Ordering Information

Catalog Number	Product Description
161-0319	**Biotinylated SDS-PAGE Standards**, Broad Range,250 µl
161-0306	**Biotinylated SDS-PAGE Standards**, Low Range, 250 µl
161-0311	**Biotinylated SDS-PAGE Standards**, High Range, 250 µl
161-0322	**Biotinylated SDS-PAGE Standards Kit**, Broad Range, AP*
161-0321	**Biotinylated SDS-PAGE Standards Kit**, Broad Range, HRP*
161-0307	**Biotinylated SDS-PAGE Standards Kit**, Low Range, HRP*
161-0308	**Biotinylated SDS-PAGE Standards Kit**, Low Range, AP*
161-0312	**Biotinylated SDS-PAGE Standards Kit**, High Range, HRP*
161-0313	**Biotinylated SDS-PAGE Standards Kit**, High Range, AP*
170-6528	**Avidin-HRP**, 2 ml
170-6533	**Avidin-AP**, 1 ml
170-6431	**Horseradish Peroxidase Conjugate Substrate Kit**
170-6432	**Alkaline Phosphatase Conjugate Substrate Kit**

* Each kit contains 250 µl Biotinylated Standards, 1 ml Avidin-AP or 2 ml Avidin-HRP, and complete instructions.

Bio-Rad Laboratories, 2000 Alfred Nobel Dr., Hercules, CA 94547

LIT395 Rev D

Biotinylated SDS-PAGE Standards, Low, High, and Broad Range

Catalog Numbers
161-0306
161-0311
161-0319

BIO-RAD

Biotinylated SDS-PAGE Standards, Low, High, and Broad Range

Bio-Rad's Biotinylated SDS-PAGE Standards are a mixture of biotinylated proteins that can be used for accurate molecular weight determinations of immune detected proteins. The standards have been blended to give equal intensities when detected with avidin-HRP and HRP Color Development Reagent.

The Biotinylated SDS-PAGE Standards and the sample proteins are run on an SDS polyacrylamide gel and electrophoretically transferred to nitrocellulose, PVDF, or Zeta-Probe® membranes. The protein samples and standards are exposed to the same reagents during detection. This prevents distortions that may occur if the standards lane is treated separately.

The molecular weights of the standards are not significantly altered by biotinylation. The consistent molecular weights of the standards give accurate molecular weight determinations every time without increasing the number of steps or length of time of immune detection.

Specifications

MW Ranges

Low	14,400–97,000 daltons
High	45,000–200,000 daltons
Broad	6,500–200,000 daltons

Protein	**MW** (daltons)	**Low Range**	**High Range**	**Broad Range**
Myosin	200,000		X	X
β-galactosidase	116,250		X	X
Phosphorylase b	97,400	X	X	X
Bovine serum albumin	66,200	X	X	X
Ovalbumin	45,000	X	X	X
Carbonic anhydrase	31,000	X		X
Soybean trypsin inhibitor	21,500	X		X
Lysozyme	14,400	X		X
Aprotinin	6,500			X

Contents Approximately 130 µg total protein in a buffer of 50% glycerol, 150 mM NaCl, 3 mM NaN_3

Volume 250 µl

Storage -20 °C

Shelf life 1 year at -20 °C

Applications Dilute 1:4 for HRP color development; 60–100 applications per vial

Dilute 1:20 for AP color development; 300–500 applications per vial

Protocol

1. When using HRP conjugates, dilute standards 1:4 in sample buffer.* When using AP conjugates, dilute the standards 1:20 in sample buffer. Heat for 5 minutes at 95 °C. Cool and load 10 µl/well for mini-gels. Load 10–15 µl/well for full length gels (16–20 cm).
2. After electrophoretic blotting of the proteins, detection of the biotinylated standards is performed after the blocking and primary antibody incubation steps. The avidin conjugates are used in a 1:3,000 dilution in antibody buffer (1% gelatin in TTBS†). This solution should contain the appropriate dilution of blotting grade second antibody conjugate, protein A, or protein G conjugate. Incubate the membrane 1 hour with gentle agitation at room temperature.
3. Remove the conjugate solution, and wash the membrane twice for 5 minutes in Tris buffered saline with 0.05% Tween-20 (TTBS)† with gentle agitation. Wash twice for 5 minutes in TBS.

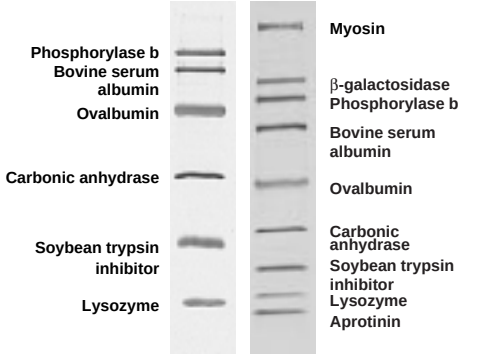

Fig. 1. Biotinylated SDS-PAGE Standards, low and broad range. A. Low range biotinylated SDS-PAGE standard run on a 12% gel, blotted to nitrocellulose, and detected with Avidin-HRP. **B.** Broad range biotinylated SDS-PAGE standards run on a 4–20% gradient gel, blotted to nitrocellulose, and detected with Avidin-AP.

4. Prepare the color development solution immediately before use. Immerse the membrane in the solution. Stop the development by washing the membrane in distilled water for 10 minutes. Change the water at least once during this time.

***Sample buffer (SDS-PAGE reducing buffer)**

Distilled water	4.0 ml
0.5 M Tris-HCl, pH 6.8	1.0 ml
Glycerol	0.8 ml
10% (w/v) SDS	1.6 ml
β-mercaptoethanol	0.4 ml
0.1% (w/v) Bromophenol blue	0.2 ml
	8.0 ml

Note: Addition of a reducing agent such as BME is important because there is no reducing agent in the buffer as supplied.

†Tris buffered saline (TBS)

(20 mM Tris, 500 mM NaCl, pH 7.5)

Tris base	4.84 g
NaCl	58.44 g

Dissolve Tris and NaCl in 1.8 L distilled water. Adjust the pH to 7.5 with HCl, and adjust the volume to 2 L with distilled water. For TTBS add 0.5 ml Tween-20 to 1 L of TBS (0.05% Tween-20).

Protein References

Protein	Reference
Rabbit muscle myosin	Woods, E. F., Himmelfarb, S. and Harrington, W. F., *J. Biol. Chem.*, **238**, 2374 (1963).
E. coli β-galactosidase	Fowler, A. V. and Zabin, I., *Proc. Nat. Acad. Sci. USA*, **74**, 1507 (1977).
Rabbit muscle phosphorylase b	Titani, K, et al., *Proc. Nat. Acad Sci. USA*, **74**, 4762 (1977).
Bovine serum albumin (BSA)	Brown, J. R., *Fed. Proc.,* **34,** 591 (1975).
Hen egg white ovalbumin	Warner, R. C., **Egg Proteins**, in The Proteins, Vol. IIA, p. 435, (Neurath, H. and Bailey, K. eds.) Academic Press, New York (1954).
Bovine carbonic anhydrase	Davis, R. P., **Carbonic Anhydrase**, in: The Enzymes, Vol. V, p. 545 (Boyer, P. D. ed.) Academic Press, New York (1971).
Soybean trypsin inhibitor	Wu, Y. V. and Scheraga, H. A., *Biochemistry*, **1**, 698 (1962).
Hen egg white lysozyme	Jolles, P., *Angew. Chem., Intl. Edit.*, **8**, 227 (1969).
Bovine pancreatic trypsin inhibitor (aprotinin)	Kassell, B. and Laskowski, M., *Biochem. Biophys. Res. Comm.*, **20**, 463 (1965).