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SDS MOLECULAR WEIGHT MARKERS IN A DISCONTINUOUS BUFFER

July 1988 Technical Bulletin No. MWS-877L

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INTRODUCTION

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. An approximately linear relationship is obtained if the logarithms of the molecular weights of standard polypeptide chains are plotted against their respective electrophoretic mobilities (R_f). The procedure for SDS molecular weight determination as outlined in this bulletin is a modification of the method of Laemmli[1]. The components in the MW-SDS-70L and MW-SDS-200 Molecular Weight Marker Kits are also useful when applied to the methods of Weber and Osborn[2] and Davies and Stark[3] as described in Technical Bulletin No. MWS-877.

MW-SDS-70L and MW-SDS-200 Kits

The proteins supplied in these kits have a molecular weight range common to most proteins and their subunits. These protein markers have been cited extensively in the literature and are characterized by a single band although myosin in the MW-SDS-200 Kit may demonstrate a second band remaining at the origin. Mixtures have been formulated to yield well-defined bands, which after SDS gel electrophoresis and staining with Brilliant Blue R (Coomassie Brilliant Blue R), Product No. B 0630, are approximately equal in color intensity. For general use, it is recommended that the MW-SDS-200 Kit be used according to the method described in Technical Bulletin No. MWS-877.

REFERENCES

1. Laemmli, U.K., Nature **227**:680 (1970)
2. Weber, K., Osborn, M., J. Biol. Chem. **244**:4406 (1969)
3. Davies, G., Stark, G., Proc. Nat. Acad. Sci. USA, **66**:651 (1970)

MOLECULAR WEIGHT PROTEIN STANDARDS AND KITS

MW-SDS-70L Kit For molecular weight range 14,000-70,000
[O-5°C] Contains one vial of each of the seven proteins below, 1 vial Stock No. SDS-7, and Technical Bulletin No. MWS-877L

INDIVIDUAL PROTEINS INCLUDED IN THE MW-SDS-70L KIT

Product No.	Proteins	Approx. Mol. Wt
A 7517	ALBUMIN, Bovine (25 mg/vial)	66,000
A 7642	ALBUMIN, Egg (Ovalbumin [25 mg/vial])	45,000
G 5262	GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE, Rabbit muscle (5 mg/vial)	36,000 (subunit)
C 2273	CARBONIC ANHYDRASE, Bovine Erythrocytes (5 mg/vial)	29,000
T 9011	TRYPSINOGEN, Bovine Pancreas, PMSF Treated (25 mg/vial)	24,000
T 9767	TRYPSIN INHIBITOR, Soybean (5 mg/vial)	20,100
L 6385	α-LACTALBUMIN, Bovine Milk (5 mg/vial)	14,200
Stock No. SDS-7	DALTON MARK VII-L vial contains 3.5 mg of a lyophilized mixture of the above seven proteins.	

MW-SDS-200 Kit For molecular weight range 30,000-200,000
[O-5°C] Contains one vial of each of the six proteins below, 1 vial Stock No. SDS-6H, and Technical Bulletin No. MWS-877.

INDIVIDUAL PROTEINS INCLUDED IN THE MW-SDS-200 KIT

Product No.	Proteins	Approx. Mol. Wt.
C 2273	CARBONIC ANHYDRASE, Bovine Erythrocytes (5 mg/vial)	29,000
A 7642	ALBUMIN, Egg (Ovalbumin, [25 mg/vial])	45,000
A 7517	ALBUMIN, Bovine Plasma (25 mg/vial)	66,000
P 4649	PHOSPHORYLASE B, Rabbit Muscle (0.5 mg/vial)	97,400 (subunit)
G 8511	β-GALACTOSIDASE, *Escherichia coli* (0.5 mg/vial)	116,000 (subunit)
M 3889	MYOSIN, Rabbit Muscle (0.5 mg/vial)	205,000 (subunit)
Stock No. SDS-6H	This vial contains 3.0 mg of a lyophilized mixture of the above six proteins.	

PREPARATION OF REAGENTS FOR USE WITH MW-SDS-70L and MW-SDS-200 KITS

NOTE: The procedure will require a full day. Use distilled or deionized water.

A. SEPARATING GEL BUFFER

Prepare solution by combining:

Tris, T 1503 ..... 36.3 g
N, N, N′, N′-Tetramethylethylenediamine, T 8133 ..... 0.23 ml

Dissolve and dilute to approximately 90 ml with water.
Adjust to pH 8.9 at 25°C with concentrated HCl.
Dilute with water to a final volume of 100 ml.
Solution is stable for at least 2 weeks when stored in refrigerator at 0-5°C.

B. STACKING GEL BUFFER

Prepare solution by combining:

Tris, T 1503 ..... 5.98 g
N, N, N′, N′-Tetramethylethylenediamine, T 8133 ..... 0.46 ml

Dissolve and dilute to 80 ml with water.
Adjust to pH 6.7 at 25°C with concentrated HCl.
Dilute with water to a final volume of 100 ml.
Solution is stable for at least 2 weeks when stored in refrigerator at 0-5°C.

C. SEPARATING GEL SOLUTION

Prepare solution by combining:

Acrylamide, A 8887 ..... 28.0 g
N, N′-Methylenebisacrylamide, M 7256 ..... 0.74 g

Dissolve and dilute with water to a final volume of 100 ml.
Remove insoluble material by filtration.
Solution is stable for at least 1 month when stored in a dark bottle in refrigerator at 0-5°C.

D. STACKING GEL SOLUTION

Prepare solution by combining:

Acrylamide, A 8887 ..... 10.0 g
N, N′-Methylenebisacrylamide, M 7256 ..... 2.5 g

Dissolve and dilute with water to a final volume of 100 ml.
Remove insoluble material by filtration.
Solution is stable for at least 1 month when stored in a dark bottle in refrigerator at 0-5°C.

E. SDS SOLUTION

Prepare solution by combining:

Sodium dodecyl sulfate
(SDS, Lauryl sulfate) L 5750 ..... 0.21 g

Dissolve and dilute with water to a final volume of 100 ml.
Solution is stable for at least 2 weeks when stored at 25°C.
(Note: Solution may become cloudy at temperatures below 20°C but clarity may be restored by warming to 25-30°C and mixing.)

F. 2X SAMPLE BUFFER

Prepare solution by combining:

Tris, T 1503 ..... 1.51 g
Glycerol, G 7757 ..... 20.0 ml

Dissolve with 35 ml of water.
Adjust pH to 6.75 with concentrated HCl, then add:

Sodium dodecyl sulfate
(SDS, Lauryl sulfate) L 5750 ..... 4.0 g
2-Mercaptoethanol, M 6250 ..... 10.0 ml
Bromphenol Blue, B 0126 ..... 0.002 g

Dilute with water to a final volume of 100 ml.
Solution is stable for at least 1 month when stored frozen at -20°C.

G. ELECTRODE BUFFER

Prepare solution by combining:

Tris, T 1503 ..... 6.05 g
Glycine, G 7126 ..... 28.8 g

Sodium dodecyl sulfate
(SDS, Lauryl sulfate) L 5750 ..... 2.0 g

Dissolve and dilute with water to a final volume of 2.0 liters.
Final pH should be about 8.3.

H. FIXATIVE SOLUTION

Prepare solution by combining:

Methanol ..... 400 ml
Glacial acetic acid ..... 70 ml
Water ..... 530 ml

I. STAINING REAGENT

Prepare solution by dissolving:

Brilliant Blue R (Coomassie Brilliant Blue R) B 0630 ..... 1.25 g

Dissolve in 500 ml Reagent H.
Store tightly capped at room temperature.
This reagent is stable for several months.

PREPARATION OF SAMPLE

The unknown proteins may be prepared by diluting an appropriate amount (0.05-1.0 ml) of protein solution at 2 mg/ml with an equal volume of 2X Sample Buffer (Reagent F). Protein solutions should not contain potassium nor high concentrations (greater than 0.2 Molar) salts; brief dialysis (2-4hrs) against 0.1% NaCl should render the solution suitable for dilution with the 2X Sample Buffer.

Alternatively, the unknown proteins may be prepared by desalting, lyophilizing, and dissolving a weighed sample with 2X Sample Buffer diluted 1:1 with water to give 1 mg/ml.

PREPARATION OF SDS MOLECULAR WEIGHT MARKERS

Reconstitute contents of vial with volume of 1X Sample Buffer (Reagent F diluted with an equal volume of water) indicated below.

Protein	Optimum Concentration (mg/ml)	Amount of 1X Sample Buffer (ml)
Albumin, Bovine	0.71	35
Albumin, Egg	1.0	25
Glyceraldehyde-3-Phosphate dehydrogenase	0.67	7.5
Carbonic Anhydrase	0.5	10
Trypsinogen	1.0	25
Trypsin Inhibitor, Soybean	1.0	5
α-Lactalbumin	0.5	10
SDS-7		1.5

Carbonic Anhydrase	0.5	10
Albumin, Egg	1.0	25
Albumin, Bovine	0.71	35
Phosphorylase b	0.5	1
β-Galactosidase	0.5	1
Myosin	1.25	0.4
SDS-6H		1.5

All proteins must be incubated at 100°C for 60 seconds in Sample Buffer prior to electrophoresis. Aliquots may be frozen at -20°C for future use.
Sample size 10μl per gel.

PREPARATION OF ELECTROPHORESIS GELS

The gel tubes should have an inner diameter of 0.5 cm and be long enough to hold a 10 cm gel.

1. a) To prepare 12 of the 11% gels for use with proteins having molecular weights of 14,000-70,000, mix the following:
   - 3 ml Separating Gel Buffer (Reagent A)
   - 9.5 ml Separating Gel Solution (Reagent C)
   - 11.5 ml SDS Solution (Reagent E) containing 17 mg of Ammonium Persulfate (A 6761) freshly prepared.

   b) To prepare 12 of the 7% gels for use with proteins having molecular weights of 30,000-200,000, mix the following:
   - 3 ml Separating Gel Buffer (Reagent A)
   - 6 ml Separting Gel Solution (Reagent C)
   - 11.5 ml SDS Solution (Reagent E) containing 17 mg of Ammonium Persulfate (A 6761) freshly prepared.
   - 3.5 ml Water

2. Mix solution thoroughly.

3. Carefully dispense 1.6 ml of solution into each gel tube.

4. Before the gel hardens, carefully layer about 0.05 ml water on top of each gel solution without disturbing the surface.

5. Once polymerization has occurred, which should take about 15 minutes, rinse the tops of the gels with about 0.5 ml water three times.

6. To prepare the stacking gel, mix the following:
   - 1 ml Stacking Gel Buffer (Reagent B)
   - 2 ml Stacking Gel Solution (Reagent D)
   - 4 ml SDS Solution (Reagent E)
   - 1 ml water containing 8 mg of Ammonium Persulfate (A 6761) freshly prepared.

7. Rinse the tops of the gels with approximately 0.25 ml of stacking gel solution.

8. Remove as much of the stacking gel solution from the tube as possible then apply 0.20 ml of fresh stacking gel.

9. Before the gel hardens carefully layer about 0.05 ml water on top of each gel solution without disturbing the surface.

10. Once polymerization has occurred, which should take about 15 minutes and will be indicated by the gel becoming white and opaque, rinse the tops of the gels with a few drops of water.

ELECTROPHORESIS

Electrophoresis should be carried out at constant current of 1 milliamp/gel with positive electrode in lower chamber

1. Decant water from top of gel and layer Electrode Buffer (Reagent G) on top of each gel to fill tube.
2. Underlay 10 μl sample on gel.
3. Apply constant current at 1 milliamp/gel until marker dye (Bromphenol Blue) is 1 cm from anodic end of gel (about 5 hours).
4. Remove gels from tubes by squirting water from a syringe between the gel and glass wall, then use a pipet ball to exert pressure.

STAINING AND DESTAINING

1 Mark center of Bromphenol Blue dye front with a piece of fine wire.

2. Immerse gels in fixative solution (Reagent H) for at least 10 hours. (It is recommended that several changes of fixative solution be made.)
3. Stain gels in staining reagent (Reagent I) for at least 3 hours Overnight staining is preferred.

4 Destain gels by diffusion against several changes of Reagent H with destaining time in Reagent H to be at least 15 hours but not to exceed 25 hours. Gels may then be transferred to 7% Glacial acetic acid for storage, scanning, or measurement. (Gels will swell somewhat in about 3 hours.) Extended destaining with Reagent H will lead to decolorization of some protein bands.

5. Record the migration distance of tracking dye and of the blue protein zones from the top of the Separating Gel.

RESULTS

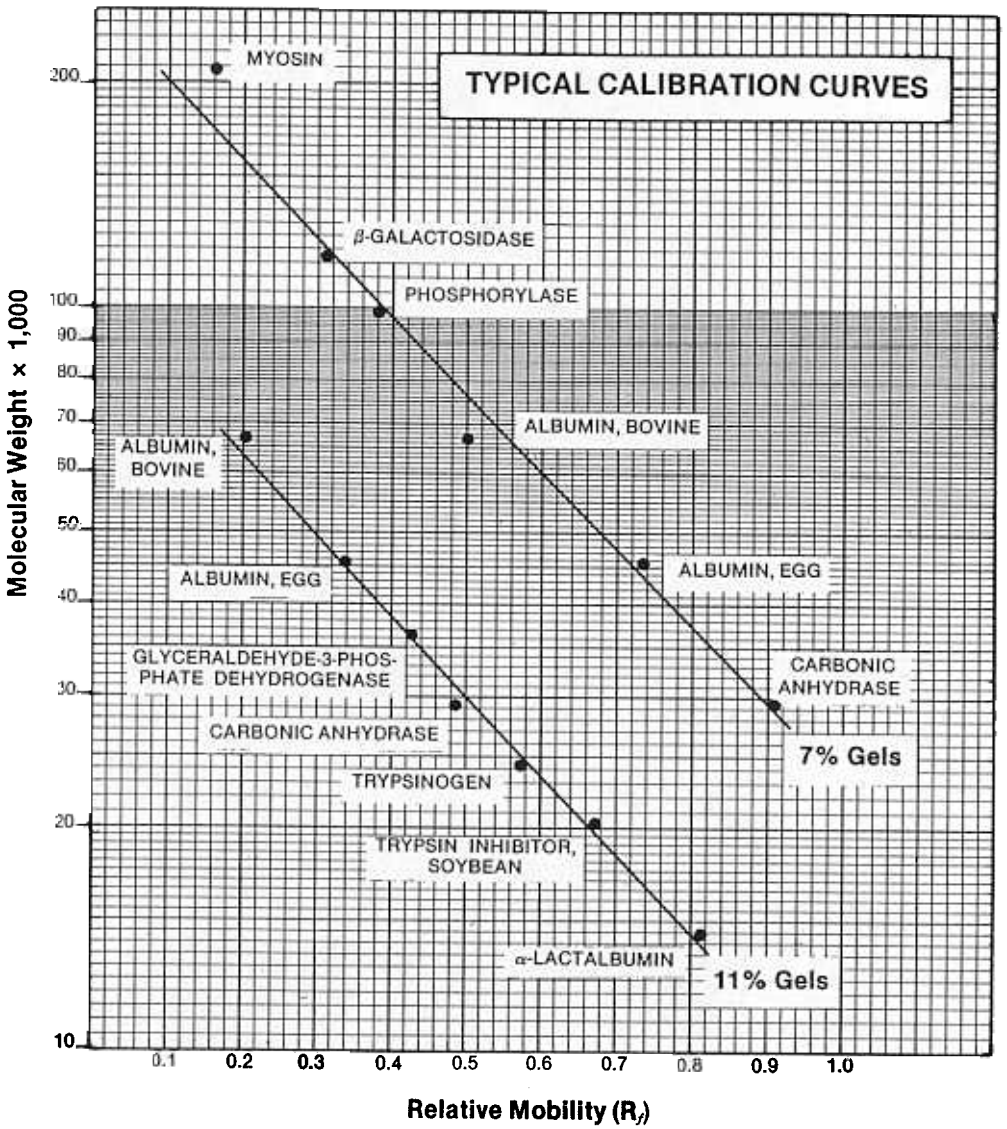
To determine the relative mobility (R_f) of a protein, divide its migration distance from the top of the Separating Gel to the center of the protein band by the migration distance of the Bromphenol Blue tracking dye from the top of the Separating Gel.

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

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



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