Troubleshooting

Review the following information to troubleshoot your experiments with MagicMark™ XP Western Standard. For troubleshooting western blotting and detection, refer to the manufacturer's recommendations.

Observation	Cause	Solution
Weak or no signal	Detection reagents not functional	Verify that the detection reagents are working well. Optimize the antibody concentration to obtain best results.
	Low amount of standards loaded	Load a higher amount of standard on the gel.
	Poor or incomplete transfer	Optimize western transfer.
	Enzyme-conjugated antibody may not bind efficiently with MagicMark™ proteins	Use unconjugated primary antibody, followed by the addition of enzyme-conjugated secondary antibody. **Note:** The Anti-*myc*-AP/HRP and Anti-V5-AP/HRP Antibodies from Life Technologies do not bind to MagicMark™ proteins.
Smeary, non-distinct bands	Overloading	Decrease amount of standard loaded.
	Antibody is too concentrated	Follow the manufacturer's recommended dilution or determine the optimal antibody concentration by dot-blotting.

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MagicMark™ XP Western Protein Standard

Catalog numbers	Size
LC5602	250 µL
LC5603	50 µL

Store at –10°C to –30°C

Pub. Part no. LC5600.pps MAN0001518 **Rev. Date:** 29 November 2011

Description

The MagicMark™ XP Western Protein Standard allows you to directly visualize protein standard bands on a blot without the need to modify proteins or use special detection reagents. MagicMark™ XP Western Standard proteins are expressed in *E. coli* from a construct containing repetitive units of a fusion protein forming the size variation and an IgG binding site.

The important features of the standard are:

- MagicMark™ XP consists of nine recombinant proteins in the range of 20–220 kDa (page 3)
- Suitable for western blotting and molecular weight estimation
- Supplied in a ready-to-use format
- Visualized with alkaline phosphatase or peroxidase conjugated antibody using chromogenic, chemiluminescent, or fluorescent substrates
- Visualized also with SimplyBlue™ SafeStain or other Coomassie stains on SDS-PAGE gels

Specifications

Contents:	MagicMark™ XP Western Protein Standard
Storage Buffer:	125 mM Tris-HCl, pH 6.8; 10 mM DTT; 17.4% glycerol; 3% SDS; and 0.025% bromophenol blue
Storage:	Store at –10°C to –30°C. To avoid repeated freezing and thawing, aliquot in small volumes and store.

Product Use For research use only. Not for human or animal therapeutic or diagnostic use.

4

Troubleshooting

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MagicMark™ XP Western Protein Standard

Catalog numbers LC5602 LC5603 Size 250 µL 50 µL

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Storage: Store at –10°C to –30°C. To avoid repeated freezing and thawing, aliquot in small volumes and store.

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Directions for Use

The MagicMark™ XP Western Standard is supplied ready to use. There is no need to heat or reduce the standard prior to loading.

1. Load 1–10 µL of the standard on an appropriate SDS-PAGE mini-gel. We recommend testing different amounts of the standard to determine the optimal amount of standard to use under your experimental conditions.

   Note: The amount of standard to use depends on the binding affinity of MagicMark™ XP for your antibody species (see **Affinity of MagicMark™ XP Standard to Antibodies** on page 3) and the sensitivity of your detection system.
2. Load your samples and perform electrophoresis.

Blotting

1. Transfer proteins to a suitable membrane.
2. Perform the blocking step, primary antibody incubation step, and (if necessary) secondary antibody incubation step with the blot using your method of choice.
3. Visualize proteins using a colorimetric or chemiluminescent detection system using the manufacturer's recommendations. After detection, you should observe standard protein bands as shown on the next page.

Note: The MagicMark™ XP Western Standard proteins may not align with pre-stained or unstained markers. See Focus™ (2003), **25.3**, pg 35-39.

Staining

Stain the gel with SimplyBlue™ SafeStain or other Coomassie stains and then destain the gel.

Pre-mixing with Pre-stained Standard

To monitor the electrophoresis run, pre-mix standards as follows:

- 5 µL MagicMark™ XP Western Standard with 5 µL Novex® Sharp Pre-stained Standard (Cat. no. LC5800)

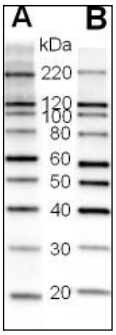
or

- 10 µL MagicMark™ XP Western Standard with 5 µL SeeBlue® Pre-stained Standard (Cat. no. LC5625)

Affinity of MagicMark™ XP Standard to Antibodies

Species	Affinity of MagicMark™
Human, Horse, Cow	++++
Pig, Rabbit	+++
Goat, Sheep, Hamster, Guinea Pig, Rat, Mouse	++
Chicken	+

Example



5 µL of MagicMark™ XP Western Protein Standard was loaded on a NuPAGE® Novex® 4–12% Bis-Tris Gel (A) or a Novex® 4–20% Tris-Glycine gel (B), blotted onto a nitrocellulose membrane, and detected using the WesternBreeze® Anti-Rabbit Chemiluminescent Kit.

Product Qualification

The Certificate of Analysis (CofA) provides detailed quality control information for each product. The CofA is available on our website at **www.lifetechnologies.com/support**, and is searchable by product lot number, which is printed on each box.

2

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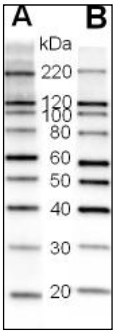
3

Affinity of MagicMark™ XP Standard to Antibodies

Species	Affinity of MagicMark™
Human, Horse, Cow	++++
Pig, Rabbit	+++
Goat, Sheep, Hamster, Guinea Pig, Rat, Mouse	++
Chicken	+

Example



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