ProductInformation

MOLECULAR WEIGHT MARKER FOR PEPTIDES
For SDS Polyacrylamide Gel Electrophoresis
Molecular Weight Range: 2,500-17,000

Product No**. MW-SDS-17S**
Store at 2-8°C

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 that of standard proteins having known molecular weights. The relationship of the log molecular weight of a series of proteins with their electrophoretic mobility's (Rf) is approximately linear.

Studies have lead to a reassignment of the observable fragments resulting from the cleavage of horse heart myoglobin[1]. The Molecular Weight Marker for Peptides has been developed to provide a useful molecular weight determination range between 2,500-17,000 when using the discontinuous electrophoresis system of Schägger and von Jagow[2], or a range of 6,000-17,000 when using a modification of the earlier continuous system of Swank and Munkres[3].

The discontinuous procedure of Schägger and von Jagow has been shown in our laboratories to give consistent resolution of polypeptides with molecular weights below 6,000, when compared to the modified Swank-Munkres procedure. The Schägger-von Jagow procedure gives less than 25% average deviation from known molecular weight standards in the range 6,000-2,500, although deviations of >50% have been observed with some peptides. In the mol. wt. 6,000-2,500 range, unique structural features have an increasingly important effect on observed electrophoretic mobility.

When using the continuous procedure of Swank and Munkres, we have observed substantial discrepancies using polypeptides in the 6,000-2,500 range and have also observed poorer band definition. Another direct method should be used to confirm molecular weights below 6,000.

The Molecular Weight Marker for Peptides 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 of myoglobin forms polypeptides of accurately known structures and therefore precise molecular weights. The mixture yields well-defined bands that, after electrophoresis and staining, are approximately equal in color intensity.

Myoglobin Fragments

	Fragment II		Fragment I		Fragment III	
Amino acid residue no.	1		55		131	153
Molecular weight		6,210		8,160		2,510

Component
Contains one vial of a mixture of the seven polypeptides listed below, 2.2 mg/vial

Myoglobin Fragments	Mol. Wt.
Myoglobin (Polypeptide backbone 1-153)	16,950
Myoglobin (I + II, 1-131)	14,440
Myoglobin (I + III, 56-153)	10,600
Myoglobin (I, 56-131)	8,160
Myoglobin (II, 1-55)	6,210
Glucagon	3,480
Myoglobin (III, 132-153)*	2,510

* Not detected using Swank and Munkres procedure.

Instructions for the Schägger-von Jagow Procedure

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

Preparation Instructions

Sample Buffer: Prepare solution by combining

20% SDS, Product No. L3771	4.0 ml
Glycerol, Product No. G7757	2.4 ml
2-Mercaptoethanol, Product No. M7154	0.4 ml
Brilliant Blue G, Product No. B0770	2.0 mg
1 M Tris HCl, pH 6.8	1.0 ml

Dilute with water to a final volume of 20.0 ml. The brilliant blue G serves as the marker dye for electrophoresis.

Preparation of Molecular Weight Marker and Samples

Reconstitute the vial of Molecular Weight Marker for Peptides with 2.2 ml of Sample Buffer to give a recommended concentration of 1 mg/ml.

Prepare unknown samples at a concentration of 1 mg/ml in Sample Buffer.

Incubate all samples and the Molecular Weight Marker at 40°C for 30 minutes. Allow samples and marker to cool to room temperature prior to loading on the gel.

Recommended gel loading volume is 10 µl for the Molecular Weight Marker.

Procedure

Procedure for Electrophoresis

SDS-polyacrylamide gels can be made following the method outlined in the Schägger-von Jagow[2] procedure or using the manufacturer's recommended protocols for the electrophoresis unit. Tris-tricine precast gels can also be purchased from Sigma for convenience (Product Nos. P3466 and P8091). Sigma's precast gels are inexpensive, reproducible and save the time necessary to cast a gel. After loading the Molecular Weight Marker for Peptides and unknown samples on the gel, run electrophoresis on the gel at a recommended 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. Stop the electrophoresis when the dye front is within 1 cm of the bottom of the gel. Mark the dye front on the gel by making a small hole with a glass disposable pipette.

Fixing, Staining and Destaining

Remove the gel carefully and immerse in a fixative solution (50% methanol, 10% acetic acid). Fix 0.75 mm thickness gels for 30 minutes and 1.5 mm thickness gels for 60 minutes. Stain the fixed gel for 1-2 hours in Brilliant Blue R stain (0.025% Brilliant Blue R in 10% acetic acid). Destain the gel using 10% acetic acid until the background reaches the desired clarity. Several changes of destaining solution will be necessary. After destaining, the gel is ready for visualization, analysis and photography. See Results section for standard curve preparation.

Note: After lengthy exposure in the destaining solution there may be fading of bands, particularly of low molecular weight species.

Instructions for the Modified Swank-Munkres Procedure

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

Preparation Instructions

1. Acrylamide solution: Prepare solution by combining:

Acrylamide, Product No. A8887	50 g
N,N'-Methylene-bis-acrylamide, Product No. M7279	5 g
Urea, Product No. U6504	192 g

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

2. 1 M Trizma buffer: Prepare solution by dissolving 121.1 g of Trizma Base, Product No. T1503, 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 is used to adjust the pH of Sample Buffer (Solution 3). Suitable for use in the absence of visible microbial growth

3. Sample Buffer: Prepare solution by combining

Lauryl sulfate, sodium, Product No. L3771	2.0 g
Urea, Product No. U6504	96.1 g

   Dissolve in 100 ml water, then add:

2-Mercaptoethanol, Product No. M7154	2.0 ml
Concentrated phosphoric acid (H_3PO_4)	0.125 ml

   Titrate to pH 6.8 with 0.1 M Trizma buffer (1:10 dilution of 1 M Trizma buffer) and dilute to 200 ml with water. Suitable for use in the absence of visible microbial growth.

4. Stock Buffer: Prepare solution by combining

Lauryl sulfate, sodium, Product No. L3771	10.0 g
Trizma Base, Product No. T1503	200 g

   Dissolve in 650 ml water, then add:

Concentrated phosphoric acid (H_3PO_4)	62.5ml

   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 1 M Trizma Buffer. After adjusting the pH, bring the volume of the solution up to 1.0 liter with water. Suitable for use in the absence of visible microbial growth.

5. TEMED Solution: Dilute 0.3 ml of N,N,N',N'-tetra-methylethylenediamine, Product No. T8133, to 4.0 ml with water.

6. Ammonium Persulfate Solution: Dissolve approx. 200 mg ammonium persulfate, Product No. A3678, in 10 ml water. Prepare fresh daily.

7. Fixative Solution: Prepare by combining:

5-Sulfosalicylic acid, Product No. S2130	15 g
Trichloroacetic acid, Product No. T4885	50 g

   Dissolve and dilute to 500 ml with water.

8. Destaining Solution: Prepare by combining:

Methanol	50 ml
Glacial acetic acid	75 ml

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

9. Staining Solution: Dissolve 125 mg of brilliant blue R, Product No. B0149, in 500 ml of Destaining Solution. Filter before use. The reagent is stable for several months stored tightly capped at room temperature.

10. Saturated Bromphenol Blue Solution: Suspend 40 mg of bromphenol blue, Product No. B6131, in 10 ml of water. Solution may be stored for 3-4 months at room temperature.

<u>Preparation of Molecular Weight Marker and Samples</u>

Reconstitute the vial contents with 2.2 ml of Sample Buffer to give a recommended concentration of 1 mg/ml.
Prepare unknown samples at a concentration of 1 mg/ml in Sample Buffer.
Incubate all samples and the Molecular Weight Marker at 60°C for 15 minutes. Allow samples and Molecular Weight Marker to cool to room temperature. Add 50 µl of saturated bromphenol blue solution (#10) to each sample and the marker mixture. Aliquots may be frozen at -20°C for future use. Recommended gel loading volume is 10-25 µl for the Molecular Weight Marker.

<u>Preparation of 12.5% Acrylamide Electrophoresis Gels</u>

The following volumes will prepare 40 ml, which is sufficient for one standard slab gel (16 cm x 16 cm x 1.5 mm)

1. Mix:

Acrylamide solution (#1)	35.2 ml
Stock buffer (#4)	4.0 ml

   Deaerate for 1 minute under vacuum.

2. Add:

TEMED solution (#5)	0.4 ml
Ammonium persulfate solution (#6)	0.4 ml

   Mix solution carefully to avoid introducing air.

3. Dispense solution into gel apparatus and insert well-forming comb.

Procedure

Procedure for Electrophoresis

After loading the molecular weight markers and unknown samples on the gel, run electrophoresis on the gel at a recommended constant current of 70 mA/gel for a 16 cm x 16 cm x 1.5 mm gel. Alternatively, samples may be run overnight at a reduced current of 25 mA/gel. Stop the electrophoresis when the dye front is within 1 cm of the bottom of the gel. Mark the dye front on the gel by making a small hole with a glass disposable pipette.

Staining and Destaining

Fix the gel prior to staining by immersing the gel in Fixative Solution for 1-2 hours. Stain the gel in Staining Solution for a minimum of 4 hours. Overnight staining is preferred and will yield better results. Destain the gel in Destaining Solution for 2 hours, replacing the Destaining Solution every 30 minutes, until the background reaches the desired clarity. Efficiency may be increased by performing the destaining procedure in a water bath at 60°C. Gels can be stored in Destaining Solution. After destaining, the gel is ready for visualization, analysis and photography. See Results section for standard curve preparation.

Results

To determine the relative mobility (Rf) of a polypeptide, measure the migration distance of the polypeptide from the top of the separating gel and divide by the migration distance of the bromphenol blue tracking dye.

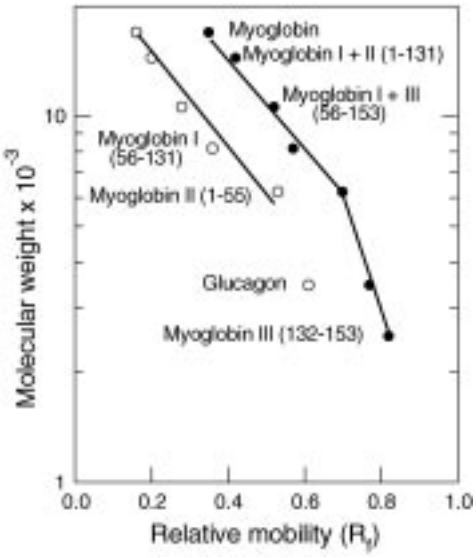
$$Rf = \frac{\text{Distance of polypeptide migration}}{\text{Distance of tracking dye migration}}$$

Generate a calibration curve by plotting the Rf values (x-axis) against the log of the known molecular weights (y-axis). If semilog paper is used, the log of the molecular weight does not need to be calculated.

Estimate the molecular weight of an unknown protein or polypeptide from the 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 Molecular Weight Marker for Peptides.
Discontinuous procedure of Schägger-von Jagow (●-●)
Continuous procedure of Swank-Munkres (O-O)

References

1. Kratzin, H.D. *et al.,* Anal. Biochem. **183**:1-8 (1989)
2. Schägger, H. and von Jagow, G., Anal. Biochem. **166**:368-379 (1987)
3. Swank, R.T. and Munkres, K.D., Anal. Biochem., **39**:462-477 (1971)

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