

### Accessory Products

The following products are available separately and may be used with the HiMark™ Pre-stained HMW Protein Standard.

Product	Quantity	Cat. no.
NuPAGE® Novex 3–8% Tris-Acetate Gels	1 box	EA0375BOX
NuPAGE® Novex 7% Tris-Acetate Gels	1 box	EA0355BOX
NuPAGE® Tris-Acetate SDS Running Buffer (20X)	500 mL	LA0041
NuPAGE® Large Protein Blotting Kit	1 kit	LP0001
NuPAGE® Large Protein Staining Kit	1 kit	LP0002
NuPAGE® Large Protein Sensitive Staining Kit	1 kit	LP0003
HiMark™ Unstained HMW Protein Standard	250 µL	LC5688
NuPAGE® Transfer Buffer (20X)	1 L	NP0006-1

#### Limited Use Label License: Research Use Only

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## HiMark™ Pre-Stained High Molecular Weight Protein Standard

**Cat. no.** LC5699      **Size** 250 µL      **Store at** –30°C to –10°C  
**Pub. Part no.** LC5699.pps      **MAN0001521**      **Rev. Date:** 27 October 2011

### Description

The HiMark™ Pre-Stained High Molecular Weight (HMW) Protein Standard allows you to determine the apparent molecular weight of high molecular weight proteins on NuPAGE® Novex Tris-Acetate Gels with the Tris-Acetate SDS buffer system.

The important features of the standard are:

- Consists of 9 protein bands in the range of 30–460 kDa
- Designed for use with NuPAGE® Novex 3–8% and 7% Tris-Acetate Gels under denaturing conditions\*
- Supplied in a ready-to-use format
- Allows you to easily visualize protein molecular weight ranges during electrophoresis and evaluate western transfer efficiency

\***Note:** HiMark™ Pre-Stained Standard can also be used with NuPAGE® Novex 4–12% Bis-Tris Gels with NuPAGE® MOPS Running Buffer and Novex® 4% Tris-Glycine Gel. However, to obtain the best results with HMW proteins, use NuPAGE® Novex Tris-Acetate Gels.

### Specifications

**Contents:** 250 µL of HiMark™ Pre-Stained HMW Protein Standard  
**Storage Buffer:** Tris-HCl, Formamide, SDS, Phenol Red  
**Storage:** Store at –30°C to –10°C  
**Stability:** 4 months at –20°C

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

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## Directions

The HiMark™ Pre-Stained HMW Protein Standard is supplied in a ready-to-use format. There is no need to heat or add reducing agent.

1. Load the standard using the loading volumes listed below to obtain the best results. Use an appropriate percentage of the gel to resolve proteins.

Gel	Electrophoresis / Blotting
Mini-Gel (1.0-mm thick, 10-well)	10 µL
Mini-Gel (1.5-mm thick, 10-well)	12 µL

2. To obtain good resolution of all 9 bands of the protein standard, run the gel until the phenol red tracking dye reaches the bottom of the gel.

**Note:** The HiMark™ Pre-Stained HMW Protein Standard is designed for use with NuPAGE® Novex Tris-Acetate Gels, NuPAGE® Novex Bis-Tris Gels, or Tris-Glycine Gels.

After electrophoresis, you should observe the protein standard bands as shown on the next page.

### Blotting

To obtain higher transfer efficiency during the transfer of high molecular weight proteins, follow the recommendations below. For a detailed blotting protocol, refer to the NuPAGE® Large Protein Analysis System manual available from [www.lifetechnologies.com/support](http://www.lifetechnologies.com/support) or by contacting Technical Support.

#### Nitrocellulose Membranes

Perform transfer with 1X transfer buffer with **10%** methanol using the recommended transfer conditions based on the gel type. Use 1:1000 NuPAGE® Antioxidant for efficient transfer of reduced proteins.

#### PVDF Membranes

Perform transfer with 1X transfer buffer with **20%** methanol using the recommended transfer conditions based on the gel type. Use 1:1000 NuPAGE® Antioxidant for efficient transfer of reduced proteins.

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## Example

The apparent molecular weight of protein bands in the HiMark™ Pre-Stained HMW Protein Standard in several buffer systems is shown below. The protein bands have different mobilities in various SDS-PAGE buffer systems.

An example of 10 µL HiMark™ Pre-Stained HMW Protein Standard analyzed on a NuPAGE® Novex 3–8% Tris-Acetate Gel is shown below.



	Apparent Molecular Weight (kDa)			
	Band no.	NuPAGE® Tris-Acetate	NuPAGE® 4–12% Bis-Tris/MOPS	4% Tris-Glycine
1				
2				
3				
4	1	460	420	500
5	2	268	247	279
6	3	238	214	251
7	4	163	165	154
8	5	117	107	121
9	6	71	64	—
	7	55	51	—
	8	41	39	—
	9	31	28	—


### 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](http://www.lifetechnologies.com/support), and is searchable by product lot number, which is printed on each box.

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