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APPENDICES

APPENDIX A

Preparation for polyacrylamide gel electrophoresis

1. Stock reagents

30% Acrylamide, 0.8% bis-acrylamide, 100ml

acrylamide	29.2 g
N,N′-methylene-bis-acrylamide	0.8 g

Adjusted volume to 100 ml with distilled water

1.5 M Tris-HCl pH 8.8

Tris (hydroxymethyl)-aminomethane	18.17 g

Adjusted pH to 8.8 with 1M HCl and adjusted volume to 100 ml with distilled water

2 M Tris-HCl pH 8.8

Tris (hydroxymethyl)-aminomethane	24.2 g

Adjusted pH to 8.8 with 1M HCl and adjusted volume to 100 ml with distilled water

0.5 M Tris-HCl pH 6.8

Tris (hydroxymethyl)-aminomethane	6.06 g

Adjusted pH to 6.8 with 1 M HCl and adjusted volume to 100 ml with distilled water

1 M Tris-HCl pH 6.8

Tris (hydroxymethyl)-aminomethane	12.1 g

Adjusted pH to 6.8 with 1M HCl and adjusted volume to 100 ml with distilled water

Solution B (SDS PAGE)

2 M Tris-HCl pH 8.8	75 ml
10% SDS	4 ml
distilled water	21 ml

Solution C (SDS PAGE)

1 M Tris-HCl pH 6.8	50 ml
10% SDS	4 ml
distilled water	46 ml

2. Non-denaturing PAGE

7.0% Separating gel

30% acrylamide solution	2.33 ml
1.5 M Tris-HCl pH 8.8	2.50 ml
distilled water	5.15 ml
10% $(NH_4)_2S_2O_8$	50 µl
TEMED	5 µl

For preparative gel, 25 µl of 10% $(NH_4)_2S_2O_8$ and 2.5 µl of TEMED were added.

5.0% stacking gel

30% acrylamide solution	1.67 ml
0.5 M Tris-HCl pH 6.8	2.50 ml
distilled water	5.80 ml
10% $(NH_4)_2S_2O_8$	50 µl
TEMED	10 µl

Sample buffer

For analytical gel

1 M Tris-HCl pH 6.8	3.1 ml
glycerol	5.0 ml
1% bromophenol blue	0.5 ml
distilled water	1.4 ml

For preparative gel

0.5 M Tris-HCl pH 6.8	1.0 ml
glycerol	0.8 ml
0.5% bromophenol blue	0.4 ml
distilled water	5.8 ml

One part of sample buffer was added to four parts of sample.

Electrophoresis buffer, 1 litre

(25 mM Tris, 192 mM glycine)

Tris (hydroxymethyl)-aminomethane	3.03 g
Glycine	14.40 g

Dissolved in distilled water to 1 litre. Do not adjust pH with acid or base (final pH should be 8.3).

3. SDS-PAGE

7.5% separating gel

30% acrylamide solution	2.5 ml
solution B	2.5 ml
distilled water	5.0 ml
10% $(NH_4)_2S_2O_8$	50 µl
TEMED	10 µl

5.0% stacking gel

30% acrylamide solution	0.67 ml
solution C	1.0 ml
distilled water	2.3 ml
10% $(NH_4)_2S_2O_8$	30 µl
TEMED	5 µl

Sample buffer

1 M Tris-HCl pH 6.8	0.6 ml
50% glycerol	5.0 ml
10% SDS	2.0 ml
2-mercaptoethanol	0.5 ml
1% bromophenol blue	1.0 ml
distilled water	0.9 ml

One part of sample buffer was added to four parts of sample. The mixture was heated 5 minutes in boiling water before loading to the gel.

Electrophoresis buffer, 1 litre

Tris (hydroxymethyl)-aminomethane	3.0 g
Glycine	14.4 g
SDS	1.0 g

Adjusted volume to 1 litre with distilled water (pH should be approximately 8.3).

APPENDIX B

Preparation for isoelectric focusing gel electrophoresis

Monomer-ampholyte solution

30% Acrylamide solution	0.9 ml
1.0% Bis-acrylamide solution	1.25 ml
Ampholyte pH 3-10	0.243 ml
Distilled water	1.39 ml
50% Sucrose	1.186 ml
TEMED	2 µl
0.02 M $(NH_4)_2S_2O_8$	39.5 µl

Fixative solution, 100 ml

Sulfosalicylic acid	4 ml
Trichloroacetic acid	12.5 g
Methanol	30 ml

Immerse gels in this solution for 30 minutes.

Staining solution, 100 ml

Ethanol	27 ml
Acetic acid	10 ml
Coomassie brilliant blue R-250	0.04 g
$CuSO_4$	0.5 g
Distilled water	63 ml

Dissolve the $CuSO_4$ in water before adding the alcohol. Either dissolve the dye in alcohol or add it to the solution at the end.

Immerse the gel in the stain for approximately 1-2 hours.

Destaining solution

First destaining solution

Ethanol	12 ml
Acetic acid	7 ml
$CuSO_4$	0.5 g
Distilled water	81 ml

Dissolve the cupric sulfate in water before adding the alcohol. Immerse the gel in two or three changes of this solution until the background is nearly clear.

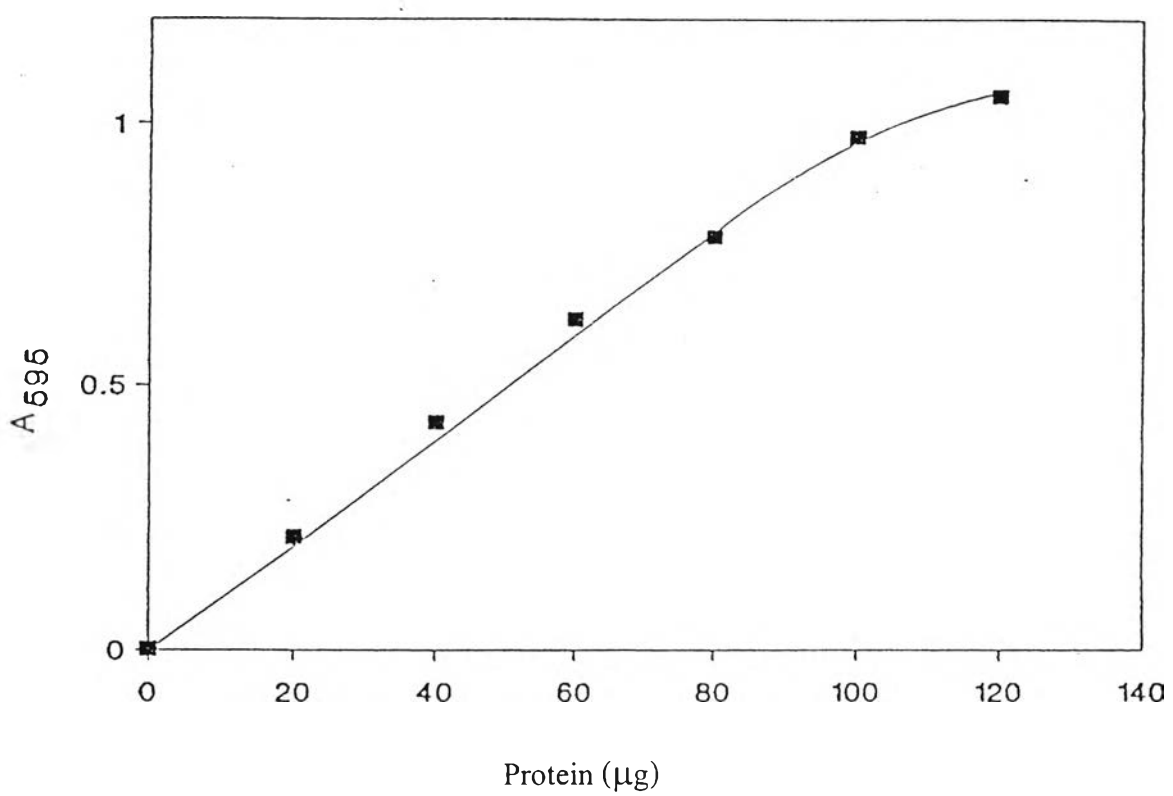
Second destaining solution

Ethanol	25 ml
Acetic acid	7 ml
Distilled water	68 ml

Immerse the gel in this solution to remove the last traces of stain and $CuSO_4$.

APPENDIX C

Standard curve for protein determination by Bradford's method



APPENDIX D

Calculation of SBE activity

Blk = CPM of reaction mixture without SBE

X = CPM of SBE products

Y = CPM of ^{14}C in 50 mmol G1P

Incubation time = 60 minutes

$$\text{SBE activity} = \frac{X - Blk}{Y} \times 50 \times 10^{3} \times \frac{1}{60} \quad \mu\text{mol/min}$$

BIBLIOGRAPHY

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