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LIFE SCIENCES

CERTIFICATE OF ANALYSIS

Unstained Protein Molecular Weight Marker

#SM0431 2x1000 µl

(for 400 mini gel applications 5 µl per well or for 200 large gel applications 10 µl per well)

Lot: _ **Expiry Date: _**

Store at -20°C

In total 2 vials. BSA included

Description

Unstained Protein Molecular Weight Marker is suitable for accurate sizing of proteins by SDS-PAGE. It is a mixture of 7 purified proteins ranging in size from 14.4 kDa to 116.0 kDa when analyzed by SDS-PAGE and stained with Coomassie Blue or silver (1).

Storage Buffer

62.5 mM Tris-HCl (pH 7.0 at 25°), 1 mM EDTA, 2% (w/v) SDS, 50 mM DTT, 30 mM NaCl, 1 mM NaN_3, 0.01% (w/v) bromophenol blue and 50% (v/v) glycerol.

Application

Accurate molecular weight determination of proteins in SDS-PAGE and Western blots.

Recommendations for Loading

1. Thaw the marker at room temperature for a few minutes to dissolve precipitated solids. Vortex gently.
2. It is recommended to divide the Marker into several aliquots to avoid contamination of the stock solution.
3. Heat an aliquot of the ladder for 10 minutes at 95°C for complete denaturation of proteins. Cool and mix.
4. Load the following volumes of the ladder on a SDS-polyacrylamide gel:
   - 5 µl per well for mini gel;
   - 10 µl per well for large gel.

   Use the same volumes for Western blotting applications. The loading volumes listed above are recommended for gels with a thickness of 0.75-1.0 mm. The loading volume should be doubled for 1.5 mm thick gels.

MW, kDa	Protein	Source	Ref.
116.0	β–galactosidase	*E.coli*	2
66.2	Bovine serum albumin	bovine plasma	3
45.0	Ovalbumin	chicken egg white	4
35.0	Lactate dehydrogenase	porcine muscle	5
25.0	REase Bsp98I	*E.coli*	6
18.4	β–lactoglobulin	bovine milk	7
14.4	Lysozyme	chicken egg white	8

8-16% Tris-glycine SDS-PAGE

QUALITY CONTROL

Electrophoresis of 5 µl of the Unstained Protein Molecular Weight Marker in a 8-16% Tris-glycine SDS gel resolves 7 individual bands of equal intensities. Bands are visualized with PageBlue™ Protein Staining Solution (#R0571).

Quality authorized by: Jurgita Zilinskiene

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Notes

- To avoid overloading gels which will be subsequently silver stained, dilute the ladder in protein loading buffer just prior to use:

Water, nuclease-free (#R0581)	35.5 µl
4X DualColor™ Protein Loading Buffer (#R1011)	12.5 µl
20X Reducing Agent (#R1011)	1 µl
Protein ladder	1 µl

  Load 5 µl of the diluted ladder per well for a mini gel/blot and 10 µl per well for a large gel/blot.
- Store denatured marker at -20°C.
- Because of the SDS presence in storage buffer the Marker should not be used in a native polyacrylamide gel electrophoresis for determining native molecular weights of proteins.
- If additional bands are observed in the gel image of the protein ladder, this might be caused by DTT oxidation in the storage buffer. Add freshly prepared DTT solution to a final concentration of 50 mM.

References

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3. Brown, J.R., Structure of bovine serum albumin, Fed. Proc., 34, 591, 1975.
4. Warner, R.C., Egg proteins, Proteins, II A., 435, (Neurath, H., Bailey, K., eds.), Academic Press, N.Y., 1954.
5. Castellino, F.J., Barker, R., Examination of the dissociation of multichain proteins in guanidine hydrochloride by membrane osmometry, Biochemistry, 7, 2207-2217, 1968.
6. Unpublished results.
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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.