BIOTINYLATED SDS MOLECULAR WEIGHT MARKERS

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INTRODUCTION

Protein blotting[1,2] to solid phase substrates has facilitated current methods of identifying and characterizing proteins in complex biological mixtures.

Biotinylated molecular weight markers have been developed for use as standards for the estimation of the molecular weights of blotted proteins.[3] Visualization is accomplished using streptavidin-peroxidase, eliminating the need to cut out separate lanes for general protein stains, and can be performed simultaneously with immunostaining procedures.

Sigma's Biotinylated SDS (Sodium dodecyl sulfate) Molecular Weight Standard Mixture contains six biotinylated proteins that have been blended to give approximately equal intensities when detected on nitrocellulose with Streptavidin-peroxidase (S 5512) and a color development reagent. In addition, our standards are compatible with several other conjugate detection systems including those using Streptavidin-alkaline phosphatase[4] and Streptavidin-sulforhodamine 101 (Texas Red).[5]

These standards may be used with either the modified Laemmli[6] procedure (Sigma Technical Bulletin MWS-877L) or the modified Weber and Osborn[7] procedure (Sigma Technical Bulletin MWS-877). The electrophoretic mobilities of the biotinylated standards used in this product are not significantly altered by biotinylation, but it is not recommended that these standards be used for precise determination of molecular weight.

REFERENCES:

1. Towbin, H., Staehelin, T., and Gordon, J., Proc. Natl. Acad. Sci. **76**:4350 (1979)
2. Burnette, W.N., Anal. Biochem. **112**:195 (1981)
3. Della-Penna, D., Christoffersen, R., Bennett, A., Anal. Biochem. **152**:329 (1986)
4. Billingsley, M., Pennypacker, K., Hoover, C., and Kincaid, R., Biotechniques **5**:31 (1987)
5. Berman, J., and Basch, R., J. Immunol. Meth. **36**:335 (1980)
6. Laemmli, U.K., Nature **227**:680 (1970)
7. Weber, K., and Osborn, M., J. Biol. Chem. **244**:4406 (1969)

BIOTINYLATED SDS MOLECULAR WEIGHT STANDARDS AND KIT

MW-SDS-100B KIT

-0°C

For molecular weight range 14,000—100,000
Contains: One vial of the Biotinylated Standard Mixture (SDS-6B)
One vial containing 0.5 mg of Streptavidin-peroxidase (S 5512)
One vial containing 2.5 g of 4-Cl-1-naphthol (C 8890)

Individual proteins included in the SDS-6B biotinylated mixture:

Biotinylated Protein	Approx. Molecular Weight	Reference for Amino Acid Sequence
Phosphorylase b (rabbit muscle)	97,400 (subunit)	Titani, K., et al., Proc. Nat. Acad. Sci. **74**:4762 (1977)
Catalase (bovine liver)	58,100 (subunit)	Schroeder, W.A., et al., Arch. Biochem. Biophys. **214**:397 (1982)
Alcohol Dehydrogenase (horse liver)	39,800 (subunit)	Jörnval, H., Eur. J. Biochem. **16**:25 (1970)
Carbonic Anhydrase (bovine erythrocytes)	29,000	Sciaky, M., et al., Biochimie **58**:1071 (1976)
Trypsin Inhibitor (soybean)	20,100	Koide, T., et al., Eur. J. Biochem. **32**:401 (1973)
Lysozyme (chicken egg white)	14,300	Canfield, R., J. Biol. Chem. **238**:2698 (1963)

Each vial contains approx. 0.1 mg of a lyophilized mixture of the above six proteins.

SOLUTIONS FOR ELECTROPHORESIS

Preparation of SDS polyacrylamide gels is described in Sigma Technical Bulletin MWS-877L (11% gels by a modified Laemmli[6] procedure) or Sigma Technical Bulletin MWS-877 (10% gels by a modified Weber and Osborn[7] procedure). These bulletins are available upon request.

Sample buffer:

(for modified Laemmli procedure)

0.063 M Tris-Cl, pH 6.75
10% (v/v) Glycerol
2% (w/v) SDS
5% (v/v) 2-Mercaptoethanol
0.001% (w/v) Bromphenol Blue

(for modified Weber and Osborn procedure)

0.1 M Sodium Phosphate, pH 7.0
6 M Urea
1% (w/v) SDS
1% (v/v) 2-Mercaptoethanol
0.015% Bromphenol Blue

ELECTROPHORESIS

Preparation of Standards:

Reconstitute the contents of the SDS-6B vial in 1.0 ml of fresh sample buffer and incubate at 37°C for 2 hours. Store reconstituted biotinylated standards in several aliquots at −20°C. Avoid repeated freeze-thaw cycles.

Sample Loading:

Optimal sample size will depend on the well size and gel thickness. (10 µl is sufficient for a well 6 mm wide and 1.5 mm thick.)

Electrophoresis:

Perform the SDS polyacrylamide gel electrophoresis as described in the instrument instruction manual. If using the modified Laemmli system (Technical Bulletin No. MWS-877L), note that when using gel concentrations of less than 11% some of the lower molecular weight standards will co-migrate with the tracking dye.

ELECTROPHORETIC TRANSFER

Transfer the proteins from the polyacrylamide gel to nitrocellulose (0.2 mm pore size) using an appropriate transfer apparatus. Nitrocellulose is offered in many pre-cut sizes in the Equipment section of our catalog.

The following transfer buffer (Towbin[1]) is recommended for blotting:

3.029 g Tris (T 1503) and 14.5 g Glycine (G 7126) are dissolved in 800 ml water (the pH should be approx. 8.3), then add 200 ml methanol.

SOLUTIONS FOR NITROCELLULOSE MEMBRANES

A. PHOSPHATE BUFFERED SALINE (PBS), pH 7.5
Prepare by combining:

Potassium phosphate, anhydrous, monobasic KH_2PO_4 (P 5379)	0.908 g
Potassium phosphate, anhydrous, dibasic K_2HPO_4 (P 8281)	2.32 g
Sodium Chloride (S 9625)	2.06 g

Dissolve and dilute to 2000 ml with water.
The pH should be approximately 7.5 at 25°C.
(Stable for at least 1 week when stored at 0-5°C.)

B. BLOCKING SOLUTION
Prepare solution by dissolving:

Bovine Serum Albumin (A 4503)	6.0 g

Dissolve in 200 ml of PBS (Solution A)
(Stable for at least 1 week when stored at 0-5°C.)

C. WASH SOLUTION (TPBS)
Prepare solution by combining:

Tween 20 (P 1379)	0.1 ml
PBS (Solution A)	200 ml

(Stable for at least 1 week when stored at 0-5°C.)

D. COUPLING SOLUTION
Prepare solution by combining:

Blocking Solution (Solution B)	50 ml
PBS (Solution A)	100 ml

(Prepare and use the same day.)

E. STREPTAVIDIN-HRP SOLUTION
Prepare solution by dissolving:

Streptavidin-peroxidase (S 5512)	0.5 mg

Dissolve in 0.5 ml of PBS (Solution A)
(Store in several aliquots at −20°C.)

F. 4-CHLORO-1-NAPHTHOL SOLUTION
Prepare solution by dissolving:

4-Chloro-1-naphthol (C 8890)	0.05 g

(Use due care!)
Dissolve in 15 ml of cold methanol
(Make fresh daily.)

G. COLOR DEVELOPMENT SOLUTION

Prepare solution by combining:

Cold 30% H_2O_2 (H 1009)	0.06 ml
PBS (Solution A at room temperature.)	85 ml
Solution F	15 ml

(Mix vigorously and use immediately.)

VISUALIZATION OF THE MARKERS

All steps performed at 25°C. In general use 0.5 ml of solution for each cm^2 of nitrocellulose. Note that antibody probes specific to the protein of interest can be added to the same solution as the streptavidin-peroxidase making it possible to do both reactions simultaneously (see Step 5).

1. Place nitrocellulose membrane into a plastic tray containing Blocking Solution (Solution B) and gently agitate, using a shaker platform, for 45 min.

2. Rinse twice by gentle agitation for 5 min each in TPBS (Solution C).

3. Transfer membrane into a tray containing Solution D. Add appropriate amount of antibody and incubate for 1 hr. with gentle agitation. (The antibody is for immundetection of the specific protein of interest in adjacent lanes. If immunochemical detection methods will not be used omit this step and go directly to step 5.)

4. Rinse twice by gentle agitation for 5 min each in TPBS (Solution C).

5. Transfer membrane into a tray containing Solution D. Add appropriate amount of secondary antibody (if any) and 0.01 ml of Streptavidin-HRP (Solution E) for each 40 ml of Solution D and incubate for 1 hr. with gentle agitation.

6. Rinse twice by gentle agitation for 5 min each in TPBS (Solution C).

7. Rinse for 5 min in PBS (Solution A).

8. Prepare Color Development Solution (Solution G). Immerse membrane in the solution and agitate gently for 30 min or until the desired color intensity has developed.

9. Stop development by immersion in dH_2O for 10 minutes with at least 1 change. Dry and store in the dark.

RESULTS

To determine the relative mobility (R_f) of a protein, divide its migration distance from the top of the gel to the center of the protein band by the migration distance of the Bromphenol Blue tracking dye from the top of the gel.

$$R_f = \frac{\text{distance of protein migration}}{\text{distance of tracking dye migration}}$$

The R_f values (abscissa) are plotted against the known molecular weights (ordinate) on semi-logarithmic paper.

Estimate the molecular weight of unknown protein from calibration curve.

NOTE: The typical calibration curve depicted in this bulletin cannot be used to derive laboratory test results. Each laboratory must prepare its own calibration curve.