invitrogen
by Thermo Fisher Scientific

PRODUCT INFORMATION

iBright™ Prestained Protein Ladder

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Lot: XXXXXXXX Expiry Date: YYYY-MM-DD

Components	#LC5605	#LC5615
iBright Prestained Protein Ladder	25 µL	2 × 250 µL

Storage Buffer: 62.5 mM Tris•H_3PO_4 (pH 7.5 at 25°C), 1 mM EDTA, 2% (w/v) SDS, 10 mM DTT, 1 mM NaN_3 and 33% (v/v) glycerol.

Storage: Upon receipt store at -20°C. Product is shipped on dry ice.

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For Research Use Only. Not for use in diagnostic procedures.

Description

Invitrogen™ iBright™ Prestained Protein Ladder is a mixture of 12 proteins ranging from 11 kDa to 250 kDa (11, 15, 26, 30, 34, 43, 55, 70, 80, 95, 130 and 250 kDa). The protein ladder produces 10 well-defined blue pre-stained bands by SDS-PAGE and 2 unstained bands (30 kDa and 80 kDa) containing IgG binding sites, which can be used for confirmation of Western blot success (see website for product images). The prestained bands of the iBright protein ladder can be visualized by SDS-PAGE or fluorescently viewed using a fluorescent detection instrument (e.g., Amersham™ Typhoon™ or LI-COR™ Odyssey™ imagers). For an easy reference, the 55 kDa protein band has a greater intensity than the other prestained proteins in the ladder.

The 30 kDa and 80 kDa immunodetectable bands contain IgG binding sites that can be visualized simultaneously with your target protein using the same antibody conjugate and protocol (see website for product images). These two immunodetectable bands are compatible with chemiluminescent or fluorescent detection methods for Western blot analysis. The iBright protein ladder can be visualized on gels using Thermo Scientific™ PageBlue™, SimplyBlue™ SafeStain, NBS Biologicals™ SafeBlue™, or other Coomassie stains, or directly on the membrane using Novex™ Reversible Membrane or Pierce™ Reversible protein stains. The iBright protein ladder provides convenient visualization during electrophoresis and transfer, and clear, sharp confirmation of Western blot detection of your target protein.

The protein ladder is conveniently packaged and ready to use with no heating, diluting, or additional reducing agent required.

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Important Product Information

- Do not boil the protein ladder.
- Use a Coomassie stain (e.g., PageBlue stain, SimplyBlue SafeStain, etc.) or reversible membrane stain (e.g., Pierce Reversible protein stain) to view the 30 and 80 kDa bands with the visible bands.
- In low-percentage gels (< 10%), the low molecular weight proteins in the ladder may migrate with the dye front.
- Large proteins (> 100 kDa) in the ladder may require longer transfer times or higher transfer voltages for Western blotting.
- The mobility of prestained proteins can vary in different SDS-PAGE buffer systems; however, they are suitable for approximate molecular weight determination when calibrated against unstained standards in the same system.
- An additional low molecular weight band of free dye is visible when using fluorescent detection.
- The apparent molecular weight variance of the 10 prestained proteins is ~5%.
- Intensity of 30 kDa and 80 kDa immunodetectable bands depends on secondary antibody concentration and substrate sensitivity.
- Primary antibodies with low starting concentrations may result in insufficient chemiluminescent detection of the Western blot positive control bands. If unstained 30 kDa and 80 kDa bands produce weak or no signal, spike the diluted primary antibody with the corresponding Rabbit IgG or Mouse IgG to a concentration of 1-5 µg/mL, prior to secondary antibody incubation. Follow with respective secondary (GAM/GAR) incubation to increase the intensity of Western blot positive control bands in the iBright Prestained Protein Ladder.

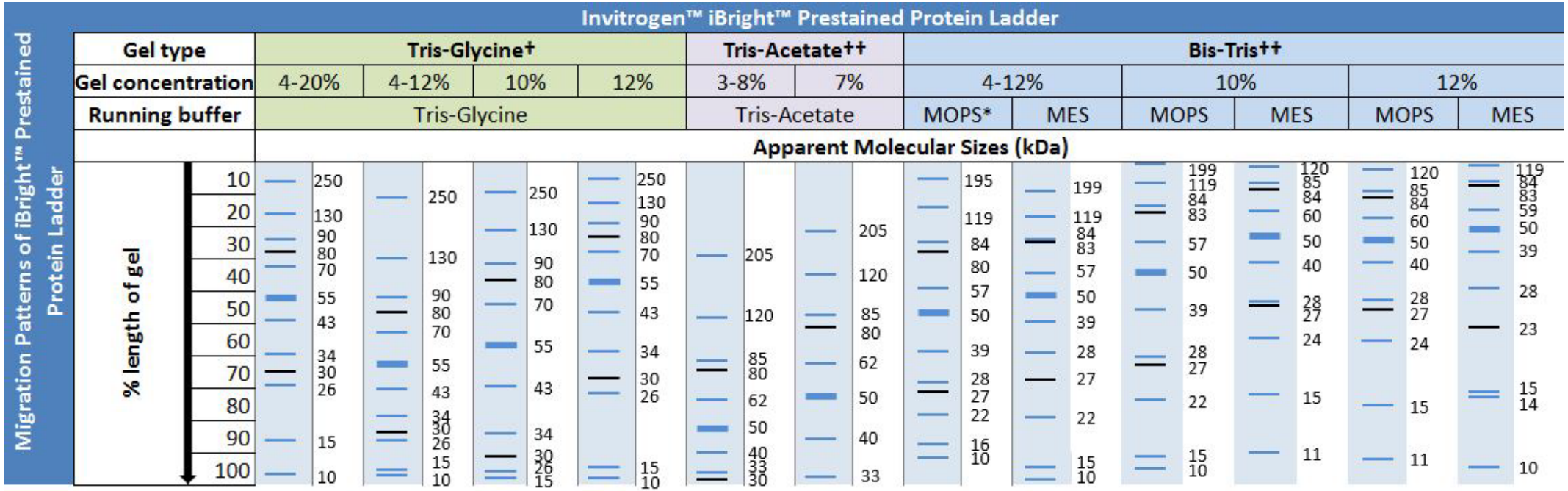
Procedure for using iBright protein ladder in polyacrylamide gel electrophoresis

1. Thaw the ladder at room temperature.
2. Vortex gently to ensure the solution is homogeneous.
3. Load the ladder on the gel (see Table 1 for recommended volumes).
4. Return unused protein ladder to -20°C and store up to one year.
5. Visualize the ladder via fluorescent detection using a fluorescent imager (e.g., Typhoon or LI-COR Odyssey imagers).
6. Detect the 30 kDa and 80 kDa ladder bands on membranes using the same alkaline phosphatase or horseradish peroxidase-conjugated antibody with chemiluminescent substrates, or fluorescently-labeled antibodies as used for your target protein.

Table 1. Volumes of Invitrogen iBright Prestained Protein Ladder to load for different applications.

Gel type	Visual detection (1.0 mm gel thickness)	Fluorescent detection	Chemiluminescent detection
Mini gel	1-3 µL (12-well & 20-well) 2 µL (26-well)	1-3 µL (12-well & 20-well) 2 µl (26-well)	1-3 µL (12-well & 20-well) 2 µL (26-well)
Midi gel	2-4 µL (12-well & 20-well) 2 µL (26-well)	2-3 µL (12-well & 20-well) 2 µL (26-well)	2-3 µL (12-well & 20-well) 2 µL (26-well)

Table 2. Example migration patterns for various gel types.



† Migration patterns were determined using respective Novex™ Wedgewell

†† Migration patterns were determined using respective NuPAGE™ precast gels.

* Migration pattern for 4-12% Bis-Tris in MOPS buffer measured using 4-12% Bolt™ Bis-Tris precast gel.

Related Thermo Scientific Products

34580	SuperSignal™ West Pico PLUS Chemiluminescent Substrate, 500 mL
34075	SuperSignal™ West Dura Chemiluminescent Substrate, 100 mL
34096	SuperSignal™ West Femto Chemiluminescent Substrate, 200 mL
32430	Stabilized Goat Anti-Mouse IgG (H+L), Peroxidase Conjugated (10 µg/mL), 2 mL
32460	Stabilized Goat Anti-Rabbit IgG (H+L), Peroxidase Conjugated (10 µg/mL), 2 mL
46430	Restore™ PLUS Western Blot Stripping Buffer, 500 mL
46640	SuperSignal™ Western Blot Enhancer
37542	StartingBlock™ (TBS) Blocking Buffer
37578	StartingBlock™ (PBS) Blocking Buffer

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