SIGMA®
ELECTROPHORESIS

SDS MOLECULAR WEIGHT MARKERS
2,500-17,000 Daltons

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INTRODUCTION

Electrophoresis in polyacrylamide gels in the presence of sodium dodecyl sulfate (SDS), an anionic detergent, is useful for separating protein subunits and determining their molecular weights.

The molecular weight of a protein can be determined by comparing its electrophoretic mobility with those of known protein markers. An approximately linear relationship is obtained if logarithms of the molecular weights of standard polypeptide chains are plotted against their respective electrophoretic mobilities.

Two procedures for SDS molecular weight determination are described in this bulletin. Procedure No. 1 is a modification of the discontinous procedure of Schägger and von Jagow.[1] Procedure No. 2 is a modification of the continuous procedure of Swank and Munkres.[2]

Recent studies have lead to a reassignment of the observable fragments resulting from cleavage of horse heart myoglobin.[3] Molecular Weight Marker Kit, Catalog No. MW-SDS-17S, has been developed to provide a useful molecular weight determination range between 2,500-17,000 when using either the discontinous electrophoresis system reported in 1987 by Schägger and von Jagow, or the earlier continuous system of Swank and Munkres.

The discontinuous procedure (Procedure No. 1) has been shown in our laboratories to yield consistent resolution of polypeptides with molecular weights below 6,000; it also yields less than 25% average deviation from known molecular weight standards in the range 2,500-6,000. In this range unique structural features have an increasingly important effect on observed electrophoretic mobility and discrepancies in excess of 50% have been observed.

When using the continuous procedure (Procedure No. 2) we have observed substantial discrepancies using polypeptides in the 2,500-6,000 range and have also observed poorer band definition. Another direct method should be used to confirm molecular weights.

The reagent in the Molecular Weight Marker Kit, Catalog No. MW-SDS-17S, is recommended for use only with the methods outlined in this technical bulletin.

REAGENT

The Molecular Weight Marker reagent is a mixture of seven polypeptides, six derived from the cleavage of myoglobin plus naturally occurring glucagon, which has been added after neutralization of the cleavage conditions. The cleavage forms polypeptides of accurately known structures and hence precise molecular weights. The mixture yields well defined bands which, after electrophoresis and staining, are approximately equal in color intensity.

For molecular weight determination in the range 2,500-17,000.
Contains one vial of a mixture of the 7 polypeptides listed below (2.2 mg/vial)

MYOGLOBIN FRAGMENTS

	Fragment II		Fragment I		Fragment III	
Amino Acid Residue No.	1		55		131	153
Molecular Wt.		6,210		8,160		2,510

	Mol. Wt.
Myoglobin (Polypeptide backbone 1-153)	16950
Myoglobin (I+II, 1-131)	14440
Myoglobin (I+III, 56-153)	10600
Myoglobin (I, 56-131)	8160
Myoglobin (II, 1-55)	6210
Glucagon (Product No. G 4250)	3480
Myoglobin (III, 132-153)*	2510

*Not detected using continuous Procedure No. 2

REAGENTS REQUIRED BUT NOT PROVIDED:

Reagent	Product No.
Acrylamide	A 3553
Ammonium Persulfate	A 3678
Brilliant Blue R	B 0149
Brilliant Blue G	B 0770
Bromphenol Blue	B 6131
Glycerol	G 8773
Lauryl Sulfate, Sodium	L 3771
2-Mercaptoethanol	M 7154
N,N'-Methylene-bis-Acrylamide	M 7279
5-Sulfosalicylic Acid	S 3147
Trizma Base	T 8404
Trichloroacetic Acid	T 4885
Tricine	T 6272
N,N,N',N'-Tetramethylenediamine (TEMED)	T 9281
Urea	U 6504
Methanol	M 3641
Glacial Acetic Acid	A 6283
Concentrated Phosphoric Acid	P 6560

PROCEDURE NO. 1

DISCONTINUOUS PROCEDURE OF SCHÄGGER AND VON JAGOW

Visualizing components	2,500-17,000
Working Range	2,500-17,000

REAGENT PREPARATION

HPLC grade or deionized water is recommended for all solutions described below. Solutions are adjusted for pH at 25°C.

ACRYLAMIDE SOLUTION

Prepare solution by combining:

	Product No.	
Acrylamide	A 3553	48 g
N,N'-Methylene-bis-Acrylamide	M 7279	1.5 g

Dissolve in water to a final volume of 100 ml. Solution is stable one month if stored at 4°C in an amber bottle. Gentle warming may be required for complete dissolution after refrigeration.

GEL BUFFER

Trizma Base	T 8404	36.34 g
Lauryl Sulfate, Sodium (SDS, Sodium Dodecyl Sulfate)	L 3771	0.30 g

Dissolve in 60 ml water. Use gentle warming if required. Adjust pH to 8.45 with concentrated HCl. Make up to final volume of 100 ml with water.

SDS STOCK SOLUTION, 20%

Lauryl Sulfate, Sodium (SDS, Sodium Dodecyl Sulfate)	L 3771	10.0 g

Dissolve in 50 ml water. Warm gently to completely dissolve. Stable approximately two weeks at room temperature. Solution may become cloudy below 20°C but clarity can be restored by warming to 30°C and mixing gently.

TRIS•HCl, 1 M, pH 6.8

Trizma Base	T 8404	12.1 g

Dissolve in 80 ml water. Adjust pH to 6.8 with concentrated HCl. Make up to final volume of 100 ml with water.

(E) SAMPLE BUFFER

Prepare solution by combining:

	Product No.	
20% SDS (Solution C)		4.0 ml
Glycerol	G 8773	2.4 ml
2-Mercaptoethanol	M 7154	0.4 ml
Brilliant Blue G	B 0770*	2.0 mg
Tris•HCl, 1 M, pH 6.8 (Solution D)		1.0 ml

Make up to final volume of 20.0 ml with water.
*Serves as marker dye.

AMMONIUM PERSULFATE SOLUTION

Prepare fresh daily. Dissolve 100 mg Ammonium Persulfate, Product No. A 3678, in 1.0 ml of water.

(G) ANODE BUFFER

Trizma Base	T 8404	121.1 g

Dissolve in 1.0 L water. Adjust to pH 8.9 with concentrated HCl. Make up to final volume of 5.0 L with water.

CATHODE BUFFER

Prepare solution by combining:

Trizma Base	T 8404	12.11 g
Tricine	T 6272	17.92 g
Lauryl Sulfate, Sodium (SDS, Sodium Dodecyl Sulfate)	L 3771	1.0 g

Dissolve in 1 L water. The pH of the solution should be approximately 8.2.

(I) FIXATIVE SOLUTION

Prepare solution by combining:

Methanol	M 3641	50 ml
Glacial Acetic Acid	A 6283	10 ml

Make up to 100 ml with water.

STAINING SOLUTION

Brilliant Blue G	B 0770	50 mg

Dissolve in 200 ml 10% Glacial Acetic Acid. Stir for 30 minutes and filter. Stain may be used twice.

DESTAINING SOLUTION

Glacial Acetic Acid	A 6283	100 ml

Combine with 900 ml water. Solution should be discarded after one use.

SAMPLE PREPARATION

Prepare unknown samples at a concentration of 1 mg/ml in Sample Buffer (Solution E).

Preparation Of Molecular Weight Markers Solution

Reconstitute Molecular Weight Marker Vial, Catalog No. MW-SDS-17S, with 2.2 ml of Sample Buffer (Solution E).

Treatment Of Samples Prior To Electrophoresis

Incubate all samples and Molecular Weight Markers Solution for 30 minutes at 40°C. Allow to cool to room temperature.

PREPARATION OF ELECTROPHORESIS GELS

	Stacking Gel Soln (1-2 cm) 4%T 3%C*	Spacer Gel Soln (2-3 cm) 10%T 3%C*	Separating Gel Soln (10 cm) 16.5%T 3%C*
Acrylamide Solution (Solution A)	1.0 ml	6.1 ml	10.0 ml
Gel Buffer (Solution B)	3.1 ml	10.0 ml	10.0 ml
Glycerol			3.2 ml
Water	8.4 ml	13.9 ml	6.8 ml

$$\%T = \frac{[\text{Acrylamide (g)} + \text{Bis-Acrylamide (g)}] \times 100}{100 \text{ ml}}$$

$$\%C = \frac{\text{Bis-Acrylamide (g)} \times 100}{\text{Acrylamide (g)} + \text{Bis-Acrylamide (g)}}$$

PROCEDURE

Pipet solutions as indicated above. Deaerate.

2. Complete the preparation of Separating Gel Solution by adding 0.1 ml Ammonium Persulfate (Solution F) and 0.01 ml TEMED, Product No. T 9281. Mix with gentle swirling.
3. Complete the preparation of Spacer Gel Solution by adding 0.1 ml Ammonium Persulfate (Solution F) and 0.01 ml TEMED, Product No. T 9281. Mix with gentle swirling.
4. Quickly pour Separating Gel Solution to a height of 10 cm. Carefully overlay with Spacer Gel Solution another 2 cm. This operation should be completed within 15 minutes. Finally, overlay the Spacer Gel carefully with water.
5. After separating and spacer gels have polymerized, remove the water overlay. Add 0.1 ml Ammonium Persulfate (Solution F) and 0.01 ml TEMED, Product No. T 9281, to 12.5 ml of Stacking Gel Solution. Mix by gentle swirling and pour onto the spacer gel.
6. Insert the well-forming comb into the stacking gel, ensuring there is space between the base of the wells and the spacer layer.
7. After the gel has set, allow to equilibrate by leaving overnight at 4°C.

ELECTROPHORESIS

1. Rinse wells with water, then with Cathode Buffer (Solution H).
2. Underlay 10 µl of Molecular Weight Markers Solution per well using a Hamilton microliter syringe, Product No. S 8891. Load the samples. For small proteins (2.5-5 KDa) an amount of 5-10 µg should be applied because of partial loss during the staining-destaining procedure.
3. Apply constant current of 20 mA for 1 hour, allowing the samples to completely enter the stacking gel. Then increase the current to 30 mA and maintain throughout the remainder of the run, typically 16 hours.
4. Apply current until the marker dye is within 1 cm of the anodic end of the gel.
5. Remove the gel carefully and immerse in the Fixative Solution (Solution I) for:

   30 minutes for 0.75 mm gels
   60 minutes for 1.5 mm gels
6. Transfer to Staining Solution (Solution J) for 1-2 hours depending on thickness of gel (1.5 mm = 2 hours).
7. Transfer to Destaining Solution (Solution K) for 2 hours, renewing the solution every 30 minutes.
8. After the destaining stage the gel is ready for visualization, analysis or photography. After lengthy exposure in the Destaining Solution there can be fading, particularly of low molecular weight species.

CALCULATIONS

To determine the relative mobility (Rf) of a polypeptide, divide its migration distance (from top of gel to center of the polypeptide band) by the migration distance of the marker dye from top of gel.

$$Rf = \frac{\text{Polypeptide Migration Distance}}{\text{Marker Dye Migration Distance}}$$

Plot the Rf values (abscissa) against the known molecular weights (ordinate) on semi-logarithmic graph paper. An inflection point occurs at the 6210 molecular weight polypeptide (see graph on p. 10). Estimate the molecular weight of the unknown polypeptide from your 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.

PROCEDURE NO. 2

CONTINUOUS PROCEDURE OF SWANK & MUNKRES

Visualizing components	3,450-17,000
Working range	6,000-17,000

REAGENT PREPARATION

HPLC grade or deionized water is recommended for all solutions described below. Solutions are adjusted for pH at 25°C.

(A) ACRYLAMIDE SOLUTION

Prepare solution by combining:

	Product No.	
Acrylamide	A 3553	50 g
N,N'-Methylene-bis-Acrylamide	M 7279	5 g
Urea	U 6504	192 g

Dissolve in water to a final volume of 352 ml. Solution is stable at least one month at room temperature.

(B) TRIZMA BASE, 1 M

Prepare solution by dissolving 121.1 grams of Trizma Base, Product No. T 8404, in 800 ml water, then dilute to a final volume of 1.0 liter with water. (A 1:10 dilution of a portion of this solution will be useful later.) Suitable for use in the absence of visible microbial growth.

(C) SAMPLE BUFFER

Prepare solution by combining:

Lauryl Sulfate, Sodium (SDS, Sodium Dodecyl Sulfate)	L 3771	2.0 g
Urea	U 6504	96.1 g

Dissolve in 100 ml water, then add:

2-Mercaptoethanol	M 7154	2.0 ml
Concentrated Phosphoric Acid (H_3PO_4) (Sp.gr. 1.75)	P 6560	0.125 ml

Titrate to pH 6.8 with a 0.1 M solution of Trizma Base (see Solution B) and dilute to 200 ml with water. Suitable for use in the absence of visible microbial growth.

(D) STOCK BUFFER

Prepare solution by combining:

Lauryl Sulfate, Sodium (SDS, Sodium Dodecyl Sulfate)	L 3771	10.0 g
Trizma Base	T 8404	200 g

Dissolve in 650 ml water and add:

Concentrated Phosphoric Acid	P 6560	62.5 ml

Addition of the acid causes an elevation in temperature which affects the pH of the buffer. The solution should be allowed to cool to room temperature before making the final pH adjustment.

Adjust the pH of the Stock Buffer to 6.8 with Trizma Base, 1 M (Solution B). Finally, make the volume of the solution up to 1.0 liter with water. Suitable for use in the absence of visible microbial growth.

(E) N,N,N',N'-TETRAMETHYLETHYLENEDIAMINE SOLUTION (TEMED)

Prepare fresh daily.

Dilute 0.3 ml of TEMED, Product No. T 9281, to 4.0 ml with water.

AMMONIUM PERSULFATE SOLUTION

Prepare fresh daily.

Dissolve approximately 200 mg Ammonium Persulfate, Product No. A 3678, in 10 ml water.

FIXATIVE SOLUTION

Prepare by combining

	Product No.	
5-Sulfosalicylic Acid	S 3147	15 g
Trichloroacetic Acid	T 4885	50 g

Dissolve and dilute to 500 ml with water.

STAINING SOLUTION

Brilliant Blue R (Coomassie Brilliant Blue R)	B 0149	125 mg

Dissolve in 500 ml of Solution I (see below) and filter before use. The reagent is stable for several months stored tightly capped at room temperature.

(I) DESTAINING SOLUTION

Prepare solution by combining:

Methanol	M 3641	50 ml
Glacial Acetic Acid	A 6283	75 ml

Dilute to 1 liter with water. Solution should be discarded after one use. Store unused solution tightly capped at room temperature.

(J) SATURATED BROMPHENOL BLUE SOLUTION

Suspend 40 mg of Bromphenol Blue, Product No. B 6131, in 10 ml of water. May be stored at room temperature for 3-4 months.

SAMPLE PREPARATION

Prepare unknown samples at a concentration of 1 mg/ml in Sample Buffer (Solution C).

Preparation Of Molecular Weight Markers Solution

Reconstitute vial contents with 2.2 ml of Sample Buffer (Solution C).

Treatment Of Samples Prior To Electrophoresis

Incubate samples and Molecular Weight Markers Solution at 60°C for 15 minutes and allow to cool to room temperature. Add 50 µl of Saturated Bromphenol Blue Solution (Solution J) to each sample. Aliquots may be frozen at −20°C for future use. Sample size = 10–25 µl per well.

PREPARATION OF ELECTROPHORESIS GELS, 12.5% IN ACRYLAMIDE

Sufficient Gel Solution for the required number and size of gels may be prepared as shown below. (40 ml is sufficient for one standard slab gel 16 cm x 16 cm x 1.5 mm).

Prepare as follows:

1. Mix: Acrylamide Solution (Solution A) 35.2 ml
   Stock Buffer (Solution D) 4.0 ml
   Deaerate for 1 minute under vacuum.
2. Add: TEMED (Solution E) 0.4 ml
   Ammonium Persulfate Solution (Solution F) 0.4 ml
   Mix solution carefully to avoid introducing air.
3. Dispense solution into gel apparatus.
4. Insert well-forming comb.

ELECTROPHORESIS

Electrophoresis should be carried out at constant current. For typically sized gel (16 cm x 16 cm x 1.5 mm), a constant current of 70 mA/gel is used. Alternatively, samples may be run overnight at a reduced current of 25 mA/gel.

1. Dilute 500 ml of Stock Buffer (Solution D) to 5 liters with water.
2. Remove comb, rinse wells with water and then with diluted Stock Buffer Solution, from Step 1 above.
3. Underlay sample into wells.
4. Fill compartments of electrophoresis apparatus with diluted Stock Buffer from Step 1 above.
5. Apply required constant current until marker dye (Bromphenol Blue) is within 1 cm of anodic end of gel.
6. Remove gel carefully from the glass plates.
   Caution: Gels are quite brittle; use due care.

STAINING AND DESTAINING

1. Mark the center of the Bromphenol Blue dye front.
2. Immerse gel in Fixative Solution (Solution G) for 1-2 hours.
3. Stain gel in Staining Solution (Solution H) for at least 4 hours. Overnight staining is preferred and will yield better results.

4. Transfer to Destaining Solution (Solution I) for 2 hours, renewing the solution every 30 minutes. Efficiency may be increased by performing the destaining procedure in a water bath at 60°C.
5. Record migration distances of the tracking dye and the blue protein bands from the top of the gel.
6. Gels can be stored in Destaining Solution (Solution I).

CALCULATIONS

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

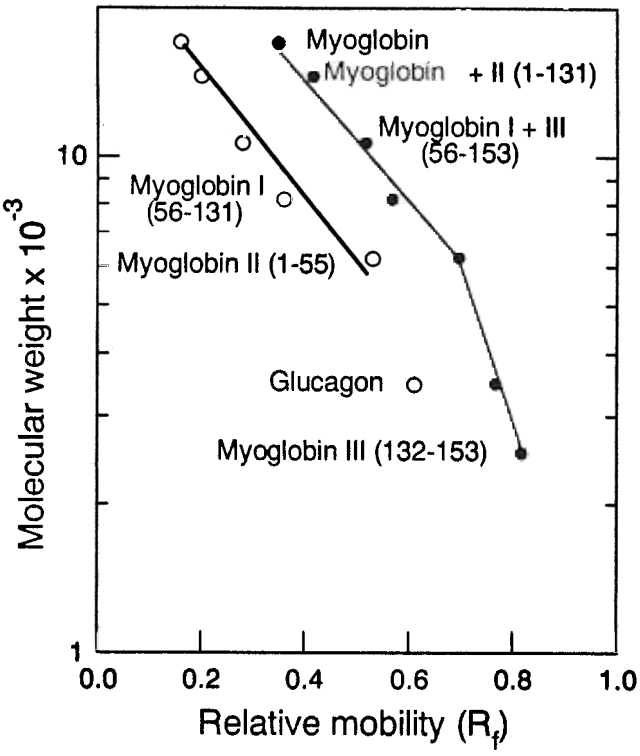
$$Rf = \frac{\text{Polypeptide Migration Distance}}{\text{Marker Dye Migration Distance}}$$

Plot the Rf values (abscissa) against the known molecular weights (ordinate) on semi-logarithmic paper (see graph below).

Estimate the molecular weight of the unknown polypeptide from your calibration curve.

TYPICAL CALIBRATION CURVE

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



Calibration curves typically obtained with peptides from the MW-SDS-17S Kit.
Procedure No. 1, Discontinuous Procedure of Schägger and von Jagow (●——●).
Procedure No. 2, Continuous Procedure of Swank and Munkres (o——o).

Catalog No.		Quantity
MW-SDS-17S [0-5°C]	**MOLECULAR WEIGHT MARKERS For SDS Gel Electrophoresis** Mixture of seven polypeptides, six derived from the cleavage of myoglobin, plus the naturally occurring glucagon which has been added after neutralization of the cleavage conditions.	1 vial

REFERENCES

1. Schägger, H., & von Jagow, G., Anal. Biochem. **166:** 368-379 (1987)
2. Swank, R.T., & Munkres, K.D., Anal. Biochem. **39:** 462-477 (1971)
3. Kratzin, H.D., Wiltfang, J., Karas, M., Neuhoff, V. and Hilschmann, N., Anal. Biochem., **183:** 1-8 (1989)