new

NuPAGE® Large Protein Kits and HiMark™ Protein Standards

Reliable separation and analysis of large proteins in SDS-PAGE.

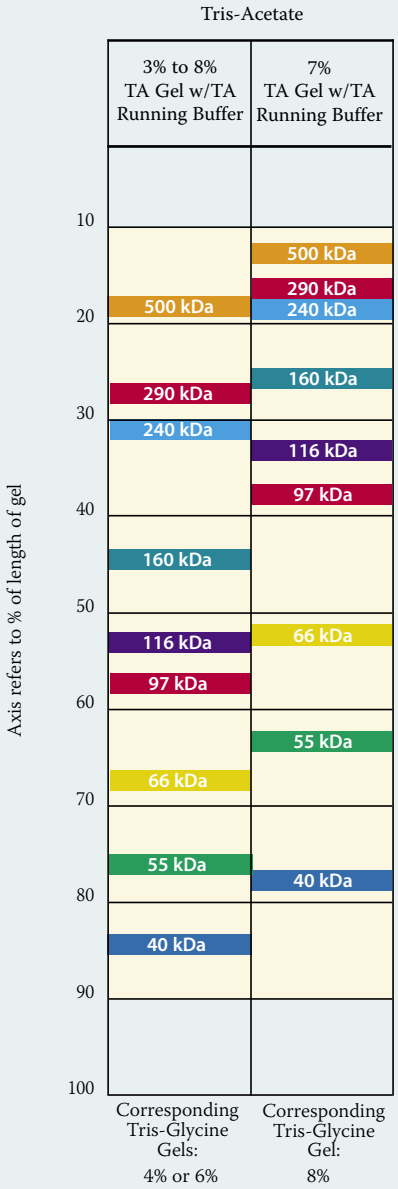
The NuPAGE® Large Protein SDS-PAGE System provides the first reliable method for separation and analysis of large proteins >250 kDa. This revolutionary system offers high-performance separation in a convenient precast, minigel format; sharp prestained/unstained high molecular weight standards with a sizing range up to 500 kDa; and necessary reagents and protocols for Western blotting or visualization.

Precast Gel Offers High Resolution. The NuPAGE® Novex Tris-Acetate Gel/SDS Buffer System is a neutral pH polyacrylamide minigel system specifically designed for clear separation of high molecular weight proteins. The system includes two percentages of NuPAGE® Tris-Acetate gels—3% to 8%, and 7% (Table 1)—and an optimized NuPAGE® Tris-Acetate SDS Buffer Kit. This combination provides a unique chemistry that increases gel stability and minimizes protein modifications, which can occur in Laemmli (Tris-Glycine) gel electrophoresis. With the Tris-Acetate Gels and preoptimized buffers, you can attain well-resolved, intact bands and consistent resolution every time you use them (Figures 1 and 2).

Molecular Weight Standards for Large Protein Analysis. The HiMark™ High Molecular Weight (HMW) Standards represent the first and only prestained and unstained high molecular weight markers designed for SDS-PAGE. The HiMark™ Prestained HMW Standard consists of nine proteins that migrate with apparent molecular weights of 31 kDa to 460 kDa in NuPAGE® Novex Tris-Acetate Gels with Tris-Acetate SDS Buffer (Figure 1B, Lanes 1 and 5). In addition to approximating molecular weight, the HiMark™ HMW Prestained Standard is suitable for monitoring the electrophoresis run and Western blotting. For a more accurate HMW estimation of your unknown large proteins, choose the HiMark™ HMW Unstained Standard. It consists of nine proteins that can be visualized with a protein stain (Figure 2A, Lane 1, and Figure 2B, Lane 4) and offers a precise sizing range of 40 kDa to 500 kDa (established in NuPAGE® Novex Tris-Acetate Gels with Tris-Acetate SDS Buffer). Both the HiMark™ HMW Prestained and Unstained Standards ensure sharp band resolution from run to run and enable you to easily establish a standard curve for estimating molecular weights.

Optimal Blotting for Large Proteins. Western detection is a highly sensitive and specific method for target protein identification. However, large proteins are difficult to transfer out of the gels and tend not to bind membranes very efficiently. The NuPAGE® Large Protein Blotting Kit provides high-resolution Tris-Acetate Gels, premixed SDS buffers, transfer buffers, precut membrane/filter paper sandwiches, and a protocol designed to overcome Western blotting issues. This factor is demonstrated by the Western blot results of oncogene and human nuclear proteins in Figure 1.

Table 1. Migration patterns of HiMark™ Unstained HMW Standard on NuPAGE® Tris-Acetate Gels under denaturing conditions.



Featured Products

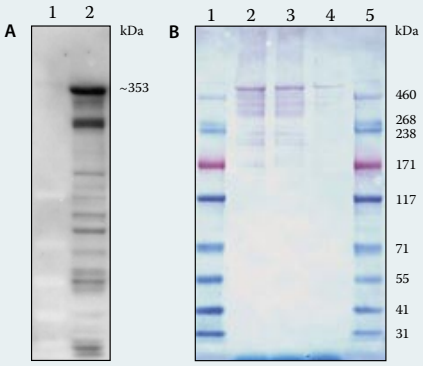
Visualization Techniques for Different Needs. For staining large proteins resolved on Tris-Acetate Gels, the NuPAGE® Large Protein Staining Kit features the ready-to-use SimplyBlue™ SafeStain for microgram/nanogram-level detection (Figure 2A). For sensitive staining of low-abundant proteins, the NuPAGE® Large Protein Sensitive Staining Kit features SilverQuest™ Silver Stain for low-nanogram-protein staining (Figure 2B). The simple, optimized protocols result in fast staining, maximal sensitivity, and minimal protein modifications for your proteins. In addition, the fluorescence-based Coomassie Fluor™ Orange Protein Gel Stain provides easy and sensitive large protein staining with dynamic linearity for quantification.

Complete Kits for Optimal Large Protein Analysis. To ensure success in your large protein studies, Invitrogen offers three detection-based starter kits to meet your needs: NuPAGE® Large Protein Blotting Kit, NuPAGE® Large Protein Staining Kit, and NuPAGE® Large Protein Sensitive Staining Kit.

To order NuPAGE® Large Protein Kits, HiMark™ Protein Standards, and related products, please visit www.invitrogen.com or contact us at locations worldwide (see page 79).

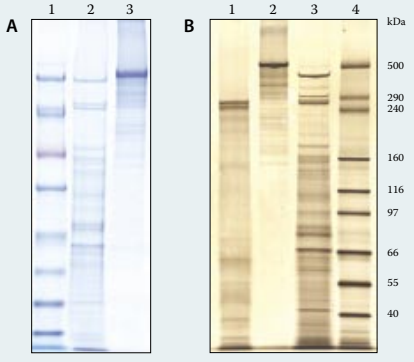
Product	Quantity*	Catalog No.
NuPAGE® Large Protein Blotting Kit†	1 kit	LP0001
NuPAGE® Large Protein Staining Kit†	1 kit	LP0002
NuPAGE® Large Protein Sensitive Staining Kit†	1 kit	LP0003
NuPAGE® Tris-Acetate SDS Buffer Kit for Tris-Acetate Gels	1 kit	LA0050
HiMark™ Prestained HMW Protein Standard	250 µl	LC5699
HiMark™ Unstained HMW Protein Standard	250 µl	LC5688
SimplyBlue™ SafeStain	1 L	LC6060
	3.5 L	LC6065
SilverQuest™ Silver Stain	1 kit	LC6070
Coomassie Fluor™ Orange Protein Gel Stain	1 L	C-33250
	5 L	C-33251
NuPAGE® Novex 7% Tris-Acetate Gel 1.0 mm, 10 well	1 box	EA0355BOX
NuPAGE® Novex 7% Tris-Acetate Gel 1.0 mm, 12 well	1 box	EA03552BOX
NuPAGE® Novex 7% Tris-Acetate Gel 1.0 mm, 15 well	1 box	EA03555BOX
NuPAGE® Novex 7% Tris-Acetate Gel 1.0 mm, 2D well	1 box	EA0356BOX
NuPAGE® Novex 7% Tris-Acetate Gel 1.5 mm, 10 well	1 box	EA0358BOX
NuPAGE® Novex 7% Tris-Acetate Gel 1.5 mm, 15 well	1 box	EA03585BOX
NuPAGE® Novex 7% Tris-Acetate Gel 1.5 mm, 2D well	1 box	EA0359BOX
NuPAGE® Novex 3%-8% Tris-Acetate Gel 1.0 mm, 10 well	1 box	EA0375BOX
NuPAGE® Novex 3%-8% Tris-Acetate Gel 1.0 mm, 12 well	1 box	EA03752BOX
NuPAGE® Novex 3%-8% Tris-Acetate Gel 1.0 mm, 15 well	1 box	EA03755BOX
NuPAGE® Novex 3%-8% Tris-Acetate Gel 1.0 mm, 2D well	1 box	EA0376BOX
NuPAGE® Novex 3%-8% Tris-Acetate Gel 1.5 mm, 10 well	1 box	EA0378BOX
NuPAGE® Novex 3%-8% Tris-Acetate Gel 1.5 mm, 15 well	1 box	EA03785BOX
NuPAGE® Novex 3%-8% Tris-Acetate Gel 1.5 mm, 2D well	1 box	EA0379BOX

Figure 1. Efficient blotting of large proteins using the NuPAGE® Large Protein Blotting Kit.



A. BRCA2 (~353 kDa) detected with HRP Chemiluminescent substrate (data contributed by V. Joukov, Dana-Farber Cancer Institute). **Lane 1:** HiMark™ Prestained High Molecular Weight (HMW) Standard. **Lane 2:** *Xenopus* egg extract. Blot probed with rabbit polyclonal antibody against *Xenopus* BRCA2. **B.** Human protein detected with WesternBreeze® Chromogenic Western Blot Immunodetection Kit. **Lanes 1 and 5:** 10 µl HiMark™ Prestained HMW Standard. **Lanes 2-4:** 500 ng, 300 ng, and 100 ng of 500 kDa human protein, respectively. HiMark™ Prestained HMW Standard and ~500 kDa human protein were separated and blotted using NuPAGE® Large Protein Blotting Kit following recommended protocol. Blot probed with a specific polyclonal antibody and detected with WesternBreeze® Chromogenic Western Blot Immunodetection Kit (anti-rabbit).

Figure 2. Analysis of various proteins using NuPAGE® Large Protein Staining Kit (A) or Sensitive Staining Kit (B).



Lane 1: 10 µl HiMark™ Prestained HMW Standard.
Lane 2: 400 ng of ~500 kDa human protein.
Lane 3: 8 µl of human kinase sample.

Lane 1: 190 ng of rat fibronectin.
Lane 2: 40 ng of ~500 kDa human protein.
Lane 3: 8 µl of 460 human kinase sample diluted 1:10.
Lane 4: 5 µl HiMark™ Unstained HMW Standard diluted 1:10.

Protein samples were separated on NuPAGE® 3% to 8% Tris-Acetate Gel with Tris-Acetate SDS buffer and stained with SimplyBlue™ SafeStain **(A)** or SilverQuest™ Silver Stain **(B)** according to the recommended protocols.

WesternBreeze® Immunodetection Chromogenic Kit –Anti-mouse	1 kit	WB7105
WesternBreeze® Chemiluminescent Kit–Anti-Rabbit	1 kit	WB7106

† Each kit includes 20 NuPAGE® Tris-Acetate gels (1.0 mm, 10 well), standard, buffers, and reagents to run and blot (LP0001) or stain (LP0002 and LP0003) 20 gels.
* 1 box contains 10 gels.