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Need of a Fast Method to De-Stain Proteins after Polyacrylamide Gel Electrophoresis

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Electrophoresis is one of the research techniques, which has extensively been used in separation and identification of proteins/ enzymes. In majority of the biochemistry and molecular biology experiments, electrophoresis of proteins and enzymes is performed using polyacrylamide gel electrophoresis (PAGE) [1,2]. In this method, sodium dodecyl sulfate (SDS) [SDS-PAGE] is used when we need to estimate molecular weight and purity of proteins [1-3].

After simple PAGE or SDS-PAGE (1D or 2D), to visualize the separated protein bands, we first stain the gels (containing proteins) with various stains (like Amido black, Coomassie Brilliant Blue R-250 (CBB R-250), Fast Green FCF, Ponceau-S or others) [2,3]. Out of these the CBB is the most common dye used to stain proteins on polyacrylamide gels [1-4] and normally it takes about 1 hr to stain the proteins. To visualize the protein bands on the gel, we have to de-stain the gel with a de-staining solution containing methanol/acetic acid to remove the characteristic background gel staining.

The main drawback with this traditional CBB staining method is that it takes very long time to get the results after staining and de-staining to observe the protein bands on the gel. Overnight de-staining periods are usually necessary to achieve a clear background. Also during de-staining multi-steps, we have to waste the de-staining solution again and again and this may contaminate our environment and leads to increase the cost of the procedure [5]. Some researchers have washed the gels with water which increases the de-staining power but time factor does not decrease. Other researchers have used high temperature (by use of microwave oven) to promote the de-staining process up to 10 min [6,7].

One of the important applications of the SDS-PAGE is to check the over-expression of proteins after modifications (mutations) of genes (for example in genetic engineering experiments, using bacteria). In this technique, the scientists first observe the over-expression of inserted gene at small scale-culture in four subcultures of bacteria [2]. To check, which lot of the bacteria has over-expressed the desire protein, we have to identify the over-expressed proteins in bacterial extract as soon as possible. This helps us to select the desired lot of bacteria for large-scale culture and production of the over-expressed protein. At present time the over-expressed protein is confirmed only by SDS-PAGE after applying the whole cell extract of bacteria on SDS-PAGE. Therefore, any method, which can de-stain the proteins as fast as possible, will be highly valuable, especially in genetic engineering procedures. I have identified a "solution" that de-stains the PAG stained with CBB R-250 in ONE minute and that de-staining solution can be used again and again, thus the cost of this solution is very less compared to the conventional method where one has to throw the de-staining solution after its use and at the same time the contamination of our environment will be minimum. The research work is in process to find out the lowest concentration of proteins, which can be detected by this method. However, it is correct to suggest that this method can easily be used to confirm the over-expressed protein in bacteria after genetic engineering method in which case the over-expressed proteins are in micromolar concentration [5].

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