiple targets via antibody stripping and reprobing.			Northern blots, dot blots
Selection Criteria		Target protein express     Wide range of molecul	ed over a broad dynamic ar weights	range	<ul><li>Target protein</li><li>&lt;20 kDa</li><li>Very low abundance</li></ul>	High-abundance target protein     Wide range of
					target protein  • Low affinity antibody	molecular weights

# 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 is 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  $\mu g$ , 6.1  $\mu g$ , 3.1  $\mu g$  of the lysate per lane. For a detailed protocol, please refer to our Protein Blotting Handbook.

#### **Ordering Information**

Description	Format	Cat. No.
Immobilon®-E PVDF Transfer Membrane, 0.45 $\mu m$ (no alcohol pre-wet step required)	8.5 cm x 10 m roll	IEVH85R
Immobilon®-P PVDF Transfer Membrane, 0.45 μm	8.5 cm x 10 m roll	IPVH85R
Immobilon®-FL PVDF Transfer Membrane, 0.45 μm	8.5 cm x 10 m roll	IPFL85R
Immobilon®-P <sup>SQ</sup> PVDF Transfer Membrane, 0.2 μm	8.5 cm x 10 m roll	ISEQ85R
Immobilon®-NC Transfer Membrane, 0.45 μm	8.5 cm x 10 m roll	HATF85R
Immobilon® NOW Dispenser for 8.5 cm x 10 cm rolls		IMDISP

Ordering Information for additional formats can be found in the appendix on page 48.

#### 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, Immobilon®-PSQ, and Immobilon®-NC 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

#### Immobilon®-PSQ 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®-PSQ higher protein binding capacity, improving retention of small proteins.

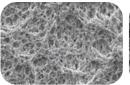
#### **Key Benefits**

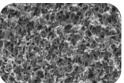
- · Higher binding capacity and retention resulting in stronger signals
- Prevents blow-through of low molecular weight proteins (<20 kDa)
- Compatible with chemiluminescent and chromogenic detection techniques

#### **Ideal for:**

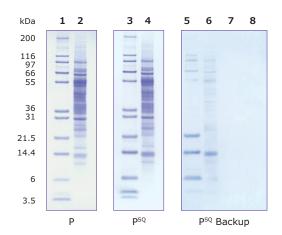
- 1. Westerns involving lysates or small proteins (<20 kDa), such as histones
- 2. Difficult Westerns due to:
  - · Low-abundance target proteins
  - Low-affinity antibodies

Substrate (WBLUC0500).





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



Immobilon®-PSQ 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®-PSQ membranes. A sheet of Immobilon®-PSQ 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®-PSQ membrane).

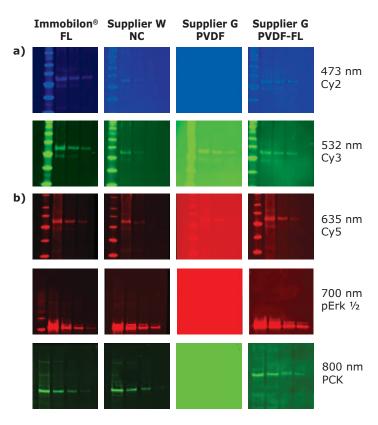
#### Immobilon®-FL transfer membrane

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

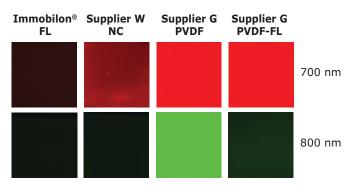
This 0.45 µm-pore membrane is the first transfer membrane specifically optimized for fluorescence-based detection of Western blots. Its extremely low background autofluorescence improves sensitivity of all fluorescence detection protocols.

#### **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 lystate (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  $\mu$ g/lane) of human brain lystate (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- $\beta$  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.

#### mPAGE® Mini Wet Transfer System

The mPAGE® Mini Wet Transfer System accommodates two gel holder cassettes for electrophoretic transfer of proteins from mini gels to PVDF or nitrocellulose blotting membrane using mPAGE® Transfer Buffer or a transfer buffer compatible with the gel chemistry chosen.



Description	Cat. No.
mPAGE® Mini Wet Transfer System	MWTS

# imunodetection



# **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.

# Traditional Western blotting relies on diffusion SNAP i.d.® 2.0 system actively pulls reagents through the membrane Antigen ... embedded in membrane Antibody trapped in membrane

#### 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.<sup>®</sup> 2.0 system in the Western blotting workflow Immunodetection in as little as 30 minutes!

	Blocking	1° Antibody Addition & Incubation	Washing	2° 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**

20 10 5 2.5 1.2

# ----

#### Anti-Huntingtin Protein (Cat. No. MAB2166)

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

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.

20 10 5 2.5 1.2

# 0----

#### 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.

12 6 3 1.5 0.7



#### Anti-erbB2 (intracellular domain) (Cat. No. 04-291)

1:200 dilution of this antibody detected erbB2 in A431 lysate (12–0.7  $\mu$ g).

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

30 20 10 5 2.5 1.25 0.6



#### 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.

Description	Format / Quantity	Cat. No.
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 $\times$ 8.4 cm and 4.5 $\times$ 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 Frame (single pack) 1/pk	SNAP2FRMN01
	Midi Blot Holding Frame (single pack) 1/pk	SNAP2FRMD01
	MultiBlot Frame (single pack) 1/pk	SNAP2FRMB01

#### **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

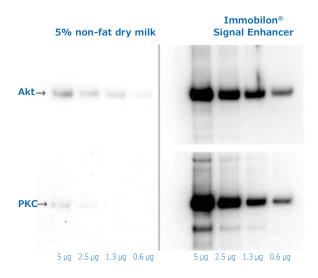
**Save primary antibody:** Use Immobilon® Signal Enhancer to reduce the amount of valuable primary antibody required

Blot A: 5% non-fat dry milk, anti-Akt antibody diluted 1:5,000



Blot B: Immobilon®

**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.

Description	Quantity	Cat. No.
Immobilon® Signal Enhancer for Immunodetection	500 mL	WBSH0500
	100 mL (trial size)	WBSH0500-100ML

# 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

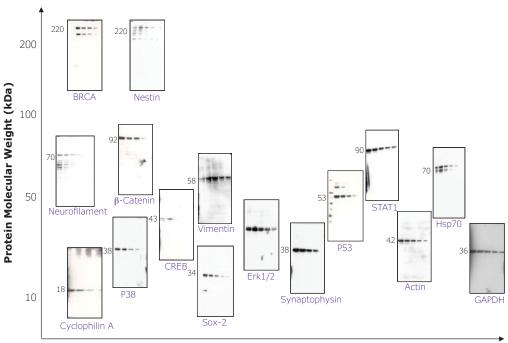
#### Immobilon® Block reagent offers:

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

#### 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.

#### Immobilon® Block reagents excel with diverse antibodies and lysates



**Relative Protein Abundance** 

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

Detection (antiphosphotyrosine)

(Highlight)

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  $\mu$ g/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.

1 2 3 4 5

2 3

4

Description	Quantity	Cat. No.
Immobilon® Block-CH Reagent, Chemiluminescence Detection	500 mL	WBAVDCH01
	100 mL (trial size)	WBAVDCH01-100ML

## **Technique Spotlight**

#### Immobilon® Block-PO noise cancelling reagent

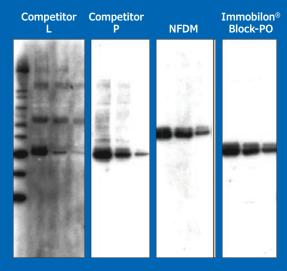
Blocking of non-specific protein binding sites on a blot is essential to decreasing the background and obtaining meaningful results. Although milk is the most commonly used blocker, the presence of phosphorylated mammalian proteins in milk can result in a very high background. For that reason, non-protein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

## How does Immobilon® Block-PO reagent improve results?

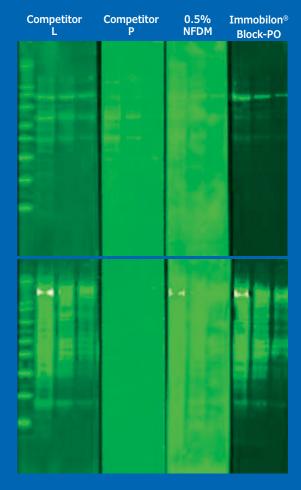
This chemical-based blocker contains phosphatase inhibitors to preserve the phosphorylation state of the blotted proteins.

#### **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<sup>®</sup> 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  $\mu$ g/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.

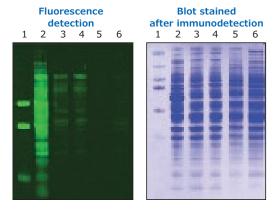
Fluorescence detection: Dilution series of EGF stimulated A431 lysate (20–2.5 µg/lane,Cat. No. 12-110) were resolved by SDS-PAGE and transferred onto Immobilon®-FL membranes. The blots were blocked, probed with either anti-phosphoserine antibody, clone 4A4 (1:400, Cat. No. 05-1000) (upper panel) or antiphosphotyrosine 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.

Description	Quantity	Cat. No.
Immobilon® Block-PO Reagent, Phosphoprotein Detection	500 mL	WBAVDP001
	100 mL (trial size)	WBAVDP001-100ML

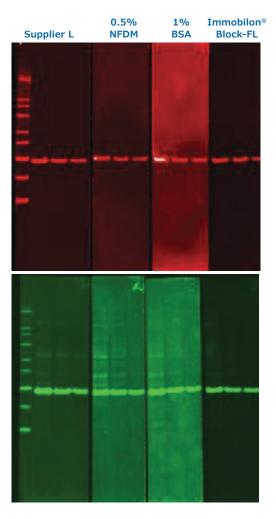
# Immobilon® Block-FL noise cancelling reagent

#### **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.

Description	Quantity	Cat. No.
Immobilon® Block-FL Reagent, Fluorescence Detection	500 mL	WBAVDFL01
	100 mL (trial size)	WBAVDFL01-100ML

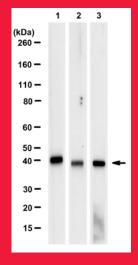
## **Technique Spotlight**

#### **ZooMAb® Antibodies**

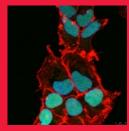
ZooMAb® antibodies represent an entirely new generation of recombinant monoclonal antibodies. They are specifically engineered to combine state-of-the-art consistency and applications performance with the most user-friendly formulation, handling, and storage features available today. With a long history 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.

# Sigma-Aldrich®

Lab & Production Materials

#### **Loading control antibodies for Western blotting**

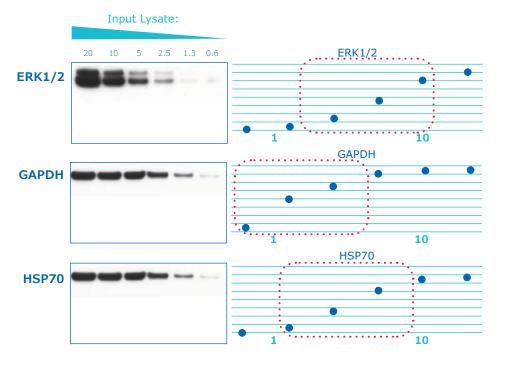
Proteins exhibiting abundant, constitutive levels in Western blot samples can be used to control for technical variability. These so-called loading controls are essential for confirming that differences in observed signal in Western blot experiments are caused due to changes in protein expression, and 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 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, SAB4200714; x-ERK1/2, 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



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

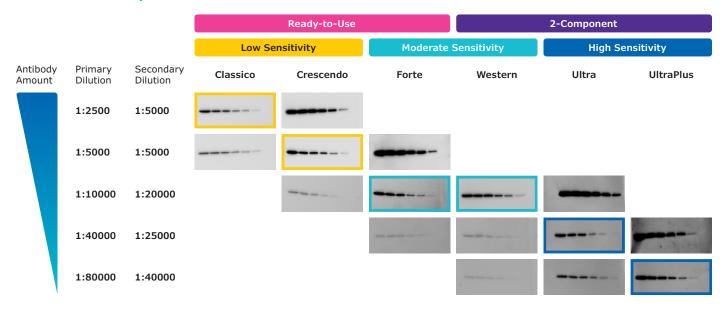
Immobilon® Classico, Crescendo and Forte substrates are a family of premixed reagents for peroxidase-based detection that offer significant advantages over other ECL reagents:

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

Immobilon® UltraPlus Western, Immobilon® ECL Ultra and Western 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 Immobilon® Chemiluminescent HRP Substrates



Two-fold dilution series of A431 cell lysate [12-110] starting at 4 µg protein was run on 10% mPAGE® gels [MP10W15] in MOPS buffer [MPM0PS] at 200V for 25 minutes. Protein was transferred to Immobilon®-P membrane [IPVH00010] for 1 hour in mPAGE® transfer buffer [MPTRB] with 10% ethanol. Immunodetection of HH3 protein (15 kDa) was performed with rabbit anti-HH3 [H0164] and goat anti-rabbit [AP132P] antibodies at listed dilutions. Blots were incubated with indicated ECL reagents for 5 minutes and images were taken with a 30s exposure on Bio-Rad® ChemiDoc system.

#### **Immobilon® Western HRP Substrates**

	Classico	Crescendo	Forte	Western	Ultra	UltraPlus	
Storage	Fridge (4 °C)	Fridge (4 °C)	Benchtop (25 °C)	Fridge (4 °C)	Fridge (4 °C)	Benchtop (25 °C)	
Format	Ready-to-Use	Ready-to-Use	Ready-to-Use	2-Component	2-Component	2-Component	
Membrane Compatibility	Nitrocellulose, PVDF	Nitrocellulose, PVDF	Nitrocellulose, PVDF	Nitrocellulose, PVDF	Nitrocellulose, PVDF	Nitrocellulose, PVDF	
Typical Signal Duration*	1 hour	1 hour	1 hour	1 hour	3 hours	3 hours	
Selection Criteria	teria and antibody or antibody abundance abundance	Low levels     of protein or	Low levels of protein <b>and</b>				
	<ul> <li>Fewer samples</li> </ul>	<ul> <li>Fewer samples</li> </ul>	of protein of protein and antibody and antibody	limited antibody	limited antibody		
			and antibody	and antibody	<ul> <li>Many samples</li> </ul>	<ul> <li>Many samples</li> </ul>	
Reagents	<ul> <li>Pierce™ ECL</li> </ul>	<ul> <li>Pierce<sup>™</sup> ECL Plus</li> </ul>	<ul> <li>SuperSignal™</li> </ul>	<ul> <li>SuperSignal™ West Pico PLUS</li> </ul>	1 3 1 3	<ul> <li>SuperSignal™</li> </ul>	<ul> <li>SuperSignal™</li> </ul>
with Similar	<ul> <li>Amersham</li> </ul>	<ul> <li>Amersham</li> </ul>	West Pico PLUS		West Femto	West Atto	
Sensitivity	ECL™ Reagent	ECL™ Reagent	<ul> <li>Amersham ECL™ Prime</li> </ul>	<ul> <li>Amersham ECL™ Prime</li> </ul>	<ul> <li>Amersham ECL™ Select</li> </ul>		
			• Clarity™ Reagent	• Clarity™ Reagent	<ul> <li>Clarity™         Max Reagent     </li> </ul>		

<sup>\*</sup>Using digital imager

# Immobilon® UltraPlus Western Blot HRP Substrate

Exceptional Sensitivity for Your Precious Proteins

## Use Immobilon® UltraPlus to visualize previously undetectable bands

The Immobilon® UltraPlus Western HRP Substrate is a two-part highly stable chemiluminescent substrate for detection of immobilized proteins conjugated with horseradish peroxidase (HRP). Immobilon® UltraPlus substrate delivers exceptional sensitivity and long signal life. This formulation permits the use of a more dilute primary antibody solution for immunoblot detection and is our highest sensitivity detection substrate at the low femtogram range.



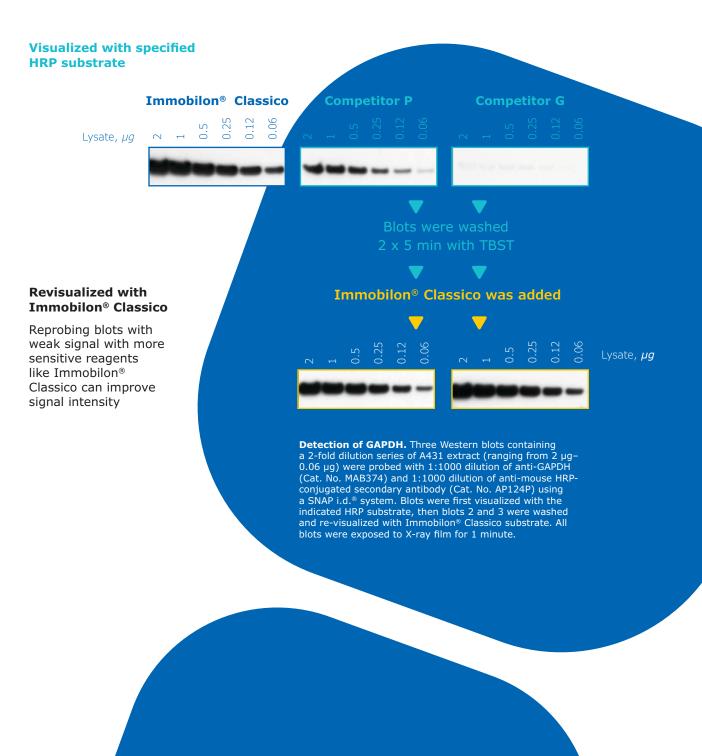
#### Decrease Antibody Use with High Sensitivity Immobilon® UltraPlus Substrate



Detection of a two-fold dilution series of recombinant human IKB alpha protein resolved by SDS-PAGE and transferred to Immobilon®-P membrane. The blots were blocked with 0.5% NFDM and incubated in diluted rabbit anti-IKB alpha (1:1,000 or 1:5,000) followed by diluted goat anti-rabbit IgG HRP. The blots were incubated with indicated chemiluminescent.

## 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.



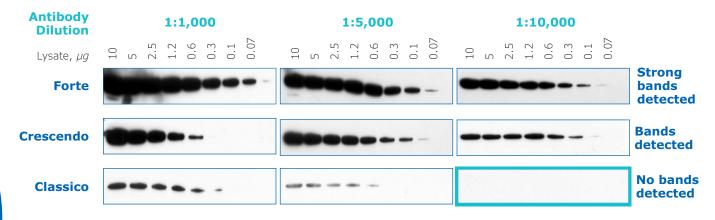
#### Optimizing Western blots with Immobilon® Western HRP substrates

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.

Description	Quantity	Cat. No.
Immobilon® UltraPlus HRP Substrate	2 x 50 mL	WBULP-100ML
	2 x 10 mL	WBULP-20ML
	2 x 5 mL (trial size)	WBULP-10ML
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

#### **ReBlot™ Plus Western blot recycling kit**

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

#### What is ReBlot™ Plus?

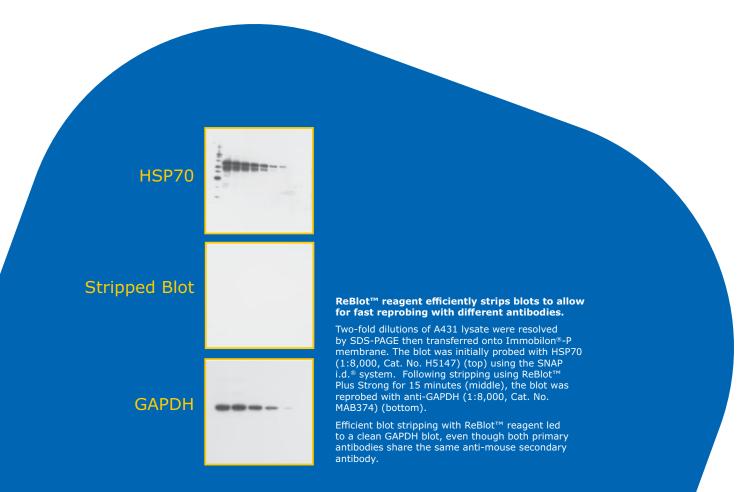
 $ReBlot^{\scriptscriptstyle\mathsf{TM}}\ Plus\ reagents\ efficiently\ strip\ probed\ blots\ of\ bound\ antibodies.\ ReBlot^{\scriptscriptstyle\mathsf{TM}}\ Plus\ reagents\ are\ available\ in\ mild\ and\ strong\ formulations.$ 

- 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)

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



# 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







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

 $<sup>\</sup>dagger$ 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 $\mu m$ or 0.45 $\mu m$ Millex $^{\otimes}$ syringe filter.
	Aggregates in HRP-conjugated secondary antibody	Filter secondary antibody solution through a 0.2 $\mu m$ or 0.45 $\mu m$ Millex $^{\otimes}$ 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 <sup>SQ</sup> 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 (continu	ued)	
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- $Q^{\otimes}$ 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	Small proteins are masked by large	Consider casein or a low molecular weight polyvinylpyrrolidone (PVP).
proteins	blocking molecules such as BSA	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) 100 mL TBS 10X 900 mL Milli-Q® water 1 mL Tween®-20 detergent (Product P9416)

### 4X SDS-PAGE sample loading buffer

[Tris-HCl1: 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 $\mu$ L 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-O® 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 Millì-Q $^{\circ}$  water and adjust pH to 6.8 Add Milli-Q $^{\circ}$  water to final volume 500 mL Filter and degas

## SDS-PAGE 10X gel running buffer [Tris]: 248 mM

Dilute to 1X for running SDS-PAGE gels

[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 No need to pH, filter, or degas

### 10X PBS

[NaCl]: 1.37 M [KCI]: 27 mM [Na<sub>2</sub>HPO<sub>4</sub>]: 100 mM [KH<sub>2</sub>PO<sub>4</sub>]: 18 mM For 1 L: 80 g NaCl (Product 71376) 2 g KCl (Product P9541) 17.8 g Na<sub>2</sub>HPO<sub>4</sub>•2H<sub>2</sub>O (Product 71643 - FW: 177.99 g/mol) 2.45 g KH<sub>2</sub>PO<sub>4</sub> (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) 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

#### mPAGE® MOPS SDS Running Buffer [Tris1: 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) 10.46 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-O® water and mix well. Add Milli-Q® water to a final volume of 1 L. Do not adjust

### 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 $^{\circ}$  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 3.0 g Tris base (Product 93362 - FW: 121.1 g/mol) 4.08 g N,N-Bis(2-hydroxyethyl)glycine (Bicine, Dissolve in 450 mL of Milli-Q® water and mix well. Add Milli-Q® water to final volume 500 mL. Do not adjust pH.

# ordering Information

### **Protein Extraction & Sample Preparation**

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
aemmli Lysis-buffer, non smelling	38733
Mammalian Protein Extraction Buffer, volume 500 mL, Cytiva	GE28-9412-79
luclear 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, Cytiva	GE80-6483-37
'eastBuster® Protein Extraction Reagent (for yeast cell lysis)	71186

## **Protein Extraction & Sample Preparation (continued)**

Description	Cat. No.
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
ReadyShield® Protease Inhibitor Cocktail for use with fungal and yeast extracts, Non-freezing solution	P0001-5ML
ReadyShield® Protease Inhibitor Cocktail for use with mammalian cell and tissue extracts, Non-freezing solution	PIC0002-5ML
ReadyShield® Protease and Phosphatase Inhibitor Cocktail for use with mammalian cell and tissue extracts, Non-freezing solution	PPC2020-5ML
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
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**

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, Cytiva	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® Gel Casting System and Accessories**

Description	Qty	Cat. No.
mPAGE® Gel Casting System		
mPAGE® Caster, 2 pk, does not include plates or combs		GCR2
mPAGE® Casting Device Kit, 0.75 mm for hand casting mini gels with 0.75 mm thickness, includes plates and combs		MGCK-75M
mPAGE® Casting Device Kit, 1 mm for hand casting mini gels with 1.0 mm thickness, includes plates and combs		MGCK-10M
mPAGE® Casting Device Kit, 1.5 mm for hand casting mini gels with 1.5 mm thickness, includes plates and combs		MGCK-15M
mPAGE® Gel Caster Accessories & Replacement Parts		
mPAGE® Combs, 0.75 mm, 10 wells	5	C75M10W
mPAGE® Combs, 0.75 mm, 15 wells	5	C75M15W
mPAGE® Combs 1.0 mm, 10 wells	5	C1M10W
mPAGE® Combs, 1.0 mm, 15 wells	5	C1M15W
mPAGE® Combs. 1.5 mm, 10 wells	5	C15M10W
mPAGE® Combs, 1.5 mm, 15 wells	5	C15M15W
mPAGE® Mini Spacer Plates, 0.75 mm	5	MSPA75
mPAGE® Mini Spacer Plates, 1.0 mm	5	MSPA10
mPAGE® Mini Spacer Plates, 1.5 mm	5	MSPA15
mPAGE® Mini Short Plates	10	MSHRT
mPAGE® Gel Caster Base	1	GCBASE
mPAGE® Gel Caster Frame	1	GCFRM
mPAGE® Gel Caster Sealing Gaskets	5	GCSEAL
mPAGE® Gel Releasers	5	GREL5
mPAGE® Casting Plate Rack	1	CPS1

## **Electrophoresis: Reagents & Buffers**

Description	Cat. No.
mPAGE® TurboMix Bis-Tris Acrylamide Gel Casting Solutions	
mPAGE® TurboMix Bis-Tris Gel Casting Kit ~10 mini gels	TMKIT-10
mPAGE® TurboMix Bis-Tris Gel Casting Kit ~60 mini gels	TMKIT-60
mPAGE® TurboMix Resolving Solution 216 mL	TMRES-216ML
mPAGE® TurboMix Stacking Solution 120 mL	TMSTK-120ML
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 <sub>2</sub> O	43816
Tris(2-carboxyethyl)phosphine hydrochloride	68957
Urea 8 M (after reconstitution)	U4883-6X25ML

## **Electrophoresis: mPAGE® Precast Gels**

mPAGE® Bis-Tris Precast Gels (10 gels per box)			
Acrylamide Percentage	10-well (80 µL/well)	12-well (60 µL/well)	15-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

## **Electrophoresis: mPAGE® Precast Gels (continued)**

Description	Qty	Cat. No.
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		MP10W12TR1
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® Precast Gel Accessories		
mPAGE® Adapter Plates	2	МРТА
mPAGE® Gel Cassette Opener	1	MPC0
mPAGE® Buffer Dam	1	MPBD

## **Electrophoresis: mPAGE® Buffers**

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

## **Protein Molecular Weight Markers (Ladders)**

Description	Cat. No.
Prestained Protein Standards	
mPAGE $^{\circ}$ Color Protein Standard, 500 $\mu$ L	MPSTD4
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, Cytiva	GERPN800E
Amersham ECL® Rainbow Marker - High range, Cytiva	GERPN756E
Amersham ECL® Rainbow Marker - Low range, Cytiva	GERPN755E
Unstained Protein Standards	
mPAGE® Unstained Protein Standard, 500 μL	MPSTD3
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

## **Protein Gel Stains**

Description	Cat. No.
Colorimetric	
ReadyBlue™ Protein Gel Stain, 1 L	RSB-1L
EZBlue™ Gel Staining Reagent	G1041
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

## 2D Electrophoresis: Auto2D® 2-D Electrophoresis

Description	Cat. No.
Auto2D® 2-D Electrophoresis	
Auto2D® 2-D Electrophoresis Device	BM-100
Electrode Chip	BM-1E
Solution Chip	BM-1S
Electrode Chip Plus	BM-1EP
Solution Chip Plus	BM-1SP
IEF Chip pH 3-10	BM-113010
IEF Chip pH 3-10NL	BM-113010NL
IEF Chip pH 4-7	BM-114070
IEF Chip pH 4-5.5	BM-114055
IEF Chip pH 5-6.5	BM-115065
IEF Chip pH 6-10	BM-116010
IEF Chip pH 7-10	BM-117010
PAGE Chip 6.5%	BM-12065
PAGE Chip 7.5%	BM-12075
PAGE Chip 10.0%	BM-12100
PAGE Chip 12.5%	BM-12125
Tris-Glycine Reagent Kit	BM-1RYSJ1
Tris-Tricine Reagent Kit	BM-1RYTJ1

## **Transfer Membrane & Blotting Papers**

Description	Format	Cat. No.
PVDF Transfer Membrane		
Immobilon®-E PVDF Transfer Membrane, 0.45 µm	8.5 cm x 10 m roll	IEVH85R
(no alcohol pre-wet step required)	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 <sup>SQ</sup> 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
Nitrocellulose Membranes		
Immobilon®-NC Transfer membrane, 0.45 μm	8.5 cm x 10 m roll	HATF85R
	33 cm x 3 m roll	HATF00010
	7 cm x 8.4 cm, 50/pk	HATF07850
	8.5 cm x 13.5 cm, 20/pk	HATF08130
Amersham Protran® Premium Western blotting membranes,	300 mm x 4 m roll	GE10600003
nitrocellulose, pore size 0.45 μm, Cytiva	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,	300 mm x 4 m roll	GE10600002
pore size 0.45 µm, Cytiva	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	300 mm x 4 m roll	GE10600001
pore size 0.2 μm, Cytiva	80 mm x 90 mm sheets, 25/pk	GE10600094
Amersham Protran® Premium Western blotting membranes,	300 mm x 4 m roll	GE10600004
nitrocellulose pore size 0.2 µm, Cytiva	80 mm x 90 mm sheets, 25/pk	GE10600097
Amersham Protran® Supported Western blotting membranes,	300 mm x 4 m roll	GE10600037
nitrocellulose pore size 0.2 µm, Cytiva	Blotting sandwich, 80 mm x 90 mm , 10/pk	GE1060013
Amersham Protran® Supported Western blotting membranes,	300 mm x 4 m roll	GE106000120
nitrocellulose pore size 0.45 µm, Cytiva	Blotting sandwich, 80 mm x 90 mm , 10/pk	GE10600119
Blotting Papers	Sistering Surfament, Go min A 50 min , 10/pk	5210000119
Immobilon® Blotting Filter Paper	7 cm x 8.4 cm sheets, 100/pk	IBFP0785C
Animobilion blocking rincer raper		IBFP0783C
Whatman® gel blotting paper Grade GR003 Cutiva	8.5 cm x 13.5 cm, 100/pk	
Whatman® gel blotting paper, Grade GB003, Cytiva	15 cm x 20 cm, 100/pk 20 cm x 20 cm, 100/pk	WHA10427812 WHA10427818

<sup>\*</sup>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

## **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 <sub>2</sub> O), ≥98.0% NaOH basis (titration)	C6070
Methanol, ≥99.6%	179957
mPAGE® Transfer Buffer Powder	MPTRB
Tris-Glycine Buffer, 10x Concentrate	T4904
Transfer Equipment	
mPAGE® Mini Wet Transfer System	MWTS

## **Immunodetection Devices**

Description	Format / Quantity	Cat. No.
SNAP i.d.® 2.0 Protein Detection System for	r 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 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, Cytiva	GEPA43009
ECL Plex™ G-A-M IgG, Cy®5, pack of 600 μg, Cytiva	GEPA45010
ECL Plex™ Gar IgG Cy®3, pack of 150 μg, Cytiva	GE28-9011-06
ECL Plex™ G-A-R IgG, Cy®5, pack of 150 μg, Cytiva	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		Т9039
Tween®20 detergent		P9416
pHast Pack™ Phosphate buffered saline, pH 7.4, powder	20 x 500 mL	PPB006-20PAK
pHast Pack™ Tris Buffered Saline pH 8.0, powder	20 x 500 mL	PPB001-20PAK

### **Detection Substrates**

Description	Quantity	Cat. No.
Immobilon Chemiluminescence Peroxidase Substrates		
Immobilon® UltraPlus HRP Substrate	2 x 50 mL	WBULP-100ML
	2 x 10 mL	WBULP-20ML
	2 x 5 mL (trial size)	WBULP-10ML
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

## **Detection Substrates (continued)**

Description	Quantity	Cat. No.
Immobilon® Chemiluminescence Peroxidase Substrates (continued)		
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, Cytiva	For 1000 cm² membrane	GERPN2109
	For 2000 cm <sup>2</sup> membrane	GERPN2209
	For 4000 cm <sup>2</sup> membrane	GERPN2106
	For 6000 cm <sup>2</sup> membrane	GERPN2134
Amersham ECL® Prime Western Blotting Detection Reagent, Cytiva	For 1000 cm <sup>2</sup> membrane	GERPN2232
	For 3000 cm <sup>2</sup> membrane	GERPN2236
Amersham ECL Select® Western Blotting Detection Reagent, Cytiva	For 1000 cm <sup>2</sup> 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

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