Good example of report on electrophoresis of gfp

Science, Biology

Aim: To separate Green Fluorescent Protein (GFP) using poly-acrylamide gel electrophoresis (PAGE).

Introduction: Bioluminescence observed in many marine organisms is due to a protein called as Green Fluorescent Protein (GFP). Since its discovery, GFP has been useful in biotechnology due to its versatility and ease of transformation in E. coli (Chalfie et al., 1994). GFP is a 26. 87 kilodaltons (kD) protein composed of 239 amino acids. It has a barrel structure with a fluorescent chromophore embedded deep in the interior of the protein. The chromophores in GFP are excited at a wavelength of 395 nm, and when the electrons of chromophores drop to a lower energy state they emit fluorescent light of 509 nm (Ormo et al., 1996).

GFP has many applications in biotechnology. It is a used as a reporter protein, where the fluorescence creates a marker, which is used in biochemical and cell biology studies. Commonly, GFP is recombined with target proteins to create chimeric reporter proteins. Recently, GFP was used as a reporter protein to study many biological samples including tumour progression, brain activity and cellular functioning.

Electrophoresis, or polyacrylamide gel electrophoresis (PAGE), is common molecular biology tool to separate and study proteins. It was developed by Laemmli, in 1970, with two gel phases – a stacking gel and a resolving gel. In the stacking gel, the percentage of polyacrylamide is low (4%) where the proteins are compacted into narrow bands without separation. Here the samples of mixed proteins are compacted in uniform narrow bands, which move further in the resolving gel. The resolving gel has a higher concentration (5–20 %) as compared to stacking gel. The proteins are

separated on the basis of their molecular weight in the resolving gel. In a vertical PAGE, the samples are loaded in wells on top of the stacking gel. These wells made using a comb. The sample proteins move downward in the gel in the presence of the electrical field toward the positive electrode. Polyacrylamide gels are used in protein separation due to their larger pore size and provide better resolution for proteins as compared to agarose gels. The proteins are separated on the basis of the molecular weights, which is expressed in kilodaltons (kD). Proteins can range from several kD to thousands of kD. The intrinsic charge of proteins can also affect the mobility of the protein and, therefore, a strongly anionic detergent - sodium dodecyl sulphate (SDS) is added. SDS coats the proteins and thus obscures the protein intrinsic charges imparting the protein with equivalent negative charge. Therefore, it allows the separation of proteins solely on the basis of their molecular weights.

Running of gel: SDS-PAGE acts like a molecular sieve and the sieving can be adjusted using the concentration of polyacrylamide. Large proteins of 100–300 kD are separated in 5% polyacrylamide gels, while small proteins of 5–30 kD can be separated in 18% polyacrylamide gels. The gel cassette is prepared the wells are set using a comb. This gel cassette is locked on a vertical electrophoresis apparatus, and running buffer is added to both the upper and lower chambers. The GFP samples are loaded in the wells, with a separate well loaded with molecular markers. The electrophoresis box is then plugged to a power supply, and the gel is run for the maximum separation of proteins. After completion of electrophoresis, the gel is stained such that the proteins appear against a clear background.

Materials:

GFP samplesMicro-centrifuge tubes

Micro-pipettes and tips (p20 and p200)Running buffer

Vertical gel electrophoresis boxPower supply

15% pre-cast gel 1 x TGS buffer

Coomassie protein stain Staining tray

95 ºC water bath Water for destaining

Thin metal spatulaMarking penProcedure:

- Label 3 microcentrifuge tubes and collect the GFP samples.

- 50 microliters of running buffer is added to each tube. It contains SDS that denatures the proteins sample as well as a tracking dye and glycerol to weigh the samples down.

- 50 microliters of GFP samples is added to the corresponding tube as labelled in step 1.

- The tubes are heated at 95 ºC for 5 minutes.

- The comb is removed, and the gel cassette is placed in the chamber.

- 200 milliliters of 1x TGS electrophoresis buffer is poured into the chamber.

- 10 microliters of the molecular standards are added in anyone of the well.

- 20 microliters of the GFP sample is loaded into a separate well.

- The tank is closed, and the power supply is connected.

- The gel is run for 30 minutes at a voltage of 200 V.

- After the running finishes, the buffer is discarded, and the gel cassette is removed. The gel is then carefully removed with the help of a thin spatula.

- The plate is transferred in a Bio-Safe Coomassie stain.

- The gel is stained for 1 hour. The gels are agitated occasionally.

- After completion of staining, the Bio-Safe Coomassie stain is discarded according to the MSDS and the gel is washed with water twice or till the background colour disappears.
- The distinct protein bands can now be observed. The GFP protein is approximately 27 kilodaltons.

Results:

References:

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