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LIFE SCIENCES

CERTIFICATE OF ANALYSIS

Spectra™ Multicolor Low Range Protein Ladder

#SM1861 250 µl

(for 50 mini gel applications 5 µl per well or
25 large gel applications 10 µl per well)

Lot: **Expiry Date:**

Store at -20°C
(or at 4°C for 2 months)

ISO 9001 ISO 14001
www.fermentas.com

Description

Spectra™ Multicolor Low Range Protein Ladder is designed specifically for small protein analysis. It is a mixture of 6 recombinant, highly purified proteins and synthetic peptides with apparent molecular weights of 1.7 to 40 kDa. The proteins are individually prestained using three different dyes.

Lot-to-lot variation of the apparent molecular weight of prestained proteins is ~5%.

The Spectra™ Multicolor Low Range Protein Ladder is ready-to-use: no heating, further dilution or addition of a reducing agent is required before use.

Applications

- Monitoring of protein migration during SDS-PAGE (1).
- Verifying Western transfer efficiency (2-4).
- Approximate sizing of proteins on SDS-polyacrylamide gels and Western blots.
- Locating a protein of interest for excision from an unstained preparative gel.

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.

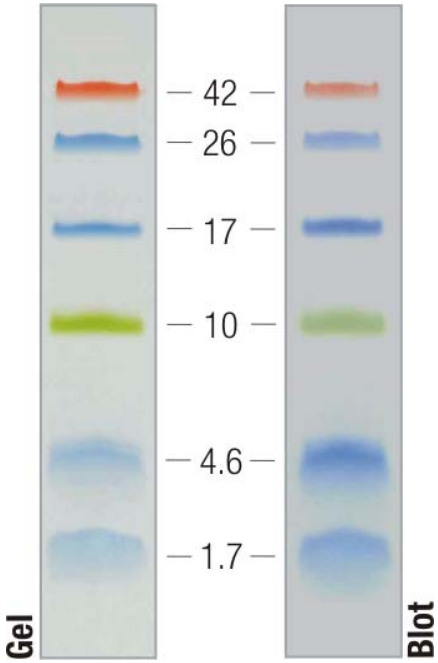
Recommendations for Loading

1. Thaw the ladder at room temperature for a few minutes to dissolve precipitated solids. **Do not boil!**
2. Mix gently, but thoroughly, to ensure that the solution is homogeneous.
3. Load the following volumes of the ladder on an SDS-polyacrylamide gel:
   – 5 µl per well for mini gel,
   – 10 µl per well for large gel.
   Use the same volumes for Western blotting.
   The loading volumes listed above are recommended for gels with a thickness of 0.75 mm. The loading volume should be doubled for 1.5 mm thick gels.

Important Note

- Tris-tricine resolving gels with higher acrylamide/bisacrylamide concentration (T≥14%) and higher level of crosslinking (C=5%) are recommended for analysis of small proteins. Such conditions result in well resolved, sharp bands of 1.7 and 4.6 kDa peptides.
- Protein fixation with 5% glutaraldehyde is required before Coomassie and silver staining (e.g., using PageBlue™ Protein Staining Solution (#R0571) and PageSilver™ Silver Staining Kit (#K0681)).
- Prestained proteins can have different mobilities in various SDS-PAGE-buffer systems. However, they are suitable for approximate molecular weight determination when calibrated against unstained standards in the same system.
  See reverse page for migration patterns in different electrophoresis conditions.

Representative lot of Spectra™ Multicolor Low Range Protein Ladder, apparent MW, kDa



18% SP-Tris-tricine SDS-PAGE
(SP – small peptide SDS-polyacrylamide gel with higher crosslinking, C=5%)

QUALITY CONTROL

5 µl of Spectra™ Multicolor Low Range Protein Ladder provide 6 individual bands in SP-SDS-PAGE (Tris-tricine buffer) and after electrophoretic transfer from the gel onto PVDF membrane.

Quality authorized by: Jurgita Zilinskiene

(continued on reverse page)

General recommendations for electrophoretic analysis of small proteins

I. SDS-PAGE

We recommend use of special electrophoresis conditions that improve resolution of small peptides (1 to 20 kDa) (5). Main differences from conventional gel include:

- higher Tris concentration in gel buffer (0.75 M instead of 0.375 M);
- pH 8.45 in both stacking and resolving gels;
- higher cross-linking of acrylamide in resolving gel (C= 5% instead of usual ~3%);
- ethylene glycol, included in the resolving gel.

A. Reagents

- Ethylene glycol
- Stock solutions of acrylamide/bisacrylamide 19:1 and 29:1
- 3 M Tris-HCl buffer containing 0.4% SDS, pH 8.45
- 40% ammonium persulfate (APS)
- TEMED
- 1X Tris-tricine-SDS Buffer (10X Buffer (#B48), diluted to 1X concentration prior to use)

B. Gel Preparation Example

Protocol provides amounts of reagents sufficient for two 0.75 mm mini gels.

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

Note. For preparation of home-made acrylamide/bisacrylamide solutions use formulas provided below:

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

C. Procedure

- Pour 3.3 ml of resolving gel solution between the glass plates with the pipette. Just afterwards, carefully apply 1.1 ml of the stacking gel solution. Avoid forming of the bubbles.

Note. Because of the difference in densities of gel solutions they do not mix with each other, a sharp interface is obtained immediately after applying of stacking gel solution.

- Insert combs. Ensure that no air burbles are left in the gels. Allow the gels to polymerize for 1 hour at room temperature.

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

- Remove combs carefully. Put the gel into the electrophoresis tank, fill the tank (bottom and top reservoirs) with fresh 1X Tris-tricine-SDS buffer, making sure that the gel wells are covered with the buffer.

- Load the samples.
- Set 200 V and run the electrophoresis for ~2 hours or until the dye front reaches the bottom of the gel.
- Disassemble the gel sandwich and proceed with gel staining or Western blot procedures.

II. Gel staining

Protein fixation with glutaraldehyde is required before staining the gel with Coomassie Brilliant Blue dye (PageBlue™ Protein Staining Solution, #R0571) or silver (PageSilver™ Silver Staining Kit, #K0681).

Note. Other compounds commonly used for protein fixation (e.g., acetic acid, isopropanol, ethanol, TCA) are not suitable; proteins will wash away during staining procedure.

Procedure

1. Add 100 ml of deionized or distilled water to the gel and wash for 1 min with gentle agitation. Discard the wash.
2. Add 50 ml of freshly prepared 5% glutaraldehyde solution (gel should be covered completely). Fix with gentle agitation for 30 min. Discard the solution.
3. Add 100 ml of deionized or distilled water to the gel and wash for 5 minutes with gentle agitation. Discard the wash. Repeat this step twice.

The gel is now ready for staining. For detailed staining protocols using Fermentas PageBlue™ Protein Staining Solution or PageSilver™ Silver Staining Kit, refer to the product manuals or www.fermentas.com.

Note. Proceed directly to the **staining step** in PageBlue™ Protein Staining Solution protocol and to **sensitizing step** in PageSilver™ Silver Staining Kit protocol.

References

1. Laemmli, U.K., Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature, 227, 680-685, 1970.
2. Burnette, W.N., "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, 1981.
3. Towbin, H., et al., Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications, Proc. Natl. Acad. Sci. USA, 76, 4350-4354, 1979.
4. Kurien, B.T. and Scofield, R.H., Protein blotting: a review, J. Imm. Meth., 274, 1-15, 2003.
5. Westermeier, R., Electrophoresis in Practice: A Guide to Methods and Applications of DNA and Protein Separations, 4th Edition, WILEY, 242-243, 2004.

PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively *for research purposes and in vitro use only*. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Please refer to www.fermentas.com for Material Safety Data Sheet of the product.