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ProductInformation

Phosphorylase b, Cross-Linked
SDS Molecular Weight Marker

Product No. **P 8906**
Store at 2-8 °C
Technical Bulletin No. MWS-877X
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TECHNICAL BULLETIN

Product Description

Electrophoresis in polyacrylamide gels, in the presence of the anionic detergent sodium dodecyl sulfate (SDS), has proven to be a useful tool for the separation of protein subunits and the determination of their molecular weights.

The molecular weight of a given protein can be determined by comparing its electrophoretic mobility with known protein markers. A linear relationship is obtained if the logarithms of the molecular weights of standard polypeptide chains are plotted against their respective electrophoretic mobilities (Rf). The procedure for SDS molecular weight determination as outlined in this bulletin is a modification of the methods of Weber and Osborn[1] and Davies and Stark[2].

We have found that the cross-linked components produce ambiguous results in the Laemmli[3] procedure and do not recommend them for use in the Laemmli method.

Reagent

Phosphorylase b, cross-linked, Product No. P 8906, from rabbit muscle
1 mg/vial
Lyophilized powder
After SDS polyacrylamide gel electrophoresis six bands are produced. Trace bands (heptamer through nonamer) may also be detected. Relative band intensity decreases as molecular weight increases.

	Approx. mol. wt.
Monomer	97,400
Dimer	194,800
Trimer	292,200
Tetramer	389,600
Pentamer	487,000
Hexamer	584,000

Preparation Instructions

Note: This procedure will require a full day. Use distilled or deionized water.

A. Sample Buffer: Prepare solution by combining:

Sodium phosphate, anhydrous, monobasic (NaH_2PO_4), Product No. S 3522	0.34 g
Sodium phosphate, anhydrous, dibasic (Na_2HPO_4), Product No. S 3397	1.02 g
Sodium dodecyl sulfate (SDS; lauryl sulfate), Product No. L 3771	1.00 g
2-Mercaptoethanol, Product No. M 7154	1.00 ml
Bromophenol blue, Product No. B 0126	0.015 g
Urea, Product No. U 6504	36.00 g

Dissolve and dilute to 100 ml with water. The pH should be approximately 7.0 at 25 °C. Keep tightly capped at all times. Solution is stable for at least 2 weeks when stored in refrigerator at 2-8 °C.

B. Stock Buffer: Prepare solution by combining:

Sodium phosphate, anhydrous, monobasic (NaH_2PO_4), Product No. S 3522	6.80 g
Sodium phosphate, anhydrous, dibasic (Na_2HPO_4), Product No. S 3397	20.45 g
Sodium dodecyl sulfate (SDS; lauryl sulfate), Product No. L 3771	2.00 g

Dissolve and dilute to 1 liter with water. The pH should be approximately 7.0 at 25 °C. Store tightly capped in refrigerator at 2-8 °C. Suitable for use in absence of visible microbial growth.

C. Acrylamide Gels
7% Solution - Use as a stock solution to be diluted to prepare 3.5% gels for slab gel systems. All % gels are as % with respect to acrylamide monomer.

To prepare acrylamide solution, mix:

Acrylamide, Product No. A 3553	15.0 g
N,N'-Methylene-bis-acrylamide, Product No. M 7279	0.4 g

Dissolve and dilute to 100 ml with water. Remove insoluble material by filtration. Solution is stable for at least 1 month when stored in a dark bottle at 2-8 °C

D. N,N,N',N'-Tetramethylethylenediamine (TEMED), Product No. T 9281
Store at room temperature.

E. Ammonium Persulfate Solution
Dissolve approximately 100 mg ammonium persulfate, Product No. A 3678, in 15 ml water. Prepare fresh daily.

F. Brilliant Blue R Staining Solution, Product No. B 6529, 0.5% (w/v) dye in ethanol:acetic acid:water (9:2:9). Store at room temperature.

G. Destaining Reagent: Prepare solution by combining:

Methanol	400 ml
Glacial acetic acid	70 ml
Water	530 ml

H. Preparation of Sample
The unknown proteins are prepared by weighing out samples so that the concentrations are about 1 mg protein/ml sample buffer (Reagent A).

I. Preparation of Phosphorylase b Cross-linked SDS Molecular Weight Marker

Reconstitute the contents of the vial with 0.25 ml of sample buffer (Reagent A) to give an optimal concentration of 4 mg/ml. Mercaptoethanol must be present in the sample buffer in order to ensure dissolution of the markers.
Aliquots may be frozen at –20 °C for future use.

All proteins must be incubated at 37 °C for 1-2 minutes in sample buffer (Reagent A) prior to electrophoresis.

If the marker is not completely dissolved after incubation, continue incubation for an additional 1-2 minutes. If still not fully dissolved, add an additional 0.25 ml of sample buffer, being sure to apply twice as much marker solution to the gels.

Sample size is 15 µl/well for 14 x 15 cm slab gels, 5-10 µl/well for 8 x 10 cm slab gels.

J. Preparation of 3.5% Electrophoresis Slab Gels
The slab gel should have a thickness of at least 1.5 mm.

<u>To prepare a standard 14 x 16 cm slab gel:</u>
Note: Use half of the following volumes for a gel of 8 x 10 cm.

1. Mix 22.5 ml stock buffer (Reagent B), 10.5 ml of acrylamide gel (Reagent C) and 9.7 ml water. Deaerate for 1 minute with a water aspirator.

2. Deaerate 2.25 ml of freshly prepared ammonium persulate solution (Reagent E) for about 15 seconds and add to above.

3. Add 0.075 ml TEMED (Reagent D).

4. Mix solution carefully to avoid introducing air.

5. Carefully pour the solution between the slab plates to the top of the plates (approx. 35 ml).

6. As soon as the gel is poured, carefully place a comb into the solution between the plates.

7. Allow the gel to harden and carefully remove the comb.

Procedure

A. Procedure for Electrophoresis

Electrophoresis should be carried out at a constant current of 50 milliamps/slab for a 14 x 16 cm gel with positive electrode in lower chamber.

Note: Adjust the current accordingly for an 8 x 10 cm gel.

1. Dilute 1500 ml stock buffer (Reagent B) with 3000 ml water. For an 8 x 10 cm gel, dilute 500 ml stock with 1000 ml water.

2. Layer buffer solution on top of each well.

3. Underlay 15 µl sample in each well. Use 5-10 µl for an 8 x 10 cm gel.

4. Fill the anode and cathode reservoirs of the electrophoresis apparatus with diluted gel buffer from Step 1.

5. Apply constant current until the marker dye (bromophenol blue) is 1 cm from anodic end of gel (about 8-9 hours for a 14 x 16 cm gel, 4-5 hours for an 8 x 10 cm gel). It is essential that the marker dye migrate the specified distance in order to resolve the high molecular weight polymers of the molecular weight marker.

6. Remove the gel from the glass plates by squirting water from a syringe between the gel and glass wall and gently working the plates apart.

B. Staining and Destaining

1. Stain the gels in brilliant blue R staining solution (Reagent F) for at least 30 minutes.

2. Destain by diffusion against several changes of destaining solution (Reagent G) until the desired background is obtained.

3. Store the gels in destaining solution (Reagent G).

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 bromophenol 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.

References

1. Weber, K.,and Osborn, M., J. Biol. Chem. **244,** 4406 (1969)

2. Davies, G.E. and Stark, G.R., Proc. Nat. Acad. Sci. USA, **66**, 651 (1970)

3. Laemmli, U.K., Nature, **227**, 680 (1970)