INSTRUCTIONS

Spectra™ Multicolor Low Range Protein Ladder

Pub. No. MAN0011776
Rev. B.0
Pub Part No. 2162357.2

26628

Number	Description
26628	**Spectra Multicolor Low Range Protein Ladder,** 250µL

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

Storage: Upon receipt store at -20°C. Product is shipped with an ice pack.

Introduction

The Thermo Scientific Spectra Multicolor Low Range Protein Ladders are a prestained mixture of six recombinant proteins ranging from 1.7kDa to 40kDa. Three different chromophores are bound to the proteins, producing a brightly colored ladder specifically designed for small protein analysis (see website for product images). The protein ladder is conveniently packaged and ready to use with no heating, diluting or additional reducing agent necessary.

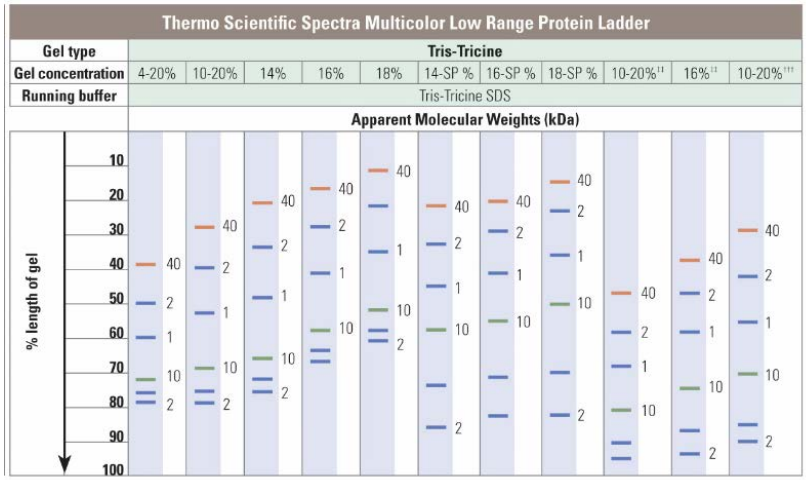
Important Product Information

- Do not boil the protein ladder.
- The molecular weights of the proteins have a lot-to-lot variation of ~5%.
- To obtain well-resolved bands of low-molecular weight proteins: Resolve proteins using gels with high concentrations of acrylamide/bisacrylamide (>14%) and crosslinking (5%); use stacking and resolving gels with pH 8.45; and add ethylene glycol to the resolving gel (see the Procedure for Using the Protein Ladder in Polyacrylamide Gel Electrophoresis for Small Proteins Section).
- Before coomassie or silver staining the gel, fix proteins with 5% glutaraldehyde.
- The mobility of prestained proteins may 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. See website for migration patterns in different electrophoresis conditions.

Procedure for Using the Protein Ladder in Polyacrylamide Gel Electrophoresis

1. Thaw the ladder at room temperature. Do not boil protein ladder.
2. Mix the solution gently and thoroughly to ensure it is homogeneous.
3. Load an appropriate volume of the ladder on the gel.
    - Mini-gel: 5µL per well (0.75-1.0mm thick) or 10µL per well (1.5mm thick)
    - Midi gel: 10µL per well (0.75-1.0mm thick) or 20µL per well (1.5mm thick)
4. Return the unused protein ladder to -20°C for up to one year or 4°C for up to two months.

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | thermofisher.com



Procedure for Using the Protein Ladder in Polyacrylamide Gel Electrophoresis of Small Proteins

Note: To improve resolution of small peptides (1-20kDa), use the following electrophoresis conditions.

A. Materials Required

- Ethylene glycol
- Acrylamide stock solution, 40%
- Bisacrylamide stock solution, 40%
- 3M Tris-HCl buffer containing 0.4% SDS, pH 8.45
- 40% ammonium persulfate (APS)
- Tetramethylethylenediamine (TEMED)
- 1X Tris-tricine-SDS Buffer (10X) (Buffer diluted to 1X concentration before use)

B. Gel Preparation Example

Note: The protocol below provides reagent amounts sufficient for two 0.75mm mini-gels (Table 1).

Table 1. Reagent amounts for gel electrophoresis of small proteins.

Component	Stacking gel (T=5%, C=3.3%)	Resolving gel (T=18%, C=5%)
Ethylene glycol	–	2.4mL
3M Tris-HCl buffer, pH 8.45	1mL	2mL
Acrylamide/Bisacrylamide (40%)	0.5mL (29:1)	3.6mL (19:1)
Ultrapure water	2.5mL	–
40% APS	4µL	8µL
TEMED (100%)	16µL	12µL
Final volume	**~4mL**	**~8mL**

Note: To prepare homemade acrylamide/bisacrylamide solutions, use the provided formulas:

$$\%T = \frac{AA(g)+BIS(g)}{mass\ of\ solution\ (g)} \times 100 \qquad \%C = \frac{BIS(g)}{AA(g)+BIS(g)} \times 100$$

AA= acrylamide
BIS= bisacrylamide
%T= acrylamide/bisacrylamide concentration
%C= crosslinking

C. Procedure

1. Pipette 3.3mL of resolving gel between the glass plates. Carefully apply 1.1mL of the stacking gel solution. Avoid forming bubbles.

   Note: The different gel solution densities will cause a layer to form between the solutions.

2. Insert the combs. Ensure the gels are free of air bubbles. Allow the gels to polymerize for 1 hour at room temperature.

   Note: For best results, maintain the gel at 4°C overnight in a plastic bag with electrophoresis running buffer to avoid drying. Do not remove the combs.

3. Carefully remove the combs. Place the gel in the electrophoresis tank and fill the top and bottom reservoirs with new 1X Tris-tricine-SDS buffer, ensuring that the gel wells are covered with buffer.

4. Load the samples.

5. Set 200V on the power supply and run the electrophoresis for ~2 hours or until the dye front reaches the bottom of the gel.

6. Disassemble the gel sandwich and proceed with gel staining or Western blot procedures.

D. Gel Staining

Note: Protein fixation with glutaraldehyde is required before staining the gel with coomassie brilliant blue dye (e.g., Thermo Scientific PageBlue Protein Staining Solution, Product No. 24620) or silver dye (e.g., Thermo Scientific Pierce Silver Stain Kit, Product No. 24612).

Note: Many protein fixation compounds (e.g., acetic acid, isopropanol, ethanol, TCA) are not suitable; proteins will wash away during the staining procedure.

1. Add 100mL of ultrapure water to the gel and wash for 1 minute with gentle agitation. Discard the wash.

2. Add 50mL of new 5% glutaraldehyde solution to completely cover gel. Fix with gentle agitation for 30 minutes. Discard the solution.

3. Add 100mL of ultrapure water to the gel and wash for 5 minutes with gentle agitation. Discard the wash and repeat twice.

   Note: Proceed to the staining step in the PageBlue Protein Staining Solution protocol or to the sensitizing step in the Pierce™ Silver Stain Kit protocol.

Related Products

Please see the catalog or website for a complete listing of protein gels and Western blotting products.

26614	**PageRuler™ Unstained Protien Ladder,** 2 × 250µL
26616	**PageRuler Prestained Protein Ladder,** 2 × 250µL
26619	**PageRuler Plus Prestained Protein Ladder,** 2 × 250µL
26630	**PageRuler Broad Range Unstained Protein Ladder,** 2 × 250µL
26632	**PageRuler Low Range Unstained Protein Ladder,** 2 × 250µL
26634	**Spectra Multicolor Broad Range Protein Ladder,** 2 × 250µL
26625	**Spectra Multicolor High Range Protein Ladder,** 2 × 250µL
26628	**Spectra Multicolor Low Range Protein Ladder,** 250µL
LC5615	**iBright™ Prestained Protein Ladder**
EC6625BOX	**Novex™ 10-20% Tricine Protein Gels, 12-well** (see thermofisher.com/proteingels for a complete listing)
XP10200BOX	**Novex 10-20% Tris-Glycine Mini Gels, 10-well** (see thermofisher.com/proteingels for a complete listing)
LC1675	**Novex Tricine SDS Running Buffer (10X)**
24615	**Imperial™ Protein Stain,** 1L
LC6060	**SimplyBlue™ SafeStain**

General References

Alegria-Schaffer, A., *et al*. (2009). Performing and optimizing Western blots with an emphasis on chemiluminescent detection. *Methods Enzymol* **463:**573-99.

Burnette, W.N. (1981). "Western blotting": electrophoretic transfer of proteins from sodium dodecyl sulfate – polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. *Anal Biochem* **112(2):**195-203.

Kurien, B.T. and Scofield, R.H. (2003). Protein blotting: a review. *J Imm Meth* **274:**1-15.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227:**680-5.

Towbin, H., *et al.* (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* **76:**4350-4.

For research use only. Not for use in diagnostic procedures.

Current product instructions are available at thermofisher.com. For a faxed copy, call 800-874-3723 or contact your local distributor.

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | thermofisher.com