Aim 43

To Separate the Proteins by Gel Electrophoresis

Introduction

The term electrophoresis is generally applied to the movement of small ions and charge molecules in solution under electric field. The rate of migration of ions depends upon the size and shapes of molecules, charge carried and applied current and resistance of the medium.

Requirements

Chemicals :- Acetic acid, methanol, water, bromophenol blue, electrode buffer (tris HCl, glycine, SDS)

Glassware :- Plastic box, beaker, pipette, measuring cylinder.

Procedure

Dissolve Glycine (14 g), tri base (3 g), SDS(1 g) in 60-70 ml of distilled water and raise the final volume to 100 ml. This is diluted 10 times to make 100 ml. before running the gel, the comb should be removed slowly to avoid any sort of disturbance in the wells. Now lower spacer is removed gently. Grease should be removed gently and completely with the help of filter paper.

The glass plates that contain the gel should fixed to the vertical gel electrophoresis apparatus by the clippers. The electrode buffer is added to upper and lower chamber. The buffer was added upto plugs attached to chambers. The wires of two chamber should be absolutely stretched to the buffer. To the upper chamber, 2 drop of bromo phenol blue is added which makes give a blue color.

The bottom of wells are marked with tiny dots to avoid puncturing of wells during loading of samples definite and equal amount(20 ml) of loading ismade in each well. The solution is loaded at the base of well. The current for the upper gel should be 17 Amp. That for the lower gel 23 Amp. As the concentration of lower gel is higher than upper. The gel should be taken out when the blue line of bromo phenol blue rich at the bottom. After its complete running the side spacer removed gently and placing a spatula between two plates. The upper pate is lefted up dicard of upper gel with help of spatual and pick up lower gel and place it in a staning solution overnight. It is prepare by mixing Methanol (100 ml) + glacial acetic acid (14 ml) + distilled water (86 ml) + coomassie brilliant blue (100 mg). and finally shaked it properly.

Precautions

1) The loading should be uniform.
2) The syringe should be washed after each loading.
3) The bubbles should be removed from lower part of gel.