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APPENDICES

APPENDIX A

Preparation of polyacrylamide gel electrophoresis

1. Stock reagents

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

Acrylamide	29.2	g
N,N'-methylene-bis-acrylamide	0.8	g

Adjust volume to 100 ml with distilled water.

1.5 M Tris-HCl pH 8.8

Tris (hydroxymethyl)-aminomethane	18.17	g

Adjust pH to 8.8 with 1 M HCl and adjust volume to 100 ml with distilled water.

2.0 M Tris-HCl pH 8.8

Tris (hydroxymethyl)-aminomethane	24.2	g

Adjust pH to 8.8 with 1 M HCl and adjust volume to 100 ml with distilled water.

0.5 M Tris-HCl pH 6.8

Tris (hydroxymethyl)-aminomethane	6.06	g

Adjust pH to 6.8 with 1 M HCl and adjust volume to 100 ml with distilled water.

1.0 M Tris-HCl pH 6.8

Tris (hydroxymethyl)-aminomethane	12.1	g

Adjust pH to 6.8 with 1 M HCl and adjust volume to 100 ml with distilled water.

Solution B (SDS PAGE)

2.0 M Tris-HCl pH 8.8	75	ml
10% SDS	4	ml
Distilled water	21	ml

Solution C (SDS PAGE)

1.0 M Tris-HCl pH 8.8	50	ml
10% SDS	4	ml
Distilled water	46	ml

2. Non- denaturing PAGE

10.0 % Separating gel

30 % Acrylamide solution	2.5	ml
1.5 M Tris-HCl pH 8.8	2.5	ml
Soluble starch	1.0	ml
Distilled water	2.14	ml
10% $(NH_4)_2S_2O_8$	100	µl
TEMED	10	µl

4.0 % Stacking gel

30 % Acrylamide solution	0.67	ml
0.5 M Tris-HCl pH 6.8	1.0	ml
Distilled water	2.39	m l
10 % $(NH_4)_2S_2O_8$	50	µl
TEMED	10	µl

5X Sample buffer

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

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

Electrophoresis buffer, 1 liter (25 mM Tris, 192 mM glycine)

Tris (hydroxymethyl)-aminomethane	3.03	g
Glycine	14.40	g

Dissolve in distilled water to 1 liter. Do not adjust pH (final pH should be 8.3).

3. SDS-PAGE

10 % Separating gel

30 % Acrylamide solution	2.5	ml
Solution B	2.5	ml
Distilled water	2.39	ml
10% $(NH_4)_2S_2O_8$	100	µl
TEMED	10	µl

4.0 % Stacking gel

30 % Acrylamide solution	0.67	ml
Solution C	1.0	ml
Distilled water	3.27	ml
10 % $(NH_4)_2S_2O_8$	30	µl
TEMED	5.0	µl

5X 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 min. in boiling water before loading to the gel.

Electrophoresis buffer, 1 liter

(25 mM Tris, 192 mM glycine)

Tris (hydroxymethyl)-aminomethane	3.03 g
Glycine	14.40 g
SDS	1.0 g

Dissolve in distilled water to 1 liter. Do not adjust pH (final pH should be 8.3).

APPENDIX B

Iodine's Solution

Iodine solution

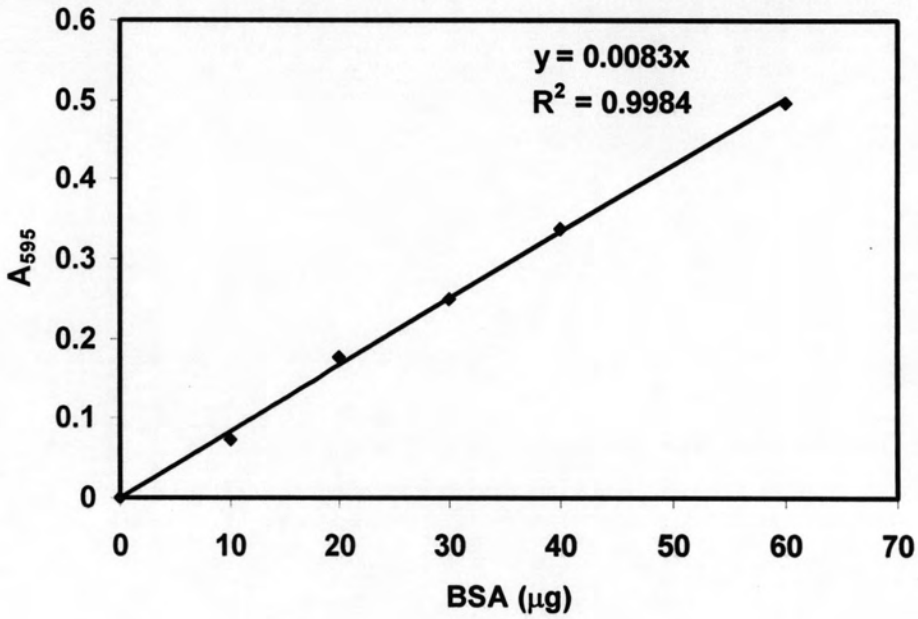
1% Potassium iodide; 0.1% Iodine

Potassium iodide	1 g
Iodine	0.1 g
Adjust to 100 ml distilled water	

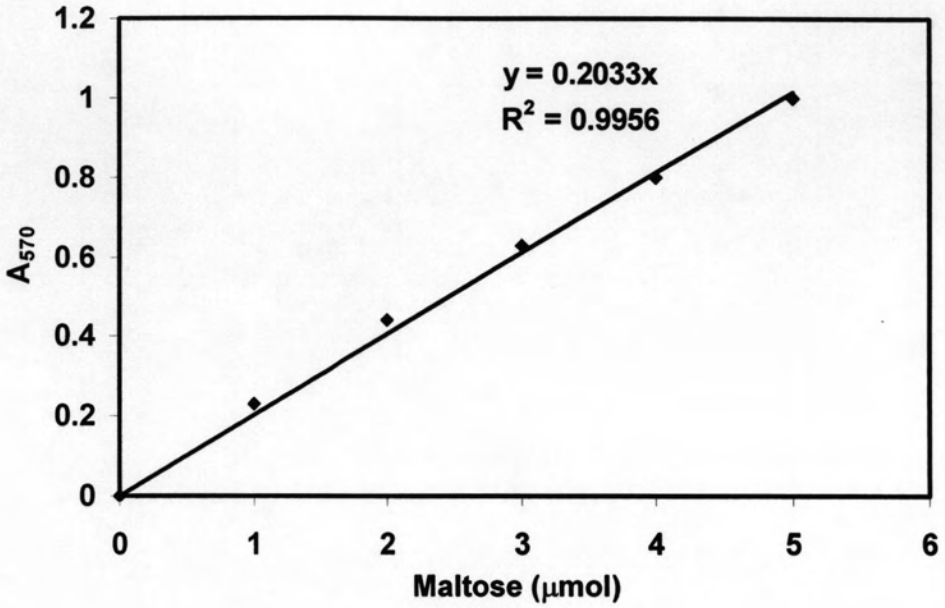
APPENDIX C

Calibration curves

1. Calibration curve of protein concentration (Bradford method)



2. Calibration curve of maltose (DNS method)



BIOGRAPHY

Miss Thippawan Kornsiripanya was born on May 4th, 1982 in Bangkok. She finished the secondary school at Krathumbaen Wisetsamutthakun School, Samuthsakorn and enrolled in the Faculty of Science Mahidol University in 2000 and graduated with B.Sc. in Biology in 2004. She continued for M.Sc. in Biochemistry, Faculty of Science Chulalongkorn University in the same year.