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LT 14. SDS-PAGE

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LT14: SDS-PAGE

SDS–polyacrylamide gel electrophoresis (SDS–PAGE) is the most widely used method for analysing protein mixtures qualitatively. It is particularly useful for monitoring protein purification and, because the method is based on the separation of proteins according to size, it can also be used to determine the relative molecular mass of proteins.

•Samples to be run on SDS–PAGE are firstly boiled for 5 min in sample buffer containing β-mercaptoethanol and SDS. The mercaptoethanol reduces any disulphide bridges present that are holding together the protein tertiary structure, and the SDS binds strongly to, and denatures, the protein. Each protein in the mixture is therefore fully denatured by this treatment and opens up into a rod-shaped structure with a series of negatively charged SDS molecules along the polypeptide chain.

•The sample buffer also contains an ionisable tracking dye, usually bromophenol blue, that allows the electrophoretic run to be monitored, and sucrose or glycerol, which gives the sample solution density thus allowing the sample to settle easily through the electrophoresis buffer to the bottom when injected into the loading well.

SDS-PAGE gel slab contains two types of gels of varying polyacrylamide gel concentration stacked on each other. These two are referred as Stacking gel and resoluting or separating gel. pH of stacking gel = 6.8 (4 % polyacrylamide concentration) and pH of separating gel = 8.8 (15 % polyacrylamide concentration).

Stacking gel: The function of stacking gel is to concentrate the protein sample into a sharp band before the protein enters the main separating gel. This is achieved by utilising differences in ionic strength and pH between the electrophoresis buffer and the stacking gel buffer and involves a phenomenon known as isotachophoresis. Under the influence of electric field, the three charged species i.e Cl^- (Chloride ion from loading buffer), SDS-protein complex and glycinate ion (from electrophoresis buffer) arrange themselves as Cl- > SDS-Protein complex > Glycinate. There is only a small quantity of protein–SDS complexes, so they concentrate in a very tight band between glycinate and Cl boundaries. Once the glycinate reaches the separating gel it becomes more fully ionised in the higher pH environment and its mobility increases. (The pH of the stacking gel is 6.8, that of the separating gel is 8.8.) Thus, the interface between glycinate and Cl leaves behind the protein–SDS complexes, which are left to electrophorese at their own rates.

Separating gel: The actual separation of protein takes place in this gel. The negatively charged protein–SDS complexes now continue to move towards the anode, and, because they have the same charge per unit length, they travel into the separating gel under the applied electric field with the same mobility. However, as they pass through the separating gel the proteins separate, owing to the molecular sieving properties of the gel. Quite simply, the smaller the protein the more easily it can pass through the pores of the gel, whereas large proteins are successively retarded by frictional resistance due to the sieving effect of the gels.

Indication of completion of electrophoresis: An indicator dye such as bromophenol blue is used to indicate the completion of electrophoresis. When the dye reaches the bottom of the gel, the current is turned off, and the gel is removed from between the glass plates.

Staining & Destaining: After removal of gel slab from glass plate it is shaken in an appropriate stain solution (usually Coomassie Brilliant Blue and then washed in destain solution. The destain solution removes unbound background dye from the gel, leaving stained proteins visible as blue bands on a clear background. A typical minigel would take about 1 h to prepare and set, 40 min to run at 200 V and have a 1 h staining time with Coomassie Brilliant Blue. Upon destaining, strong protein bands would be seen in the gel within 10-20 min, but overnight destaining is needed to completely remove all background stain. Vertical slab gels are invariably run, since this allows up to 10 different samples to be loaded onto a single gel.

Application of SDS-PAGE

It is used for protein separation and assess the purity of protein at each purification step. A pure protein should give a single band in SDS-PAGE, unless the molecules are made up of two unequal subunits. In latter case, two bands corresponding to the two unequal subunits will be seen.

Poly acrylamide Gel Eectrophoresis

PAGE

This is also used for separating proteins from mixture. It also contains two types of stacking and separating gel similar to SDS PAGE. The only difference with respect to SDS-PAGE is that PAGE does not uses detergent for denaturating protein before loading on to gel. PAGE takes place under non-denaturating condition and in native gels. Separation of proteins is based on electrophoretic mobiitis of protein in gels and sieving effect of gels.

Appliction of PAGE

Since it invoves non-denaturating condition, proteins in their native and active state can be separated. Therefore, it is best suited for application aiming to detect a particular protein on the basis of its biological activity.

Detection, estimation and recovery of proteins in gels

Detection, estimation and recovery of proteins in gels

Dye staining: The separated protein bands can be visualized using protein stains such as coomasie brilliant blue R-250 (CBB). Staining is carried out using 0.1 % (W/V) CBB in methanol-water-glacial acetic acid (helps in fixing the protein in gels to prevent wash out of protein from gel) mixture followed by destaining overnight in same mixture but devoid of dye.

Silver staining: It is highly sensitive and is based on principle of reduction of silver ions (Ag+) to metallic silver on the protein, where silver is deposited to a black or brown band. Silver staining is able to resolve minor bands on gels which remain unstained by CBB.

Estimation

Quantitative analysis (i.e measurement of the relative amount of different proteins in a sample) can be achieved by scanning densitometry------ stained gel tracks are illuminated by beam of laser and transmitted light is measured----------Absorbance vs migration distance.

Purification

Purification of protein is achieved by cutting the separated gel tracks into pieces and sequenced using protein sequencer. The protein is taken out of the gel using process of electroelution. The gel pieces are sealed into dialysis sac and placed in buffer between two electrode. On application of electric field, proteins electrophorese out of gel into buffer. Proteins are precipitated out of buffer using ethanol

Test your understanding

The pH of (i) stacking, (ii) resolving gel and (iii) tank buffer in SDS PAGE is _____ respectively.

(i). 6.80 (ii) 8.80 (iii) 8.30

(i) 8.30 (ii) 8.80 (iii) 6.80

(ii) (i). 8.30 (ii) 6.80 (iii) 8.80

None of the above

Sodium dodecyl sulfate (SDS) used in SDS PAGE is_____.

a. An anionic detergent

b. A cationic detergent

c. A non-ionic detergent

d. An anion exchanger

What is the purpose of resolution gel in SDS-PAGE electrophoresis?

a. Dispersion of protein

b. Concentration of protein sample in sharp bands

c. Separation of protein sample

d. None of the above

What is the point of distinction between PAGE & SDS-PAGE?

Name and state the function of tracking dye used in SDS-PAGE

References & Further reading

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