APPENDIX A

10% glucose (w/v)

10 g of glucose [Sigma] was dissolved in 100 ml dH_20. The solution was then diluted to obtain solutions with concentrations of 2, 4 and 6 %

10% sucrose (w/v)

20 g of sucrose [Sigma] was dissolved in 200 ml dH_20. The solution was then diluted to obtain solutions with concentrations of 2, 4 and 6 %

Chloramphenicol ($1g\ l^{-1}$)

0.1g of chloramphenicol [Sigma] was added into 100 ml dH_20. Chloramphenicol was added to the sugar solutions to a final concentration of $0.1mg\ l^{-1}$.

AOA (1mM)

0.9mg of AOA [Sigma] was dissolved in 10 ml of dH_20. Serial dilution was done to obtain solutions with concentrations of 0.1, 0.05 and 0.025 mM.

STS (0.1M)

i) 0.1 M sodium thiosulphate [BDH] was prepared by dissolving 1.58 g of sodium thiosulphate in 100 ml of dH_2O
ii) 0.1 M silver nitrate [Sigma] was prepared by dissolving 1.7 g of silver nitrate into 100 ml dH_2O

STS solution (1mM) was prepared by adding 0.1M silver nitrate to 0.1M sodium thiosulphate at a ratio of 1:4.The solution was then diluted to obtain solutions with concentrations of 0.2, 0.4, 0.6, 0.8 and 1.0 mM

Glucose Reagent

Reagent I

(i) 1.65g sodium phosphate dibasic [BDH]

(ii) 1.09g sodium phosphate [BDH]

(iii) 2.4mg peroxidase (POD) [Sigma]

(iv) 0.092g glucose oxidase (GOD) [Sigma]

(i) – (iv) were dissolved in 150 ml of dH_2O.

Reagent II

1.0 mg of dianicyanidin hydrochloride was dissolved in 2.0 ml of dH_2O.

The Glucose Reagent was obtained by adding 0.5 ml of *Reagent II* to 50.0 ml of *Reagent I*.

Standard Glucose Solution

100 mg of glucose [Sigma] was added to 25.0 ml of perchloric acid (70%) [BDH] and the volume made up to 1000 ml with dH_20.

Copper Reagent

Reagent A

(i) 25.0 g potassium sodium tartrate [BDH]

(ii) 25.0 g sodium carbonate [Sigma]

(iii) 20.0 g sodium bicarbonate [Sigma]

(iv) 200.0 g sodium sulphate [BDH]

(i) - (iv) were dissolved in 800 ml of dH_2O and then made up to 1000 ml

Reagent B

15.0 g sulphate pentahydrate [Sigma] was dissolved in 100 ml of dH_20

Copper Reagent was obtained by adding 1.0 ml of *Reagent B* to 25.0 ml of *Reagent A.*

Arsenomolybdate Solution

(i) 25.0 g ammonium molybdate [BDH]

(ii) 21.0 ml of conc. sulphuric acid [Systerm]

(iii) 3.0 g sodium hydrogen arsenate [Sigma]

Solution (i) was dissolved in 450 ml of dH_20 and (ii) was added slowly to (i) while mixing the solution. This was followed by the addition of (iii). The mixture was incubated at 37^0C for 24 hours before use.

Sodium acetate 0.2M

0.0328 g of sodium acetate [Sigma] was dissolved in 2 ml of SDW. The pH was then adjusted to 4.5.

Sodium chloride 0.2M

0.1 g of sodium chloride [Sigma] was dissolved in 10 ml of SDW.

1% Polygalacturonic acid

0.03 g of polygalacturonic acid [BDH]was dissolved in 3 ml of SDW.

DNS solution

i) 0.315 g of dinitrosalicyclicacid 0.63% [Sigma]
ii) 0.25 ml phenol 0.5% [Systerm]
iii) 0.25 g of sodium bisulfite 0.5% [Sigma]
iv) 1.07g of sodium hydroxide 2.14% [Sigma]
v) 50 ml of SDW

40% Rochelle salt

4 g of potassium sodium tartrate [BDH] was dissolved in 10 ml of SDW.

Sodium acetate 4mM

0.01 g of sodium acetate [Sigma] was dissolved in 30ml of SDW. pH was adjusted to 4.5.

1% Polygalacturonic acid

0.03g of polygalacturonic acid [Sigma] was dissolved in 3ml of SDW. pH was adjusted to 4.5.

Sodium hydroxide solution 2M

8 g of sodium hydroxide [Sigma] powder was dissolved in 100 ml of SDW.

0.01% (W/V) Pectin solution

1 g of pectin [BDH] was dissolved in 100 ml of SDW. The solution was then heated to 40ºC while continually stirring. Then pH is adjusted to 7.5 using 2M sodium hydroxide.

Potassium hydrogen phosphate(0.003M) pH7.5

i) 0.04 g of potassium hydrogen phosphate [Sigma] in 100 ml SDW.
ii) 0.05 g of K_2HPO_4dipotassium hydrogen phosphate [Sigma] in 100 ml SDW.
iii) 16.6 ml of (i) and 83.4 ml of (ii) were mixed together.

0.01% (W/V) Bromothymol blue

1 ml bromothymol blue 0.1% [Systerm] was added in 9 ml potassium hydrogen phosphate (0.003M) pH7.5.

0.15M Sodium chloride (NaCl)

0.17 g of sodium chloride [Sigma] was dissolved in 20ml SDW.

1mM Galacturonic acid monobasic ($C_6H_{10}O_7$)

0.02g of galacturonic acid monobasic [BDH] was dissolved in 100ml of SDW.

Glucose standard solution (1mg/ml)

0.1 g glucose [Sigma] dissolved in 100 ml distilled water.

DNS solution

i) 0.315 g of dinitrosalicyclic acid 0.63% [Sigma]
ii) 0.25 ml phenol 0.5% [Systerm]
iii) 0.25 g sodium bisulfite 0.5% [Sigma]
iv) 1.07 g of sodium hydroxide 2.14% [Sigma]
v) SDW 50 ml

40% Rochelle salt

4g of Potassium sodium tartrate was dissolved into 10 ml of SDW.

Protein extraction buffer

The following chemicals were added:

5 ml tris-HCl [Sigma] pH 7.5

10 ml glycerol [BDH]

0.03 g EDTA [Sigma]

2.9 g sodium chloride [Sigma]

0.5 ml triton –X [Systerm]

0.07 ml mercaptoethanol [Sigma]

dH_2O was finally added to make the volume up to 100 ml. The solution was autoclaved and stored at 4^oC until use. Right before use, 0.1 ml 10 mM Leupeptin [Sigma] and 0.015 g DTT [BDH] were added.

Bradford reagent

The following chemicals were added:
0.2 g coomasie blue G250 [Sigma]

10 ml 95% ethanol [Systerm]

20 ml 85% phosphoric acid [Systerm]

dH_2O was added to make the volume up to 200 ml. Finally the solution was filtered with a Whatman Filter paper.

SDS PAGE stacking gel

The stacking gel was prepared by adding the following:

6.1 ml dH_2O

2.5 ml 0.5M tris HCL [Sigma] pH 6.8

0.1 ml 10% sodium dodecyl sulphate [Sigma]

1.3 ml 30% bis-acrylamide [BDH]

0.05 ml ammonium persulphate [Sigma]

0.01 ml TEMED [BDH]

The solution was mixed by gentle swirling and then transferred to the casting chamber using a pasteur pipette. Water was immediately pipetted on to the acrylamide solution. The gel was then left on the bench to polymerize for at least 30 minutes. When the gel has polymerized, the gel in its casting chamber was tilted to decant the water layer. Any remaining water was dried using a Whatman paper.

10% SDS Page separating gel

The separating gel was prepared by adding the following:

4.02 ml dH_2O

2.5 ml 0.5 M tris HCL [Sigma] pH 8.8

0.1 ml 10% sodium dodecyl sulphate [Sigma]

3.33 ml 30% bis-acrylamide [BDH]

0.05 ml ammonium persulphate [Sigma]

0.005 ml TEMED [BDH]

The solution was mixed by gentle swirling and then transferred to the casting chamber using a pasteur pipette. A 0.8 mm comb was inserted. Care was taken to ensure that no bubbles were trapped beneath the comb. The gel was then left on the bench to polymerize for at least 30 minutes.

Protein Sample buffer

4 ml dH_2O

1.0 ml 0.5 M tris HCL [Sigma] pH 6.8

0.8 ml glycerol [BDH]

1.6 ml 10% sodium dodecyl sulphate [Sigma]

0.4 ml mercaptoethanol [Sigma]

0.2 ml 0.05% bromophenol blue [Sigma]

SDS PAGE 5x running buffer

15 g tris base [Sigma]

72 g glycine [BDH]

5g sodium dodecyl sulphate [Sigma]

dH_2O was added to make the volume up to 1 L

SDS PAGE Coomasie staining buffer

0.1 g coomasie blue R-250 [Sigma]

40 ml ethanol [Systerm]

10 ml acetic acid [Systerm]

dH_2O was added to make the volume up to 100 ml

SDS PAGE destaining buffer

40 ml ethanol [Systerm]

10 ml acetic acid [Systerm]

dH_2O was added to make the volume up to 100 ml.