Molecular Weight Estimations and SDS PAGE

Often questions are posed regarding apparent discrepancies between protein size as determined by gels vs. other methods, such as sequence analysis. Two factors explain most of the observed variation.

The first factor is the amount of SDS bound to the protein. SDS is employed to disrupt secondary structure and give all proteins a constant charge/ mass ratio, which is assumed to be 1.2g SDS/g protein. However, as stated in a review by Hjelmeland and Chrambach1; 'this assumption fails more frequently than is generally known.' The most common deviation from this assumption is probably a lower than normal amount of bound SDS. All else being equal, mobility would decrease, since the protein would have less of a negative charge.

A second source of error in molecular weight estimates, is that protein mobility in the gel is more a function of molecular size (which is a function of both weight and length) than of molecular mass. It's generally assumed that SDS proteins all exist in a random coil form, so the relationship between length and mass should be constant. Even assuming constant charge, if a protein has unreduced disulfide bonds or areas of incompletely disrupted secondary structure, it cannot unfold to full length and, it would tend to run faster than expected in a typical SDS gel.

These deviations from the ideal can combine in every conceivable way, making it difficult to predict a net effect on migration rate. Nevertheless, the effects can be large. Unreduced BSA will run with an apparent size of about 55kDa instead of 67kDa. Furthermore, in smaller proteins a non-ideal region will have a larger proportional effect than the same region in a large protein. For example, polypeptides of around 2kDa can give estimates which are off by a factor of 2 or more from actual size.

This is not to say that SDS derived molecular weights are invalid, just that they have limitations. Most proteins will give estimates within a few percent of their actual weight by comparing them to appropriate calibration markers. And possible deviations from 'true' molecular weight do not affect the utility of SDS gels in identification, because even 'unusual' proteins, if prepared in the same way each time should run reproducibly on a given gel type.

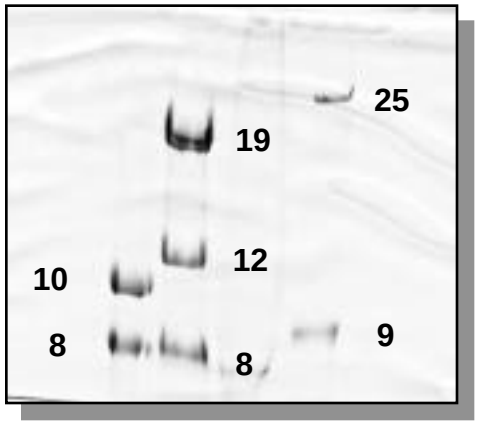
Finally, care should also be taken in inferring precise size based on published weights for calibration markers. Even common proteins may have several slightly different size estimates reported in the literature, depending upon the methods of molecular weight measurement. For a recent example see reference 2.

References:

1. Hjelmeland, L.M. and Chrambach, A., Electrophoresis 1981, 2, 1-11
2. Sallantin, M., Huet, J., Demarteau, and Pernollet, J., Electrophoresis 1990, 11, 34-36

SDS-PAGE MW

6%



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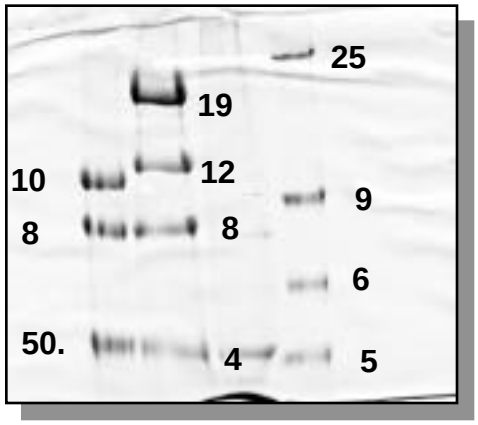

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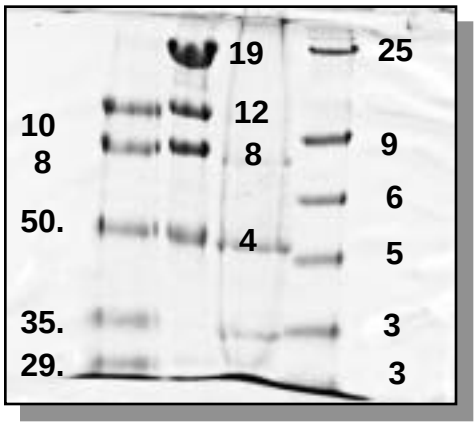
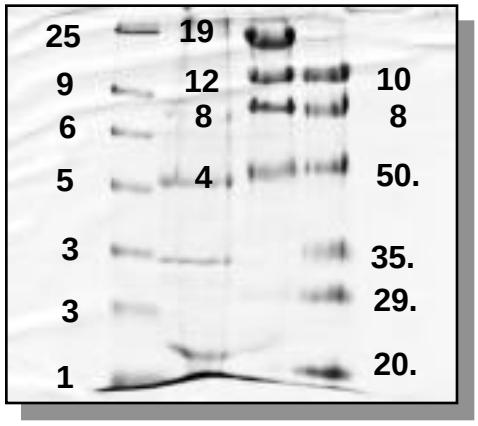
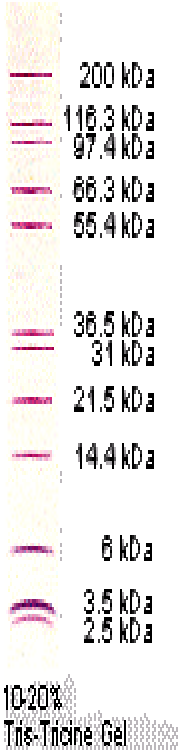


12%



1 - Novex See-Blue Wide Range
2 - BioRad High Range Prestained
2 - BioRad Low Range Prestained

Novex
Mark12™ Wide-Range Protein Standard



200 kDa
116.3 kDa
97.4 kDa
66.3 kDa
55.4 kDa

36.5 kDa
31.0 kDa
21.5 kDa

14.4 kDa

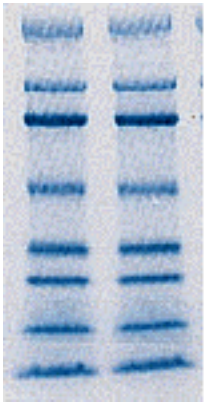
6 kDa

3.5 kDa
2.5 kDa

The NOVEX Mark12™ Standard is an unstained protein standard which allows close approximation of molecular weight over a wide molecular weight range and simplifies sample identification. .

BioRad Low Range Pre-Stained Standards

Prestained standards are used in SDS-PAGE and western blotting applications. They provide a quick and easy way to assess blotting efficiency and allow continuous monitoring of protein separations during electrophoresis. Prestained standards can be used as a control for repetitive blotting experiments, or in locating



Calibrated Molecular Weights of Prestained SDS-PAGE Standards*

PROTEIN	HIGH RANGE	LOW RANGE	BROAD RANGE
Control #	#71605	#74177	#72807A
Myosin	205,000	—	208,000
beta-galactosidase	118,000	—	115,000
Phosphorylase B	—	107,000	—
BSA	85,000	76,000	79,500
Ovalbumin	47,000	52,000	49,500
Carbonic anhydrase	—	36,800	34,800
Soybean trypsin inhibitor	—	27,200	28,300
Lysozyme	—	19,000	20,400
Aprotinin	—	—	7,200

** Molecular weights are of representative lots; actual weights may vary. The lot-specific molecular weights are included with every vial.*