## **Ordering Information**

Catalog Number

Product Description

#### Molecular Weight Standards

161-0303 **SDS-PAGE Standards,** High, 200 μl 161-0304 **SDS-PAGE Standards,** Low, 200 μl

161-0317 SDS-PAGE Standards, Broad, 200 µl

161-0314 Silver Stain SDS-PAGE Standards, Low, 200  $\mu l$ 

161-0315 Silver Stain SDS-PAGE Standards, High, 200 µl 161-0306 Biotinylated SDS-PAGE Standards, Low. 250 µl

161-0311 **Biotinylated SDS-PAGE Standards**, High, 250 µl

161-0319 Biotinylated SDS-PAGE Standards, Broad, 250 µl

161-0320 2-D SDS-PAGE Standards

161-0326 Polypeptide SDS-PAGE Standards, 200 μl

#### **Prestained Standards**

161-0305 Prestained SDS-PAGE Standards, Low, 500 µl 161-0309 Prestained SDS-PAGE Standards, High, 500 µl

161-0309 Prestained 3D3-PAGE Standards, Flight, 300 µl

161-0324~ Kaleidoscope Prestained Standards,  $500~\mu\text{I}$ 

161-0325 Kaleidoscope Polypeptide Standards, 500 µl

#### **IEF Standards**

161-0310 **IEF Standards.** pl range 4.45-9.6, 250 ul



# Silver Stain SDS-PAGE Standards, Low Range

# Catalog Number 161-0314

Product shipped on dry ice. Store at -20 °C upon arrival.



# SDS-PAGE Molecular Weight Standards,Low Range Specifications

#### Low Range

Range 14.400 to 97.400 daltons

**Contents** Rabbit muscle phosphorylase b

Bovine serum albumin Hen egg white ovalbumin Bovine carbonic anhydrase Soybean trypsin inhibitor Hen egg white lysozyme

200 ul concentrated solution

Storage -20 °C

Shelf Life 1 year at -20 °C

Applications 400 full size gels per vial 800 mini gels

Recommended 12.5%\*

gel percentages

Volume

\*Note:

The lowest recommended gel percentage for the low range standards is 10%. At gel percentages of 10% or less, one or more of the standards may migrate at the dye front, depending on running conditions and other factors. As a result, only four or five bands may be visible after staining. If this should occur, an increase in gel percentage is recommended.

Silver Stain SDS-PAGE Standards contain approximately 700 µg total protein in 50% glycerol (w/v), 300 mM NaN<sub>3</sub>, 20 mM Tris, and 4 mM EDTA. The proteins have been blended to give bands of equal intensity on SDS polyacrylamide gel systems run according to Laemmli<sup>1</sup> and stained with Bio-Rad Silver Stain or Silver Stain Plus. Different results may be obtained when alternative silver staining chemistries are used.

### Reference

- 1. Laemmli, U. K., Nature, 227, 680 (1970).
- Hames, B. D. and Rickwood, D., Gel Electrophoresis of Proteins: A Practical Approach, Second Edition, p. 17, Oxford University Press, New York (1990).

## **Protocol**

Dilute standards 1:20 in SDS Reducing Sample Buffer.\* Heat for 5 minutes at 95 °C. Cool and load 10  $\mu$ l/well for full length gels (16-20 cm) or 5  $\mu$ l/well for mini gels. These load volumes and dilutions have been optimized for development with Bio-Rad Silver Stain or Silver Stain Plus for approximately 10 minutes. If silver stain development times vary, the loading volume or dilution of the standards may need to be adjusted to optimize the band intensity.

# \* SDS Reducing Sample Buffer (Prepare immediately before use)

β-mercaptoethanol	25 µl
Stock sample buffer	475 μl
	500 μ1

#### Stock Sample Buffer (Store at room temperature)

Distilled water	4.8 ml
0.5M Tris-HCl, pH 6.8	1.2 ml
Glycerol	1.0 ml
10% (w/v) SDS	2.0 ml
0.1% (w/v) bromophenol blue	0.5 ml
	9.5 ml

Use of stock sample buffer with insufficient or old \( \beta\)-mer-captoethanol may result in doublets at the soybean trypsin inhibitor and ovalbumin bands.

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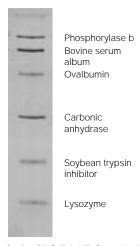
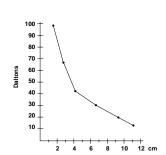


Fig. 1. Silver Stain SDS-PAGE Standards give bands of even intensities when stained with Bio-Rad's Silver Stain or Silver Stain Plus. Note that no extraneous bands are present. Low range standards run on a 12.5% SDS polyacrylamide gel and stained with Bio-Rad's Silver Stain Kit.

## **Protein Molecular Weights**

Molecular	
Weight	References
97,400	Titani, K., et. al., <i>Proc. Natl. Acad. Sci. USA</i> , <b>74</b> , 11, 4762 (1977).
66,200	Brown, J. R., Fed. Proc., 34, 591 (1975).
45,000	Warner, R. C., "Egg Proteins," in: <b>The Proteins</b> , Vol. IIA, p. 435 (Neurath, H. and Bailey, K., eds.), Academic Press, New York (1954).
31,000	Davis, R. P., "Carbonic Anhydrase," in: <b>The Enzymes,</b> Vol. V, p. 545, (Boyer, P. D. eds.) Academic Press, New York (1971).
21,500	Wu, Y. V. and Scheraga, H. A., <i>Bio-chemistry</i> , <b>1</b> , 698 (1962).
14,400	Jolles, P., Angew. Chem., Intl. Edit., 8 227 (1969).
	Weight 97,400 66,200 45,000 31,000 21,500



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Fig. 2. Curve generated by plotting the molecular weight of the low range molecular weight standards run on a 12% SDS polyacrylamide gel vs. the distance migrated from the interface of the stacking and separating gels in centimeters. An alternative method is to plot the  $\log_{10}$  relative mobility ( $R_p$ ) vs. the gel concentration, %T, (percentage total monomer, i.e. grams acrylamide plus bis acrylamide/100ml).

$$R_{\rm f} = \frac{distance\ migrated\ by\ protein}{distance\ migrated\ by\ dye}$$

The curve can be used to determine molecular weights of unknown proteins.<sup>2</sup>