MILLIPORE™

Maximize Your Western Blotting Results

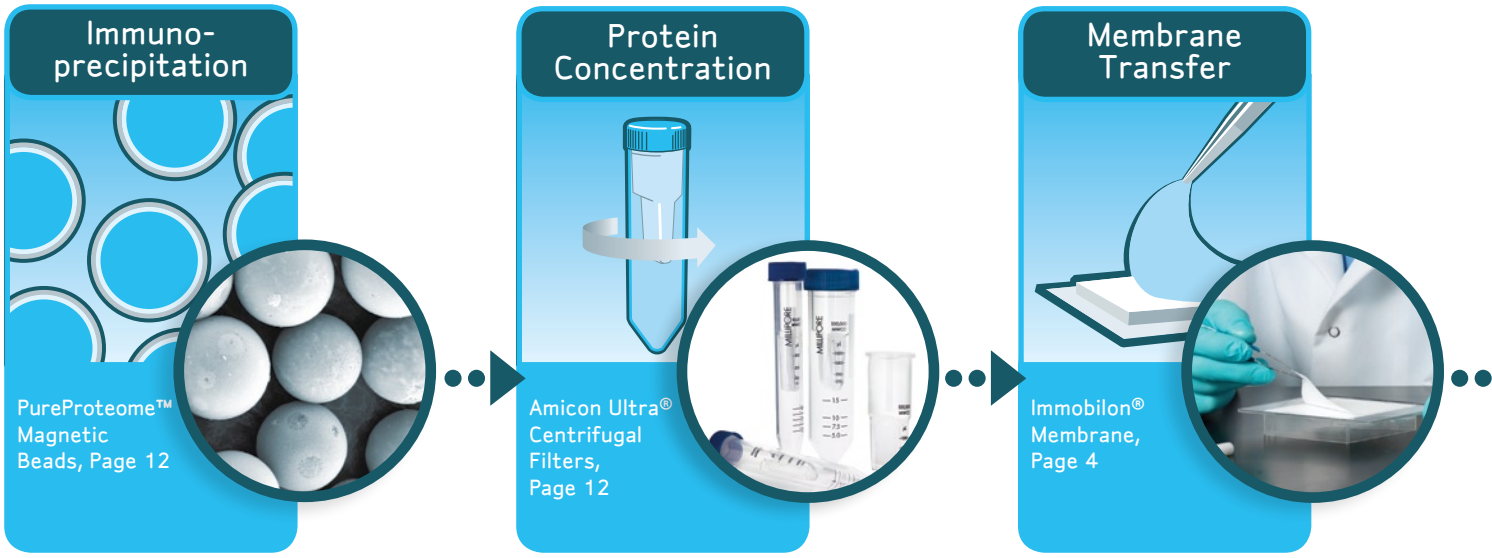
Superior products for every step of the Western workflow

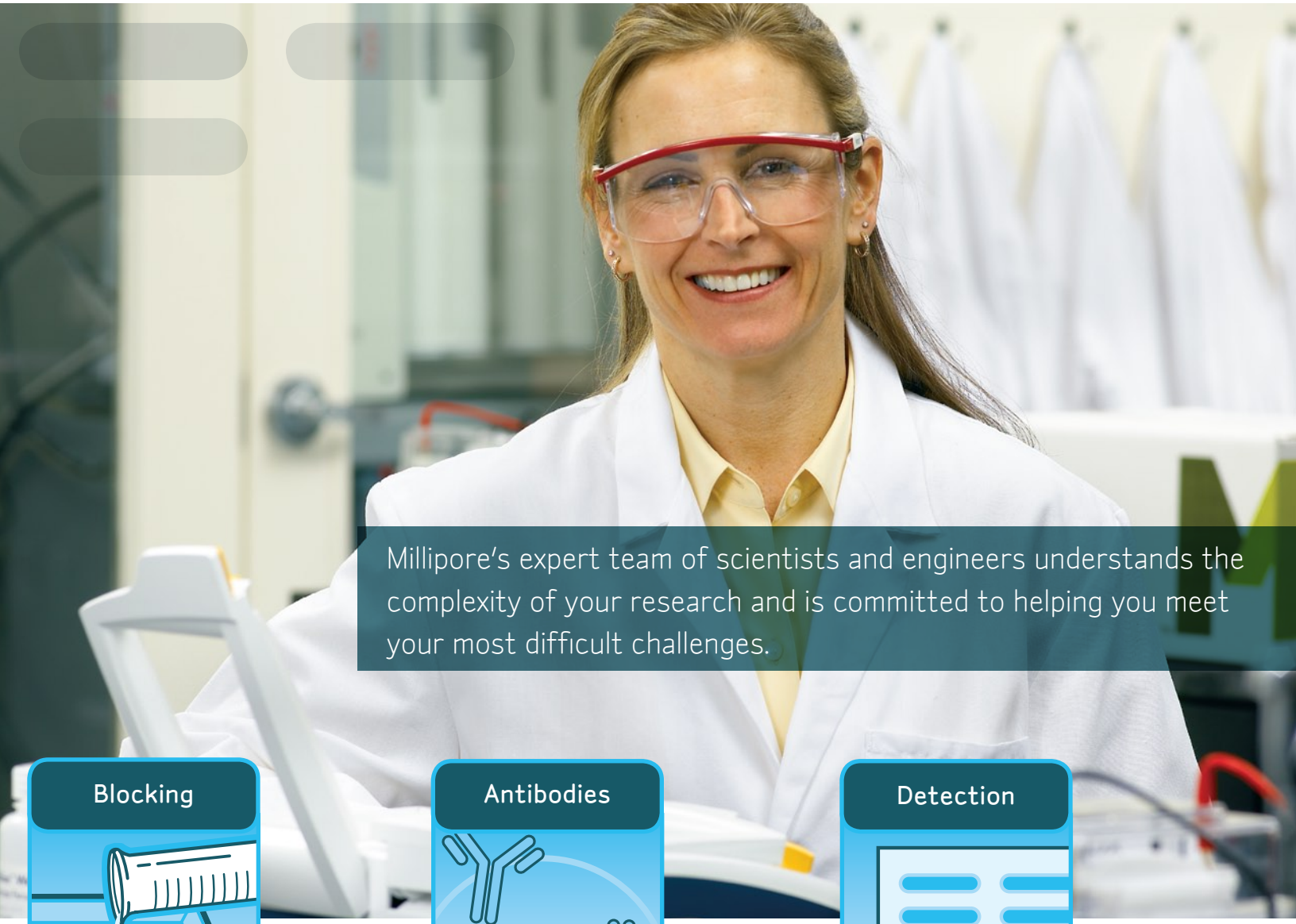
ADVANCING LIFE SCIENCE TOGETHER™
Research. Development. Production.

Fisher Scientific

Let Millipore's Western Blotting Expertise Help Keep Your Research On Track.

One way to improve the quality and consistency of results from your immunodetection protocols is to use components that have been optimized to complement each other during every critical step of the Western Blotting process. Our full line of innovative immunodetection products demonstrates our commitment to providing you with technologies and expertise that will help streamline your laboratory process while delivering the highest quality results.





Millipore's expert team of scientists and engineers understands the complexity of your research and is committed to helping you meet your most difficult challenges.

Blocking

SNAP i.d.™ Protein Detetion System, Page 6

Antibodies

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Millipore Antibodies, Page 9

Detection

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Immobilon®

Transfer Membranes

Millipore offers three varieties of PVDF transfer membranes, each optimized for different protein blotting applications. All have exceptional handling characteristics and are compatible with a variety of detection chemistries and reprobing techniques.

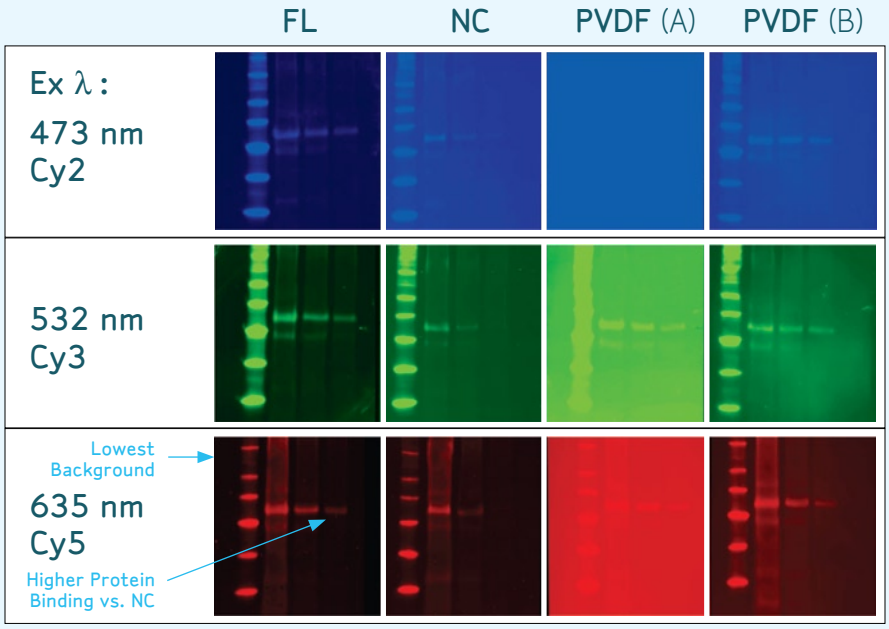
We also offer Blotting Sandwiches with pre-cut sheets of membrane and blotting filter paper interleaved for added convenience.

PVDF membranes offer:

- Excellent protein retention
- Physical strength
- Broad chemical compatibility

Immobilon-FL Membrane Shows Lowest Membrane Auto-fluorescence

Membrane Fluorescence



FL = Immobilon-FL membrane
NC = nitrocellulose
PVDF (A)
PVDF (B)

Effect of membrane auto-fluorescence on actual fluorescent Western.
SDS-PAGE separated brain tissue lysates were electroblotted on the indicated membranes and probed for GSK3b (Glycogen Synthetase Kinase 3 beta) using anti-GSK3b (rabbit polyclonal, Millipore AB8687) followed by secondary conjugated to Cy2, Cy3, or Cy5. Indicated membranes were scanned using a laser scanner (Fuji, FLA5100) equipped with Cy filter sets. Excitation wavelengths are indicated at left. Cut off filters at 510 nm, 573 nm, and 700 nm were used for Cy2, Cy3, and Cy5, respectively.

Comparison of Immobilon membrane properties

	Immobilon-P transfer membrane	Immobilon-PSQ transfer membrane	Immobilon-FL transfer membrane
Description	Optimized to bind proteins transferred from a variety of gel matrices	Uniform pore structure results in superior binding of proteins with MW <20 kDa	Optimized for fluorescence immunodetection applications
Composition	PVDF	PVDF	PVDF
Pore size	0.45 µm	0.2 µm	0.45 µm
Phobicity	Hydrophobic	Hydrophobic	Hydrophobic
Applications	Western blotting Binding assays Amino acid analysis N-terminal protein sequencing Dot/slot blotting Glycoprotein visualization Lipopolysaccharide analysis Mass spectrometry	Low molecular weight western blotting Amino acid analysis Mass spectrometry N-terminal protein sequencing	Western blotting Dot/slot blotting Fluorescence immunodetection
Detection methods	Chromogenic Chemiluminescent Radioactive	Chromogenic Chemiluminescent Radioactive	Fluorescent Chromogenic Chemifluorescent Chemiluminescent
Protein binding capacity	Insulin: 160 µg/cm^2 BSA: 215 µg/cm^2 Goat IgG: 294 µg/cm^2	Insulin: 262 µg/cm^2 BSA: 340 µg/cm^2 Goat IgG: 448 µg/cm^2	Insulin: 155 µg/cm^2 BSA: 205 µg/cm^2 Goat IgG: 300 µg/cm^2

Ordering Information

Product Description	Size	Quantity	Catalogue No.
Immobilon-P Blotting Sandwiches: PVDF 0.45 µm	7 x 8.4 cm Sheet	20/pk	IPSN07852
	8.5 x 13.5 cm Sheet	20/pk	IPSN08132
Immobilon-P: PVDF 0.45 µm	*10 x 10 cm Sheet	10/pk	IPVH10100
	26.5 cm x 3.75 m	Roll	IPVH00010
Immobilon-PSQ: PVDF 0.22 µm	*10 x 10 cm Sheet	10/pk	ISEQ10100
	26.5 cm x 3.75 m	Roll	ISEQ00010
Immobilon-FL: PVDF 0.45 µm	*10 x 10 cm Sheet	10/pk	IPFL10100
	26.5 cm x 3.75 m	Roll	IPFL00010

*Many other sizes of cut sheets are available. Please go to Millipore.com (fishersci.com) for more information on additional membrane sizes.

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

SNAP i.d.™

Protein Detection System

The SNAP i.d. Protein Detection System revolutionizes immunodetection by providing high-quality blots in under **30 minutes!**

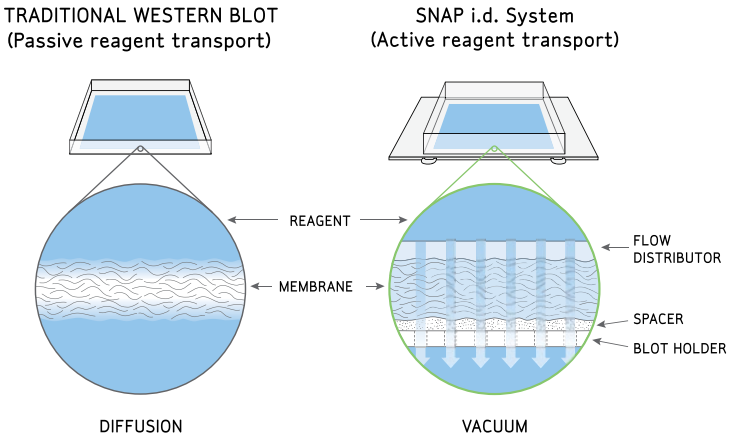
Unlike conventional western blotting, where diffusion is the primary means of reagent transport, the SNAP i.d. system applies a vacuum to actively drive reagents through the membrane.

This novel method allows you to optimize your blotting conditions in record time for maximum results. The SNAP i.d. system minimizes overblocking by using lower concentrations of blocking agents. In addition, more effective wash steps remove unwanted contaminants from the membrane.

With the SNAP i.d. system, you'll achieve incredibly low background, high signal-to-noise ratios, reproducibility from blot to blot, and sensitivities that are the same or often better than traditional immunodetection techniques.

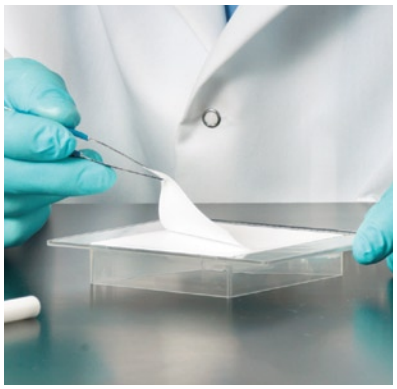
Visit **www.millipore.com/snapid** for more information.

Active reagent transport delivers superior blots in under 30 minutes.



Unlike traditional, time consuming, western blotting techniques that rely on diffusion to disperse reagents, the SNAP i.d. system uses a vacuum to quickly pull reagents completely through the membrane.

Sample Prep — **Electro-phoresis** — **Membrane Transfer**



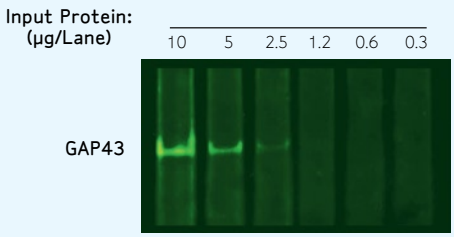
Advantages of the SNAP i.d. System

- **Dynamic –** vacuum actively drives reagents through blotting membrane.
- **Quality –** equal or better signal-to-noise ratios than standard western blotting.
- **Fast –** reduces immunodetection time from 4 hours to 30 minutes.
- **Simple –** incorporates blocking, washing, and antibody incubation steps.
- **Compatible –** works with standard gel sizes and protocols.
- **Efficient –** optimizes your protocol with a new antibody in 30 minutes.

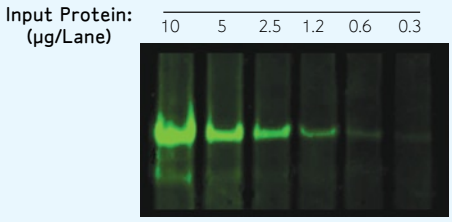
Immobilon-FL Membranes with the SNAP i.d. System

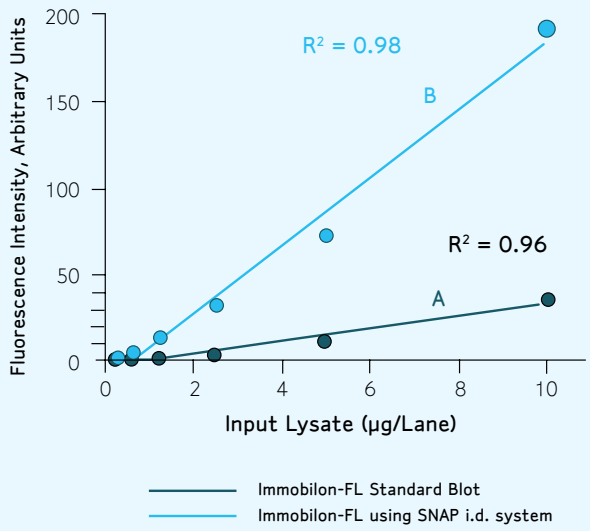
Combine the SNAP i.d. system with Immobilon-FL membranes to maximize fluorescence performance

A. Immobilon-FL Standard Blot



B. Immobilon-FL using SNAP i.d. system





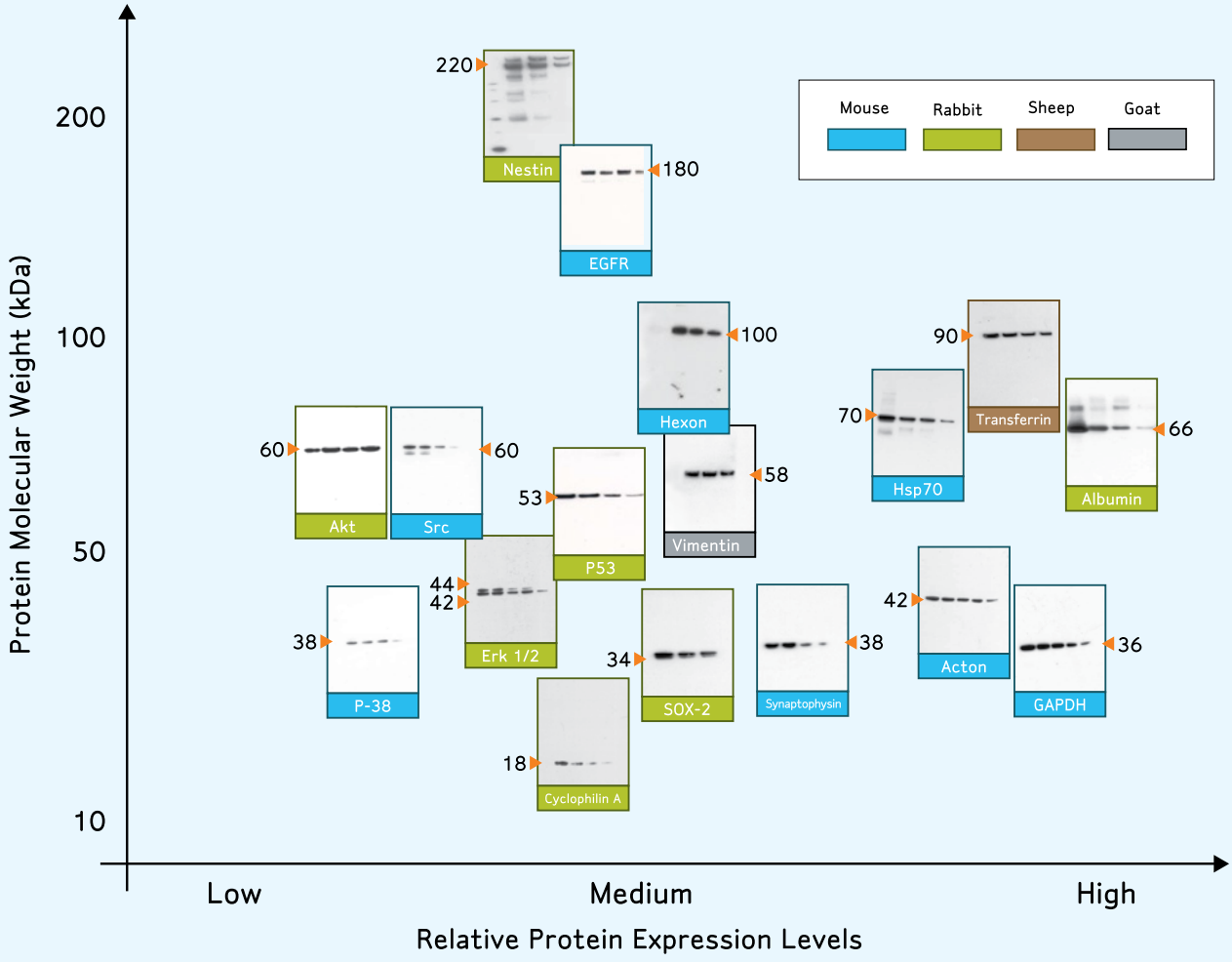
Cy3 fluorescent immunodetection of GAP43 in human brain lysates.
Blots of a 2-fold dilution series of human brain lysates were prepared as described under Materials and Methods. The blots were visualized simultaneously using a Fujifilm FLA-5100 imaging system. Representative images from multiple scanned blots are shown. Panel **A** shows the standard blot and panel **B** displays the SNAP i.d. blot. The graph shows the quantification of the immunoreactive bands from blots (A) and (B), respectively.

Blocking → **Antibody Addition** → **Detection**

SNAP i.d. System

22 minutes using the SNAP i.d. System vs. 4-20 hours using traditional methods.

Compatibility of the SNAP i.d. system with proteins of varying weights and expression levels.



Immunodetection after optimization of primary and secondary antibody:
A variety of proteins from different lysate sources (rat liver, cancer cell and stem cell) with a wide range of molecular weight (18 to 220 kDa) and relative abundance in the cell (depending on the sample type and amount loaded in the gel), were detected in the SNAP i.d. system.

Ordering Information

Product Description		Quantity	Catalogue No.
SNAP i.d. Protein Detection System			WBAVDBASE
SNAP i.d. Consumables and Accessories	Single Blot Holder	30/pk	WBAVDBH01
	Double Blot Holder	30/pk	WBAVDBH02
	Triple Blot Holder	20/pk	WBAVDBH03
	Antibody Collection Tray	20/pk	WBAVDABTR
	SNAP i.d. Blot Roller		WBAVDR0LL
	Chemical Duty Pump, 115 V/60 Hz		WP6111560
	Chemical Duty Pump, 220 V/50 Hz		WP6122050
	Vacuum filtering flask, 1 L		XX1004705
	Silicone No. 8 Perforated Stopper, 5/pk		XX1004708
	Millipore Forceps SS		XX6200006

We're Committed to Exceptional Antibodies

Now that Upstate® and Chemicon® are part of Millipore, we are continuing the tradition of developing innovative antibodies to meet all your research needs!

TRUSTED

You've trusted Millipore's lab water and sample preparation technologies for years. You'll find the same high level of quality in our antibodies

FOCUSED

Choose from our extensive portfolio of high-quality antibodies focused in specific areas of research. Whether you study Neuroscience, Cell Signaling, Nuclear Function, Cell Structure & Adhesion, or Apoptosis, you'll find what you need from our large selection of monoclonal and polyclonal antibodies.

VALIDATED

Have confidence in your research results. All Millipore antibodies are validated with appropriate techniques and documented protocols. We validate with Western Blot and more rigorous applications such as IP, IHC, ELISA, ChIP and others.

TO PLACE AN ORDER

In the U.S. and Canada, call toll-free **1-800-MILLIPORE (1-800-645-5476)**

Outside of North America, please visit **www.millipore.com/offices**

www.millipore.com/antibodies

Millipore offers the best possible service, quality and value.
This is reflected in our 100% Antibody Performance Promise.

If you are not 100% satisfied with your antibody performance:

- Contact one of our support scientists immediately.
- We'll work with you to resolve your concern or you'll receive a full product credit against future purchases.

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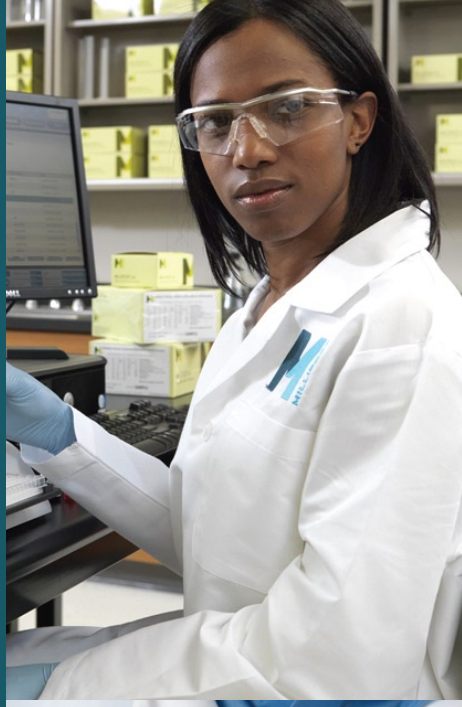
www.millipore.com/ISS



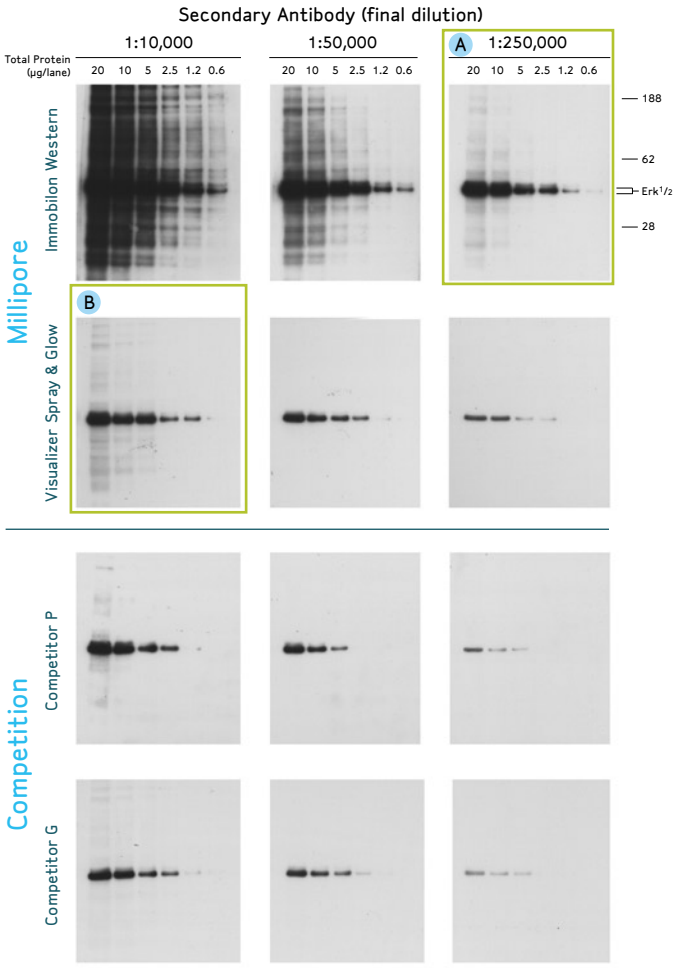
Immobilon®

Western Detection Reagents

The ultimate detection tool with exceptional performance and value. Immobilon Western Substrates are optimized for high sensitivity and low background over a broad dynamic range, at a considerably lower cost than other chemiluminescent reagents.

- **High signal intensity** and low background allow detection to the mid-femtogram level without enhancers or special buffers
- **Reduce antibody consumption** at least two- to fivefold compared to less sensitive chemiluminescent substrates
- **Compatible with both PVDF and nitrocellulose membranes,** as well as all commonly used buffers and blocking reagents

Competitive Analysis of Chemiluminescent Reagents



Chemiluminescent detection of electroblotted MAP kinase Erk1/2 is summarized. Serially diluted cell lysate samples (EGF stimulated A431 cells, catalogue number 12-110, protein concentration per lane indicated at the top) were electrophoretically separated and electroblotted onto PVDF membranes (Immobilon-P, catalogue number IPVH00010). Blots were blocked with 3% non-fat milk, catalogue number 20-200, in TBS containing 0.1% Tween®-20 (TBS-T). MAP kinase Erk1/2 was identified using affinity purified rabbit anti-Erk1/2 antibody, catalogue number 06-182, 1:4,000 final dilution, and HRP conjugated anti-rabbit IgG, catalogue number AP132P, at indicated final dilutions. All washes were done in TBS-T and blots were immunodetected under identical conditions except for the indicated chemiluminescent reagents. Treated blots were developed simultaneously from the same X-ray cassette (1 minute exposure). Molecular weight markers (kDa) are indicated at right. Notice that Immobilon Western HRP reagent produces very strong chemiluminescent signal. 10 fold less secondary antibodies are recommended to obtain desired result compared to other chemiluminescent reagents. Representative result from multiple experiments is shown.

A. Excellent sensitivity with optimization and 25x less secondary antibody

B. Good sensitivity with no optimization

Ordering Information

Product Description	Volume, mL	Catalogue No.
Immobilon Reagents Chemiluminescent HRP Substrate	50	WBKLS0050
	100	WBKLS0100
	500	WBKLS0500
Immobilon Reagents Chemiluminescent AP Substrate	25	WBKDS0025
	100	WBKDS0100

Visualizer™

Spray & Glow™ ECL Detection System

The Spray & Glow Detection System is a simple, enhanced chemiluminescent (ECL) detection reagent that comes in a convenient spray bottle. To detect your target protein using standard detection methods, simply spray the Western membrane. Combine it with the Millipore's extensive line of primary antibodies for fast, quality results!

- Convenient spray bottle format
- No mixing of solutions required

Ordering Information

Product Description	Catalogue No.
Spray & Glow Detection System Kit capacity: 50 mini-gel-sized blots for 1000 cm^2 of membrane, 100 mL	17-373MI

Amicon® Ultra

Centrifugal Filters

You can shorten spin times and improve protein recovery with Amicon Ultra centrifugal filters. These filters combine low-binding Ultracel® membrane with an innovative vertical housing — which makes them ideal for concentrating biological samples containing antibodies, antigens or pre-purified proteins from column eluents.

Fast Sample Processing

- Sample volumes of **4 –15 mL** can be concentrated in as little as **15 minutes**
- **Recoveries Typically >90%**
- **High-recovery, low-binding** Ultracel (regenerated cellulose) ultrafiltration membrane provides maximum sample recovery

Ordering Information

Model	Max. Volume	NMWL	8/Pk.	24/Pk.	96/Pk.
Amicon Ultra-4	4 mL	3 K	UFC800308	UFC800324	UFC800396
		10 K	UFC801008	UFC801024	UFC801096
		30 K	UFC803008	UFC803024	UFC803096
		50 K	UFC805008	UFC805024	UFC805096
		100 K	UFC810008	UFC810024	UFC810096
Amicon Ultra-15	15 mL	3 K	UFC900308	UFC900324	UFC900396
		10 K	UFC901008	UFC901024	UFC901096
		30 K	UFC903008	UFC903024	UFC903096
		50 K	UFC905008	UFC905024	UFC905096
		100 K	UFC910008	UFC910024	UFC910096

PureProteome™

Protein A and Protein G Magnetic Beads

Now you don't have to trade off performance for economy. PureProteome Protein A and Protein G Magnetic beads provide high binding capacity and low non-specific binding at an affordable price. These magnetic beads will allow you to easily isolate low-abundant proteins from complex samples with the highest level of purity. This capability assures optimum results from downstream assays.

- **High binding capacity** with low background
- **Economical –** up to half the cost of magnetic beads from other sources
- **Faster** and easier than alternative purification methods

Ordering Information

Product Description	Volume, mL	Catalogue No.
PureProteome Protein A Magnetic Beads	10	LSKMAGA10
	2 x 1	LSKMAGA02
PureProteome Protein G Magnetic Beads	10	LSKMAGG10
	2 x 1	LSKMAGG02
Magna GrIP™ Rack, 8 sample holder		20-400

MILLIPORE

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