Protein analysis by poly acrylamide gel electrophoresis (SDS-PAGE)

The protein patterns were analyzed using SDS-PAGE according to Laemmli, (1970) in the first dimension.

SDS-PAGE solutions

Stock (1)

Acrylamide (30 grams) and 0.8 gram bis N.N. methylene bisacrylamide were dissolved in 100 ml distilled water (each component dissolved separately). The solution was then filtered through glass filter under vacuum and kept at 4 ˚C in a dark bottle.

Stock (2)

Tris-HCl (18.2 grams) was dissolved in 50ml distilled water. The pH was adjusted with HCl to 8.8 after that the volume was completed to 100 ml distilled water and the solution stored in the refrigerator.

Stock (3)

Tris-HCl (6 grams) dissolved in 50ml distilled water. The pH was then adjusted with HCl to 6.8 after that the volume was completed to 100 ml distilled water and the solution was stored in the refrigerator.

Sodium dodecyl sulfate solution (SDS 10% W/V):

Stock solution was prepared by dissolving 1.0 grams SDS in 10ml distilled water.

Ammonium persulfate solution (APS 10% W/V): (freshly prepared)

A solution of ammonium persulfate was prepared by dissolving 1.0 gram in 10ml distilled water, just before use in gel preparation.

Preparation of 12% and 5% SDS-PAGE

The gel was prepared as shown in the following table:

Reagents	12% separating gel	5% stacking gel	For Mini-BioRad cell	
			12% separating gel	5% stacking gel
Distilled H2O	9.9 ml	11 ml	6.6 ml	7.3 ml
Stock (1)	12.0 ml	2.6 ml	4 ml	1.7 ml
Stock (2)	7.5 ml	….	5 ml	….
Stock (3)	…..	2.0 ml	…..	1.3 ml
10% SDS	300 µl	100 µl	200 µl	66 µl
10% APS*	300 µl	100 µl	200 µl	66 µl
TEMED	12 µl	8 µl	9 µl	6 µl
Volume	30.012	15.806	16.016	10.0156

* APS: ammonium persulfate.

Procedure

The gel components were mixed and the acrylamide solutions were quickly poured between the plates. The meniscus of the acrylamide solution should be far enough below the top of the notched plate to allow for the length of the comb plus 1cm.

After the gel has been set about 20-30 min, the overlay water poured off and the top of the separating gel was washed several times with distilled water. Excess water was drained with the edge of a paper towel. The stacking gel (5.0 acrylamide) solution was prepared as shown in the table and poured directly into the polymerized separating gel.

The appropriate comb was placed into the gel solution being careful not to make any bubbles. The comb was cleaned by washing with distilled water. The gel was placed in a vertical position at room temperature. The stacking gel would set in approximately 15 min.

Electrode buffer solution (2x):

Tris-HCl (6 grams), Glycine (28.8 grams) and 2 grams SDS were dissolved in 1000 ml distilled water. pH 8.8

Loading buffer (2x) (double concentration):

100 ml 0.125M (1.512g/100) Tris-HCl (pH 6.8) – 4% SDS (W/V) – 20% glycerol

(V/V) -10% 2- mercaptoethanol (V/V) – 0.002% (W/V) bromophenol blue.

Protein analysis:

Composition of lysing buffer:

100ml 0.625M (0.706 g/100ml) Tris-HCl (pH 6.8) – 2% SDS (w/v) - 10% glycerol (v/v) - 5% 2- mercaptoethanol (v/v)

Preparation of samples:

Cells (~o.1 gm fresh weight) of each treatment in addition to control were suspended in 1.0 ml lysing buffer. Heated at 100 ˚C for 5 min., centrifuged at 10,000 rpm for 30 min., and 50 µl of each extracted protein treatment was used for protein analysis.

Sample loading and application:

After the stacking gel has set, carefully remove the comb. Wash the wells immediately with distilled water to remove unpolymerized acrylamide. Straighten the teeth of the wells. 100µl of each sample was loaded directly into the bottom of the wells, using a microliter syringe washed by electrode buffer after each sample.

Electrophoresis

After sample loading, the molded gel was completed with electrode. Electrophoresis was carried out at (140V) for each well for 120 min.

Protein staining:

Staining solution:

0.5 gm Commassie R-250 brilliant blue was dissolved in 250 ml methanol, 50 ml glacial acetic acid, completed by distilled water to 500ml mixed and kept at room temperature.

Procedure

Gels were stained overnight in 200 ml of Commassie brilliant blue R-250 solution.

Destaining solution:

Destaining of protein was performed in 200 ml of destaining solution which composed of 250 ml methanol, 50 ml glacial acetic acid and 200 ml distilled water with gentle shaking. The destaining solution was changed several times until background colour was removed.

Molecular weight determination of proteins:

The LabImage (2006) program or Gel pro analyzer was used in molecular weight determination of protein.