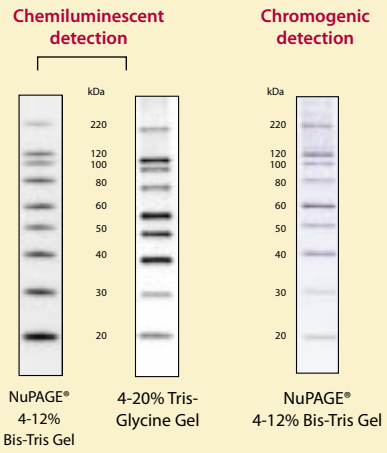
accelerate > easy molecular weight estimation directly on westerns

- Visualize marker bands directly on western blots
- Size proteins (from 20-220 kDa) easily and accurately
- Eliminate extra steps and reagents, saving time and effort

The magic behind the mark

The MagicMark™ XP Western Protein Standard employs innovative technology for easy and accurate molecular weight estimation directly on western blots. Each protein band contains an IgG binding site, allowing marker visualization using the same reagents and protocol for the target protein as your immunoassay. Using the MagicMark™ XP Western Protein Standard, you can establish calibration curves and easily size proteins ranging from 20-220 kDa (Figure 1).

Figure 1 — Sharp bands of MagicMark™ XP Western Protein Standard



5 microliters of the MagicMark™ XP Western Protein Standard was run on each indicated gel type. Chemiluminescent detection was performed using the WesternBreeze® Anti-rabbit Chemiluminescent Kit. Chromogenic detection was performed using the WesternBreeze® Anti-rabbit Chromogenic Kit.

Proven resolution for accurate results

The MagicMark™ XP Western Standard consists of a mixture of nine recombinant proteins ranging in size from 20 to 220 kDa. Bands are visualized with chemiluminescent, fluorescent, or colorimetric substrates, according to your western detection method. There are no extra procedures or additional reagents involved that can increase non-specific background on your western blots (Table 1). In addition, the non-dye modified proteins in the MagicMark™ XP Standard result in truer molecular weight estimation and sharper band resolution.

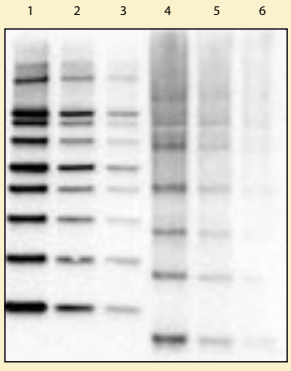
Table 1 — Comparison of various common protein molecular weight standards used on western blots

	MagicMark™ XP Standard	Biotinylated or S-protein tagged Standards	Pre-stained Protein Standards	Unstained Protein Standards
Extra reagents	None	Avidin-conjugate or S-protein conjugate	None	Coomassie®, or other stain to visualize the standard bands
Additional steps	None	Prepare standard in sample buffer; optimize avidin-conjugate or S-Protein conjugate dilution	Align, mark, and overlay the standard bands on the blot	Cut out the standard lane and stain with Coomassie® (or other) stain; align, mark, and overlay the standard bands on the blot
Outcome	Sharp bands; accurate results	Diffuse bands and non-specific background lead to inaccurate results	Mismarking of bands leads to inaccurate results	Misaligning of bands leads to inaccurate results

Outperform the rest

To demonstrate high resolution, MagicMark™ XP Standard and a western standard from another manufacturer were blotted and detected side-by-side. The results show that MagicMark™ XP bands are much sharper and cleaner (Figure 2, lanes 1-3) than the diffuse and smeary bands in the S-protein tagged standard (Figure 2, lanes 4-6), even with the additional S-protein conjugate required to detect the competitor's marker. In addition, since the MagicMark™ bands are detected using the same reagents used to detect the target proteins, you'll eliminate extra purchases, supplementary preparation steps and incubations, and awkward marking and overlay procedures thereby saving money, time, and effort.

Figure 2 — MagicMark™ XP Standard outperforms a western standard from another manufacturer



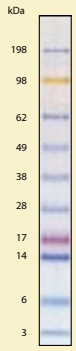
Protein standards below were electrophoresed on a NuPAGE® 4-12% Bis-Tris gel using MES SDS running buffer and transferred to a 0.45 µm nitrocellulose membrane. The blot was probed with an anti-V5 antibody (1:5,000-Invitrogen) and anti-mouse HRP (1:10,000) and S-Protein-HRP (1:5,000–**this additional reagent required to detect competitor's marker** according to manufacturer's recommendation). The blot was then detected with a luminol substrate.

lanes 1-3: MagicMark™ XP Standard 10, 5, 2 µl, respectively
lanes 4-6: Competitor's western marker 10, 5, 2 µl, respectively

Increase confidence by adding a colored standard

Invitrogen's SeeBlue® Plus2 Pre-stained Standard offers easy band identification and serves well for quick judgment of gel run and blotting efficiency. By using the SeeBlue® Plus2 Pre-stained and MagicMark™ XP Standards in the same gel run, you'll get the benefits of both. Simply load the appropriate amount of the SeeBlue® Plus2 and MagicMark™ XP Standards into two different lanes on the same gel or even into same lane, leaving more lanes available for your samples. With either approach, you'll see brightly stained SeeBlue® bands during the gel run (Figure 3) and after transferring to the solid membrane. Following the immunodetection procedure of your choice, you'll see sharp MagicMark™ XP bands develop directly on the blots (Figure 1) with your target protein.

Figure 3 — SeeBlue® Plus2 Pre-stained Standard



The SeeBlue® Plus2 Pre-stained Standard run on a NuPAGE® 4-12% Bis-Tris Gel w/ MES SDS Buffer.

Related blotting products

In addition to MagicMark™ XP Western Protein Standard, Invitrogen offers a blotting apparatus, membranes/filter papers, accessories, and detection kits to simplify blotting set-up and improve results. With the addition of Zymed® antibodies, Invitrogen is now able to provide you with everything you need for western blotting experiments.

Easy-to-use XCell II™ Blot Module

The XCell II™ Blot Module (Figure 4) fits easily into the XCell *SureLock*™ Mini-Cell for mini-gel blotting and offers fast transfer times, simple set-up, leak-proof runs, and even transfers.

Figure 4 — The XCell II™ Blot Module

Related blotting products (cont.)

Convenient pre-cut membrane/filter paper

Pre-cut, pre-assembled membrane/filter paper sandwiches (Figure 5) make setting up blotting procedures easy and convenient. Membranes perfectly fit mini-gels or E-PAGE™ gels and are available in different types (PVDF, nitrocellulose) and pore sizes to meet your needs.

Figure 5 — Pre-cut, pre-assembled membrane/filter paper sandwiches

Efficient blotting roller and incubation tray

The Blotting Roller (Figure 6) is designed for easy and efficient removal of bubbles when assembling gel/membrane sandwiches for blotting all types of gels such as mini-gels and E-PAGE™ gels. It consists of a Delrin roller attached to a stainless steel handle. The Incubation Tray/Lid (10 cm (l) x 14 cm (w) x 3 cm (d)) (Figure 6) enables convenient and even staining or immunodetection of E-PAGE™ blots. Mini incubation trays for blots of mini-gels are provided in the complete WesternBreeze® Immunodetection Kits.

Figure 6 — The Blotting roller and Incubation Tray/lid

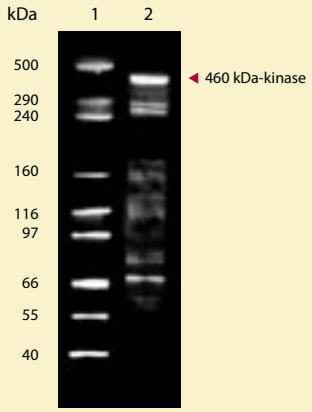
blotting roller

incubation tray

Sensitive SYPRO® Ruby Blot Stain for total-protein detection

The SYPRO® Ruby Protein Blot Stain offers ultra-sensitive detection of total proteins on blots down to ~2-8 ng protein/band and is fully compatible with subsequent immunodetection. The stain is simple to use and protein signals can be easily visualized with UV transilluminators (Figure 7) or laser scanners.

Figure 7 — Blot of HiMark™ Standard and protein sample stained with SYPRO™ Ruby Blot Stain



Standard and protein sample were separated on a NuPAGE® 3-8% Tris-Acetate Gel/SDS buffer and transferred to an Invitrolon™ PVDF membrane, then stained with SYPRO® Ruby Blot Stain according to protocol. AlphaInnotech™ 1/8-sec. exposure

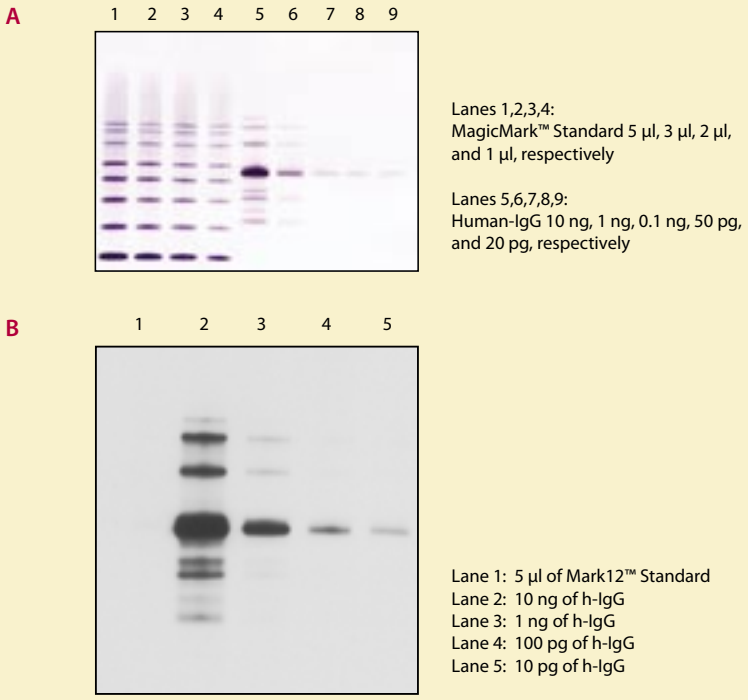
Lane 1: 5 µl of HiMark™ Standard
Lane 2: 4 µl of protein sample

Related blotting products (cont.)

Convenient and sensitive immunodetection kits

Invitrogen offers both alkaline phosphatase (AP)-based and horseradish peroxidase (HRP)-based western immunodetection kits for chemiluminescent, chromogenic, and fluorescent detection of proteins. These kits provide a complete set of reagents for sensitive detection of primary antibodies immobilized on nitrocellulose or PVDF membranes. The AP-based WesternBreeze® Kits offers picogram- to femtogram-level detection with your choice of either a chromogenic or chemiluminescent substrate (Figure 8). The HRP-based Western Blot Kits allow nanogram-level detection with either your choice of primary antibody or anti-phosphoserine antibody with chemiluminescent substrate. In addition, fluorescence-based immunodetection kits are also available for nanogram-level detection and multiplexing capability.

Figure 8 — Sensitive results with the WesternBreeze® Chromogenic and Chemiluminescent Kits (anti-rabbit)



Protein samples and standards were separated on a NuPAGE® Novex 4-12% Bis-Tris Gel (w/MES) and transferred onto a 0.45 µm nitrocellulose membrane. Immunodetection was performed according to the WesternBreeze® Chromogenic Kit (A) and Chemiluminescent Kit (B) protocols.

Broad selections of antibodies

A full line of epitope tag antibodies and research antibodies against proteins involved in cancer studies, cell cycle, neuroscience, signal transduction, and more are available from Invitrogen. For detailed information, visit www.invitrogen.com/antibodies.

Experience the magic today

For convenient and easy molecular weight estimation of proteins directly on western blots, use the MagicMark™ XP Western Protein Standard. Order today.

Product	Quantity	Cat. no.
Protein Standards		
MagicMark™ XP Western Protein Standard	250 µl	LC5602
SeeBlue® Plus2 Pre-stained Standard	500 µl	LC5925
Related Products		
XCell II™ Blot Module	1 unit	EI9051
XCell *SureLock*™ Mini-Cell and XCell II™ Blot Module	1 kit	EI0002
Nitrocellulose, 0.2 µm pore size		
8.3 cm x 7.3 cm (for mini-gels)	20 membrane/filter paper sandwiches	LC2000
8.5 cm x 13.5 cm (for E-PAGE™ or midi gels)	16 membrane/filter paper sandwiches	LC2009
Nitrocellulose, 0.45 µm pore size		
8.3 cm x 7.3 cm (for mini-gels)	20 membrane/filter paper sandwiches	LC2001
8.5 cm x 13.5 cm (for E-PAGE™ or midi gels)	16 membrane/filter paper sandwiches	LC2006
Invitrolon™ PVDF, 0.45 µm pore size		
8.3 cm x 7.3 cm	20 membrane/filter paper sandwiches	LC2005
8.5 cm x 13.5 cm	16 membrane/filter paper sandwiches	LC2007
PVDF, 0.2 µm pore size		
8.3 cm x 7.3 cm	20 membrane/filter paper sandwiches	LC2002
Blotting Roller, 8.6 cm wide	1 unit	LC2100
Incubation Tray, 10 x 14 cm	8 trays & lids/pk	LC2102
SYPRO® Ruby Protein Blot Stain	200 ml	S11791
WesternBreeze® Chemiluminescent Detection Kit (AP-based)		
Anti-Mouse	1 kit	WB7104
Anti-Rabbit	1 kit	WB7106
Anti-Goat	1 kit	WB7108
WesternBreeze® Chromogenic Detection Kit (AP-based)		
Anti-Mouse	1 kit	WB7103
Anti-Rabbit	1 kit	WB7105
Anti-Goat	1 kit	WB7107
Western Blot Kit (HRP-based) with anti-mouse and anti-rabbit secondary antibodies	1 kit	96-9045
Phosphoserine Western Blot Kit (HRP-based) with anti-phosphoserine antibody	1 kit	96-8145
DyeChrome™ Double Western Blot Detection Kit (Fluorescence-based) with total protein stain and anti-mouse AP antibody and anti-rabbit HRP antibody	1 kit	D-21887

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