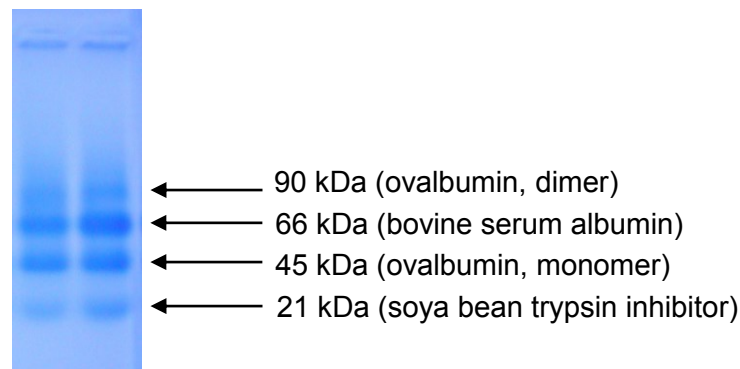


Powdered Protein Standards for Protein Electrophoresis



Molecular weights of proteins are too small to measure in grams or even nanograms. The unit for the molecular weight of proteins is a **dalton (Da)**, an atomic mass unit approximately equal to the mass of a hydrogen atom. If a protein has a molecular weight of 6000 Da it weights about the same as 6000 hydrogen atoms. Most proteins have masses in the order of thousands of Da, so the term kilodalton (kDa) is used to denote 1000 Da.

In protein electrophoresis, denatured proteins with different molecular weights move to different areas of the gel, with smaller proteins moving further than larger proteins. If protein standards of known molecular weight are run on a gel, their migration distances can be used to estimate the molecular weights of unknown proteins run on the same gel. The Scottish Initiative for Biotechnology Education (SIBE) has made a simple mixture of proteins of specific sizes, which produce the following banding pattern:



The size of unknown protein bands can be estimated by two methods:

- 1) Estimate the size by eye, i.e. it is bigger than 66 kDa but smaller than 45 kDa. Then make an approximation.
- 2) Measure the distance (mm) each band in the protein standard solution has migrated. Plot distance travelled on the x-axis and the molecular weight of each band (kDa) on the y-axis. The size of unknown proteins can then be estimated by measuring their migration distance and plotting on the standard curve.

A useful piece of information is that the average weight of an amino acid is 110 Da. So if you know the number of amino acids in a particular protein you can estimate what the molecular weight of the protein should be. Conversely, if you know the molecular weight you can estimate roughly how many amino acids are in the protein.

As a reference here are some sizes of common proteins:

Protein	Size (Da)	Size (kDa)
Myosin (a main protein in meat)	205, 000	205
Actin (a main protein in meat)	42,000	42
Rubisco large subunit (a main protein in plants)	55,000	55
Rubisco small subunit (a main protein in plants)	13,000	13
Caesin (main protein in milk and milk products)	23,500	23.5
Egg Albumin (main protein in egg)	45, 000	45

Instructions for making up the Protein Standards

Powdered Protein Standards must be kept in the fridge. They should last for 1-2 years in the fridge.

They must be made up into solution immediately before use and kept on ice until used.

1. Add 1ml of distilled water to the microtube containing the powdered protein standards.
2. Mix thoroughly.
3. Place tube in ice until use.
4. Add 0.25ml Laemmli buffer to 0.25ml of protein standard solution.
5. Boil for 5 minutes (with other protein samples) and load (10 - 20 μ l) onto gel.

LAEMMLI BUFFER

Available as part of NCBE 'Protein Power' kit. Or: mix the following ingredients well, allow to settle and make up to 100 mL with distilled or deionised water: 6.25 mL 1M Tris-HCl, pH 6.8; 20 ml 10% SDS solution; 25 ml Glycerol; 10 ml 0.1% aqueous Bromophenol blue. Makes 100 ml. May be stored indefinitely at room temperature.

Powdered Protein Standards can be obtained free from The Scottish Initiative for Biotechnology Education. Please request via e-mail, giving your full postal address.

Scottish Initiative for Biotechnology Education
University of Edinburgh
Roger Land Building G43
Alexander Crum Brown Road
King's Buildings
Edinburgh EH9 3FF

Tel: 0131 650 7123
Email: SIBE@ed.ac.uk