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APPENDICES

Appendix A

Stock solutions for ND-PAGE

a) 30% acrylamide - 0.8% Bis

acrylamide	30 g
bis-acrylamide	0.8 g
final volume	100 ml

The solution was deaired ,filtered through filter paper Whatman # 1 and stored in dark and cold place.

b) 1.5 M Tris-HCl pH 8.8

18.165 g Tris was dissolved in distilled water and adjusted to pH 8.8 with HCl before made up volume to 100 ml.

c) 0.5 M Tris-HCl pH 6.8

For 100 ml of buffer, 6.055 g of Tris was dissolved and adjusted to pH 6.8 with HCl.

d) 1.25 M Tris-HCl pH 6.8

Tris (15.14 g) was dissolved in distilled water adjusted to pH 6.8 with HCl and adjusted to final volume of 100 ml.

e) Electrode buffer (0.025 M Tris-HCl - 0.192 M Glycine, pH 8.3)

Tris	3.275 g
Glycine	14.42 g
final volume	1000 ml

The buffer was diluted to 10 folds before used in electrophoretic run as described in section 2.2.9.1 and directly use in section 2.2.9.3.

f) Sample buffer

Three parts of sample was mixed with one part of sample buffer which composed of glycerol : stock solution d) : distilled water = 2:1:2 (v/v) with trace amount of bromophenol blue before loading to the gel.

Preparation for discontinunous nondenaturing polyacrylamide gel

Reagent	separating gel			stacking gel
	(section 2.2.9.1)	(section 2.2.9.3)		
	7.5%	7.5%	12.5%	3%
30%acrylamide-0.8%bis (ml)	4.8	2.4	4.2	1.5
1.5 M Tris-HCl pH 8.8 (ml)	7.0	3.5	2.5	-
0.5 M Tris-Hcl pH 6.8 (ml)	-	-	-	3.7
distilled water (ml)	15.0	4.0	3.1	6.6
10mg% ammoniumpersulfate (μl)	200	200	200	200
TEMED (μl)	5	5	5	5
total volume (ml)	27	10	10	12.0

f) Staining solution (0.2% Coomassie blue stain)

Coomassie blue	0.8	g
methanol	200	ml
acetic acid	40	ml
distilled water	160	ml

The dye was stirred in methanol before adding acetic acid and water. The solution was filtered through the filter paper (Whatman # 1). THe solution can be used 2-3 times.

g) Destaining solution

7% acetic acid and 5% methanol

acetic acid	70 ml
methanol	50 ml

Made up to final volume 1000 ml with distilled water.

10% acetic acid and 50% methanol

acetic acid	100 ml
methanol	500 ml

Made up to final volume of 1000 ml with distilled water.

Appendix B

Preparation of SDS-polyacrylamide gel electrophoresis

Stock solutions a,b,c and d was prepared as described in Appendix A. The separating gel and stacking gel were set according to this formula.

Reagent	12.5% separating gel	3% stacking gel
30% acrylamide - 0.8% Bis (ml)	4.2	1.5
Tris-HCl pH 8.8 (ml)	2.5	-
Tris-HCl pH 6.8 (ml)	-	3.7
1% SDS (ml)	1.0	1.5
distilled water (ml)	2.1	5.1
10mg% ammonium persulfate (μl)	200	200
TEMED (μl)	5	5
total volume (ml)	10	12.0

Solubilizing medium

chemicals	volumes (ml)	final concentration
20% SDS	1	1%
Glycerol	2	4%
β-mercaptoethanol	1	1%
1.25 M Tris pH 6.8	1	62 mM
Bromophenol blue	trace	

One part of solubilizing medium was added to three parts of sample. The mixture was heated in boiling water bath for 2 min before loading to the gel.

Appendix C

Preparation of isoelectric focusing polyacrylamide gel

The gel was set with the following compositions :-

Reagent	volume (ml)
30% acrylamide	0.9
1% Bis-acrylamide	1.25
Pharmalyte pH 5-7	0.243
distilled water	1.39
50% sucrose	1.186
0.02 M Ammonium persulfate (μl)	39.5

After the run, the gel was stained and destained with the following solutions.

Staining solution 27% ethanol, 10% acetic acid, 0.04% Coomassie brillaint blue R 250 and 0.5% $CuSO_4$.

Destaining solution : composed of 12 % ethanol, 7 % acetic acid and 0.5% $CuSO_4$. The gel was immersed in a few changes of destaining solution until the background was as clear as possible with gentle agitation.

Appendix D

Peeled potato	200	g
Dextrose (or glucose)	20	g
Agar	17	g

Prepared in distilled water according to the following procedure : Peeled potato (200 g) is washed and weighed before cutting into 1 cm^3 pieces, then boiled in 500 ml of distilled water and seived through the cheesecloth. Then, 20 g of dextrose or glucose was added and mixed well with agar by heat-stirring before make up volume to 1000 ml with distilled water and autoclaved at 121°C, 15 min. After the temperature is lowered to 45-50°C , the agar mixture (PDA) is poured to petridish (15 ml-each) , or 5 ml if prepared in tube, and allowed to solidify.

Appendix E

Preparation of chemical reagents for SOD assay

1) For spectroscopic method

- 50 mM potassium phosphate buffer , pH 7.8, 0.1 mM EDTA

8.05 g K_2HPO_4 and 0.486 g KH_2PO_4 are dissolved in distilled water and adjusted to pH 7.8 before adding 0.33 g of EDTA and make up to 1000 ml with distilled water.

- 0.01 mM cytochrome c

0.025 g of cytochrome c is dissolved in 4 ml of distilled water.

- 0.05 mM xanthine

Dissolved 0.004 g xanthine in 10 ml of distilled water.

- Xanthine oxidase

Either 29 µl xanthine oxidase solution is dissolved in 900 µl by distilled water , or 5 mg of crystal xanthine oxidase is dissolved in 1 ml distilled water. Ten microlitres of this solution in 1 ml assay mixture will result in the change of absorbance at 550 nm at 0.02 OD unit/minute.

For 1 ml assay volume ; 830 µl of buffer reagent is mixed with 20 µl each of xanthine and cytochrome c before added 120 µl of distilled water or distilled water diluted-sample. Xanthine oxidase solution (10 µl) is added last to the system for starting the reaction.

2) Reagent for SOD-activity stain on ND-PAGE

- 36 mM phosphate buffer pH 7.8

5.64 g of K_2HPO_4 and 0.49g of KH_2PO_4 are dissolved together in distilled water and adjusted to pH 7.8 before make up volume to 1000 ml.

- NBT solution

0.41 g of NBT is freshy dissolved in 50 ml and kept in dark.

- 0.028 mM riboflavin/ 0.025 M TEMED solution

Riboflavin (0.0015 g) is dissolved in 100 ml phosphate buffer and 210 μl of TEMED is pipetted into 100 ml reaction mixture before the reaction is started.

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