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ProductInformation

MOLECULAR WEIGHT MARKER KIT
For Molecular weight range 14,000 – 70,000

Product No**. MW-SDS-70L**
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. The method of Laemmli[1] is the most common procedure for the molecular weight determination of protein subunits. The components in the MW-SDS-70L kit can also be used when applied to the methods of Weber and Osborn[2] and Davies and Stark[3].

The proteins supplied in these kits have a molecular weight range common to most proteins and their subunits. These standard protein markers have been cited extensively in the literature and are characterized by a single band. Mixtures have been formulated to yield well-defined bands, which after SDS gel electrophoresis and staining with Brilliant Blue R (Coomassie Blue R, Product No. B 8647) are approximately equal in color intensity.

Components

Contains one vial of each of the seven proteins below and 1 vial of SDS-7

Product No	Proteins	mg/vial	Approx. Mol. Wt.
A 7517	Albumin, Bovine	25	66,000
A 7642	Ovalbumin, Chicken	25	45,000
G 5262	Glyceraldehyde-3-Phosphate Dehydrogenase, Rabbit	5	36,000
C 2273	Carbonic Anhydrase, Bovine	5	29,000
T 9011	Trypsinogen, Bovine, PMSF treated	25	24,000
T 9767	Trypsin Inhibitor, Soybean	5	20,100
L 6385	∀-Lactalbumin, Bovine	5	14,200
SDS-7	Dalton Mark VII-L	3.5	

Preparation

Preparation of SDS Molecular Weight Markers

Reconstitute the contents of a vial of one of the individual proteins with 1X Sample Buffer (Product No. S 3401) at a recommended concentration of 1 mg/ml based on the mg/vial in the table above. Reconstitute the mixture (SDS-7L) with 1.5 ml of 1X Sample Buffer. Heat the reconstituted vials in boiling water for 1-2 minutes prior to loading on the gel. Recommended gel loading volumes are 10 µl for the mixture or 1-10 µl of the individual proteins when staining with Brilliant Blue R.

Procedure

Procedure for Electrophoresis

SDS-polyacrylamide gels can be made following the methods outlined in the references below or using or using the manufacturer's recommended protocols for the electrophoresis unit. Precast gels can also be purchased from Sigma for convenience. Sigma's precast gels are inexpensive, reproducible and save the time necessary to cast a gel. After loading the molecular weight markers and unknown samples on the gel, run electrophoresis on the gel at a recommended constant voltage of 150 volts. Stop the electrophoresis when the dye front is within 1 cm of the bottom of the gel (approx. 40 minutes for an 8 x 10 cm minigel or 60 minutes for a 10 x 10 cm minigel). Mark the dye front on the gel by making a small hole with a glass disposable pipette.

Staining and Destaining

Stain the gel for approximately 30 minutes in Brilliant Blue R stain (0.025% Brilliant Blue R, 40% methanol, 10% acetic acid, Product No. B 8647). Destain the gel using a destaining solution containing 40% methanol, 10% acetic acid until the background reaches the desired clarity. Several changes of destaining solution will be necessary. Other staining and destaining methods may also be used but will not necessarily give the same even staining pattern designed using Brilliant Blue R stain.

Results

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

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

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 from the calibration curve.

References

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

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