

Accessory Products

The following products are available separately. To learn more about NativePAGE™, visit www.lifetechnologies.com/native.

Product	Quantity	Catalog no.
NativePAGE™ Novex® 3–12% Bis-Tris Gels	1 box	BN1001BOX
NativePAGE™ Novex® 4–16% Bis-Tris Gels	1 box	BN1002BOX
NativePAGE™ Running Buffer (20X)	1 L	BN2001
NativePAGE™ Cathode Buffer Additive (20X)	250 mL	BN2002
NativePAGE™ Sample Buffer (4X)	10 mL	BN2003
SilverQuest™ Silver Staining Kit	1 kit	LC6070
Colloidal Blue Staining Kit	1 kit	LC6025

A wide variety of Tris-Glycine and NuPAGE® Tris-Acetate Gels are also available. Refer to www.lifetechnologies.com/support for more information.

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www.lifetechnologies.com



novex[®]
by *life* technologies™

NativeMARK™ Unstained Protein Standard

Catalog no. LC0725 Size 5 × 50 µL Store at **–30°C to –10°C**

Pub. Part no. LC0725.pps MAN0001514 Rev. Date 4 May 2012

Description

The NativeMARK™ Unstained Protein Standard is designed to allow you to estimate the molecular weight* of proteins when performing **native** electrophoresis, and is supplied in a ready-to-use format. Features of the NativeMARK™ Unstained Protein Standard include:

- Composition of 8 protein bands in the range of ~20–1,200 kDa
- Compatibility with visualization by Coomassie or silver staining after electrophoresis, or with Ponceau S, Coomassie, or other membrane stains after western transfer
- Compatibility with NativePAGE™ Novex® Bis-Tris Gels, and gradient Tris-Glycine, or NuPAGE® Novex® Tris-Acetate Gels

Note: The NativeMARK™ Unstained Protein Standard is designed for use with the gels listed above for native applications. Use with denaturing gels will result in inaccurate molecular weight estimation.

*Estimating the molecular weight in native electrophoresis can be significantly affected by the charge and conformation of the proteins. To more accurately estimate, use SDS-PAGE separation or mass spectrometry analysis.

Specifications

Contents: 5 × 50 µL of NativeMARK™ Unstained Protein Standard

Buffer: Bis-Tris-HCl, pH 7.0; NaCl; glycerol; Ponceau S; sodium azide

Storage: Store at –30°C to –10°C. Avoid repeated freezing and thawing. After a vial is thawed for use, store the vial at 4°C for up to one month.

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

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Directions

The NativeMARK™ Unstained Protein Standard is supplied in a ready-to-use format. For best results, use the appropriate gel type (see page 1) with the proper percentage to resolve your proteins of interest.

1. Thaw the standard on ice or at 4°C. Vortex gently to ensure the solution is homogeneous.
2. Load the following volumes of standard to obtain the best results:

Gel Type/Thickness	Volume*
Mini-Gel (1.0 mm)	5 µL
Mini-Gel (1.5 mm)	7 µL

*For silver staining or other sensitive staining methods, dilute the NativeMARK™ Standard 1:20 in 1X appropriate native sample buffer before loading (e.g., mix 0.5 µL NativeMARK™ Standard with 9.5 µL 1X native sample buffer).

3. After **native** electrophoresis, we recommend visualizing the standard proteins by Coomassie stain (SimplyBlue™ SafeStain or Colloidal Blue Staining Kit), silver stain (SilverQuest™ Silver Staining Kit), or fluorescent stain (SYPRO® Ruby Protein Stain). After staining, you should observe the protein standard bands as shown on the page 3.

Blotting

To increase the transfer efficiency of native proteins:

1. Incubate the gel in 0.1% SDS for 15 minutes before blotting with 1X Tris-Glycine Transfer Buffer (12 mM Tris base, 96 mM glycine, pH 8.3) or 1X NuPAGE® Transfer Buffer (Cat. no. NP0006)
2. Use native transfer buffer (25 mM Tris base, 25 mM glycine, pH 9.2) to allow proteins with pI <9.2 to transfer towards the anode

After transfer, the standard proteins can be stained on the membrane with Ponceau S, Coomassie, or any membrane stain of choice.

Refer to the NativePAGE™ manual, available at www.lifetechnologies.com/manuals for information on the NativePAGE™ gel blotting protocol.

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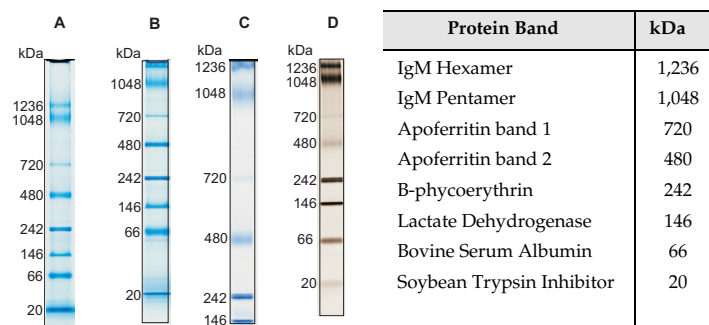
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Example



The NativeMARK™ Unstained Protein Standard (5 µL) was stained with the Colloidal Blue Staining Kit after separation on a NativePAGE™ Novex® 3–12% Bis-Tris Gel (A), NativePAGE™ Novex® 4–16% Bis-Tris Gel (B), or a NuPAGE® Novex® 3–8% Tris-Acetate Gel (C). The standard (5 µL of a 1:20 dilution) was also analyzed on a Novex® 4–12% Tris-Glycine Gel (D) and stained with the SilverQuest™ Silver Staining Kit.

Estimating Protein Molecular Weight

To estimate the molecular weight of proteins after native electrophoresis on polyacrylamide gels, plot the Rf (retardation factor) values versus log molecular weight using a linear or non-linear equation. To increase the accuracy of molecular weight estimation, we recommend using a polynomial curve fit (second-order polynomial best-fit) instead of a straight line for plotting the points.

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.

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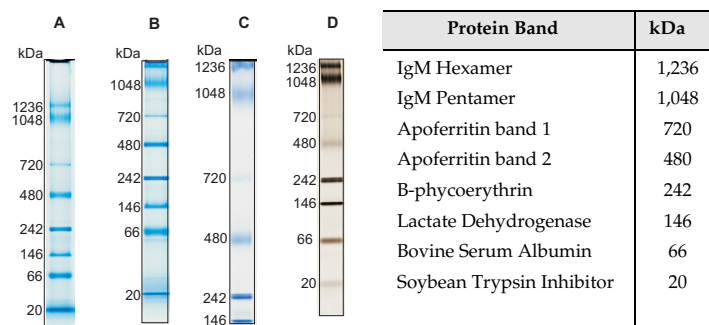
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