Aim 42

To Prepare a Polyacrylamide Gel Slab for the Separation of Protein

Introduction

PAGE (Polyacrylamide Gel Electrophoresis), is an analytical method used to separate components of a protein mixture based on their size. The technique is based upon the principle that a charged molecule will migrate in an electric field towards an electrode with opposite sign. The general electrophoresis techniques cannot be used to determine the molecular weight of biological molecules because the mobility of a substance in the gel depends on both charge and size. To overcome this, the biological samples needs to be treated so that they acquire uniform charge, then the electrophoretic mobility depends primarily on size. For this different protein molecules with different shapes and sizes, needs to be denatured (done with the aid of SDS) so that the proteins lost their secondary, tertiary or quaternary structure.The proteins being covered by SDS are negatively charged and when loaded onto a gel and placed in an electric field, it will migrate towards the anode (positively charged electrode) are separated by a molecular sieving effect based on size. After the visualization by a staining (protein-specific) technique, the size of a protein can be calculated by comparing its migration distance with that of a known molecular weight ladder(marker).

Requirements

Chemical: - Acraylamide tris-HCl, APS, SDS, TEMED, distilled water, *Glassware:* - glass plate, beaker, measuring cylindrical,

spacer comb, clip, cotton, silicon high vacuum grease, blotting paper etc.

Procedure

1**) Acrylamide stock solution preparation :-** Mix 30 g acrylamide + 135 mg bisacrylamide + few ml of D.W.. Finally, volume is raised up to 100 ml. It is then filtered.

2) 1 ml tris-HCl preparation pH 8.8 :-

12.1 g of trisma base is dissolved in 60.70 ml of distilled water. Then, adjust the pH to 8.8 by adding HCl (about 1.7 ml). Finally, raise the volume to 100 ml.

3) Tris-HCl preparation pH 6.8 :- 12.1 g of trisma base is dissolved in 60-70 ml of distilled water. Then, adjust pH to 6.8 by adding HCl (8.3 ml approx.)

4) Ammonium per sulphate (APS) :- Mix 1 % APS i.e. 50 mg APS + 50 ml of distilled water.

5) 10 % SDS i.e. 20 mg of SDS + 2 ml of distilled water.

6) Preparation of gel assembly:-

Gel plates are cleaned, dried and washed with rectified alcohol and greased three sides near the edge where spacer are to be put. Greasing is also done on to the spacer after setting them on the slab. Then, place the other glass plate forming the complete assembly. Assembly were made to stand by clips initially at the bottom followed by two side. In order to have a fixed length of gel to insert the comb, a mark is made at 20 mm below the comb.

7) Pouring of lower gel:-

Stock acrylamide (17 ml) + tris HCl (8.8 pH, 12.5 ml) +APS (1 ml) + distilled water (5.2 ml). Now degas the solution and add SDS (1 ml) and TEMED (30 ml) with the help of pipette. The solution is poured into the gel assembly, till the mark. When the mark is reached immediately with the help of syringe poured water layer above the gel in order to prevent the shrinkage of gel. After 2 hour of pouring, the lower gel. Polymerized completely and is ready for upper to be poured.

8) Pouring of upper gel: - Stock acrylamide (3 ml) + tris HCl (6.8 pH, 2.5 ml) + APS (0.5 ml) + distilled water (13.8 ml). Degas and added SDS (0.2 ml) + TEMED (20 ml). After discarding, the water layer upper gel is poured above the gel in order to prevent the shrinkage of gel. The comb is inserted immediately into it for the formation of well where the desirable sample proteins can be loaded.

Precautions

1) The plates, spacer, comb should be clean to avoid contamination.
2) Greasing should be done uniformly in straight line.
3) Degas is must to remove oxygen which inhibits the polymerization.
4) APS should be prepared fresh, as it is hygroscopic in nature.