**Protein Ladder (10–250 kDa)**

**Description:** Protein Ladder is a mixture of 12 recombinant, highly purified proteins, which resolve into clearly identifiable sharp bands from 10–250 kDa when analyzed by SDS-PAGE and stained with Coomassie Brilliant Blue R-250 (1). This ladder is intended for use as a precise size standard when performing SDS-PAGE to calculate the molecular weight of a protein of interest. The 25 and 80 kDa bands have triple the intensity of the other proteins and serve as reference indicators. The molecular weights of the Protein Ladder are confirmed by sequencing, mass spectrometry and migration in a Laemmli SDS-PAGE Tris-Glycine system (3).

**Contents:** 0.1–0.3 mg/ml of each protein in 70 mM Tris-HCl (pH 6.8 @ 25°C), 33 mM NaCl, 1 mM Na₂EDTA, 2% (w/v) SDS, 40 mM DTT, 0.01% Bromophenol Blue and 10% glycerol.

**Notes on use:**
- For silver stained gels, dilute the Protein Ladder in 1:15 in buffer (70 mM Tris-HCl, pH 6.8, 33 mM NaCl, 1 mM Na₂EDTA, 2% SDS, 40 mM DTT, 0.01% Bromophenol Blue and 10% glycerol).
- To ensure uniform mobility, load an equal volume of 1X Reducing SDS Loading Buffer into any unused wells.

**References:**

**Suggested Protocol for Preparing and Loading Protein Ladders:**
1. Thaw the Protein Ladder at room temperature. Vortex gently to ensure the solution is homogeneous.
2. Transfer the desired amount of the Protein Ladder to a separate tube. Use 5 µl for mini-gels and 10 µl for full length gels.
3. Heat the ladder at 95–100°C for 3–5 minutes.
4. Load directly onto SDS-PAGE gel and electrophorese.

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