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APPENDICES

Appendix 1 Preparation for 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

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 1 M 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 1 M 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 1 M 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) Denaturing PAGE (SDS-PAGE)

10.0 % separating gel

30% Acrylamide solution	2.50 ml
Solution B (SDS-PAGE)	2.50 ml
Distilled water	2.39 ml
10% $(NH_4)_2S_2O_8$	100 µl
TEMED	10 µl

5.0% stacking gel

30% Acrylamide solution	0.84 ml
Solution C (SDS-PAGE)	1.0 ml
Distilled water	3.1 ml
10% $(NH_4)_2S_2O_8$	50 µl
TEMED	10 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 buffers was added to four parts of sample. The mixture was heated for 5 minutes in boiling water prior to loading to the gel.

Electrophoresis buffer, 1 litre

Tris (hydroxylmethyl)-aminometane	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 2 Preparation for buffer solution

0.2 M Sodium Acetate pH 4.0, 5.0 and 6.0

CH_3COONa	1.21 g

Adjusted volume to 100 ml with distilled water. Adjusted to pH 4, 5 or 6 by 0.2 M acetic acid

0.2 M Phosphate pH 6.0

KH_2PO_4	3.28 g
K_2HPO_4	0.16 g
Distilled water	100 ml

0.2 M Phosphate pH 7.0

KH_2PO_4	1.35 g
K_2HPO_4	1.67 g
Distilled water	100 ml

0.2 M Phosphate pH 8.0

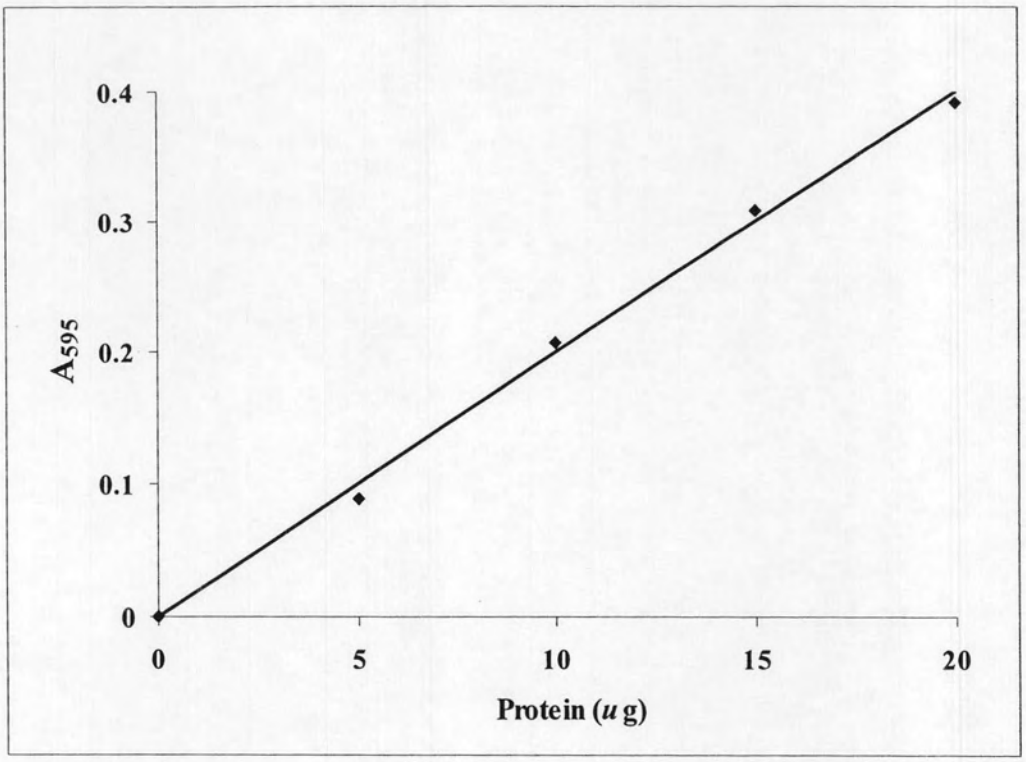
KH_2PO_4	0.48 g
K_2HPO_4	2.34 g
Distilled water	100 ml

0.2 M Tris-Glycine NaOH pH 8.0, 9.0 and 10.0

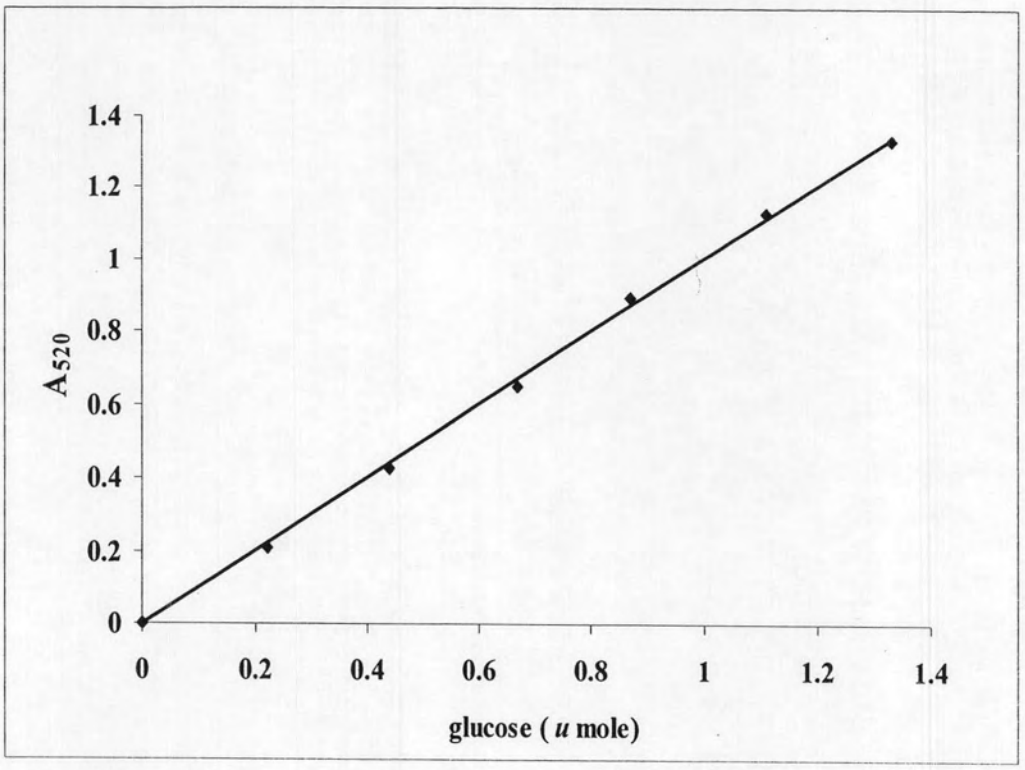
glycine	1.5 g

Adjusted to pH 8.0, 9.0 and 10.0 by 1 M NaOH and adjusted volume to 100 ml with distilled water.

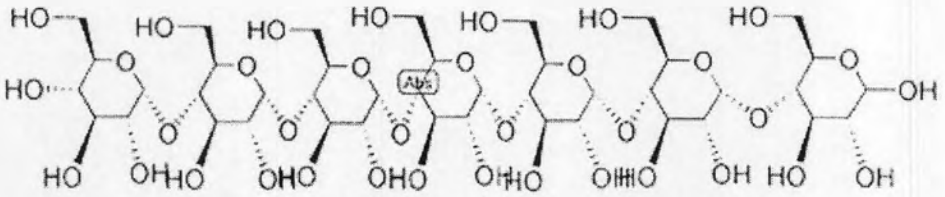
Appendix 3 Standard curve for protein determination by Bradford's method



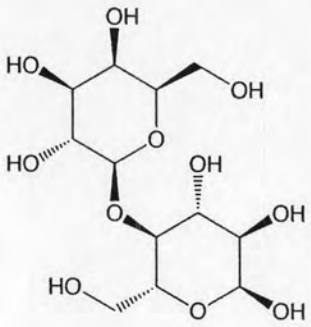
Appendix 4 Standard curve for glucose determination by Somagyi-Nelson 's method (1990)



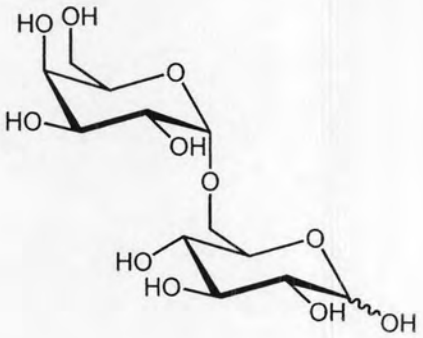
Appendix 5 Structure of saccharides acceptor.



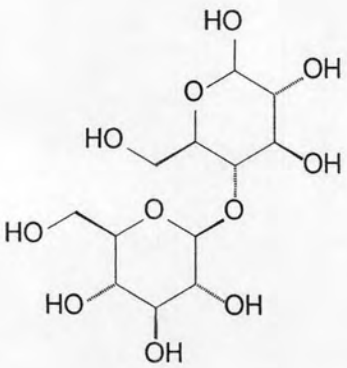
Maltoheptaose



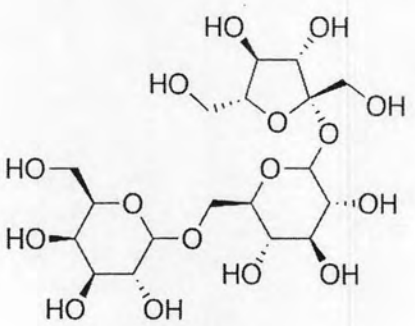
Lactose



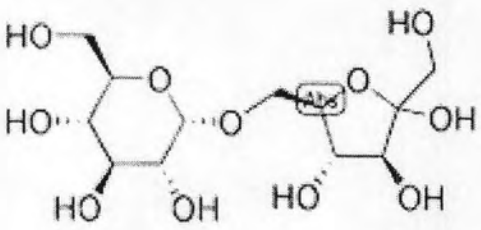
Melibiose



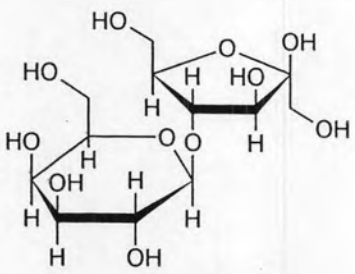
Cellobiose



Raffinose



Palatinose



Lactulose

BIOGRAPHY

Miss Pitchanan Nimpiboon was born on September 18th, 1980. She graduated with the Bachelor's degree of Science from the Department of Biotechnology at Ramkhamhang University in 2003, and continued studying for the Master degree of Science in Biotechnology program, Faculty of Science at Chulalongkorn University in 2005.