APPENDIX

APPENDIX A

1. Reducing sugar determination

Reducing sugar was determined by DNS method (Miller, 1959).

1.1 Reagents

1.1.1 DNS solution was prepared by dissolved 10 g 3, 5-Dinitrosalicylic acid in 1 N NaOH 200 ml. 300 g Sodium potassium tartrate was added, mixed until it dissolved well and made volume to 1,000 ml with distilled water.

1.1.2 Glucose stock solution 2 mg/ml

1.2 Method

1.2.1 200 µl of sample was mixed with 200 µl DNS solution in test tube.

1.2.2 Boiled the test tube containing reaction mixture in boiling water for 5 min.

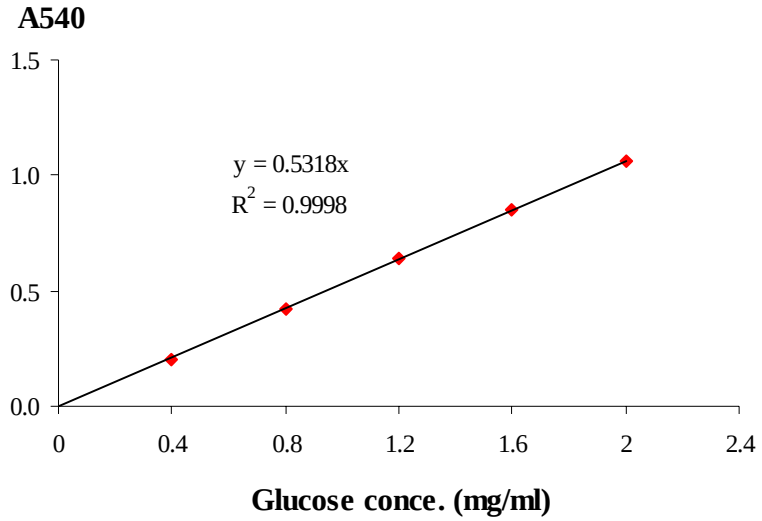
1.2.3 After 5 min boiling, cool down the test tune immediately in ice-bath.

1.2.4 Added 2 ml of distilled water and mixed well.

1.2.5 Absorbance was measured by spectrophotometer at 540 nm.

1.3 Standard curve of reducing sugar

Standard glucose and xylose solution were prepared in the concentration of 0.4, 0.8, 1.2, 1.6 and 2.0 mg/ml. The reactions were carried out with the same method as described in 1.2. The standard curves were plotted absorbance against standard glucose concentration as Appendix Figure A1.



Appendix Figure A1 Standard curve of glucose concentration

2. Protein determination

Protein concentration was measured by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard.

1.1 Reagents

2.1.1 Reagent A: 2 % Na_2CO_3 in 0.1 N NaOH

2.1.2 Reagent B: 0.5 % $CuSO_4.5H_2O$

2.1.3 Reagent C: 1 % Sodium potassium tartrate

2.1.4 Reagent D: mixed 100 ml of Reagent A, 1 ml of Reagent B and 1 ml of Reagent C.The solution should be freshly prepare before used.

2.1.5 Folin-ciocalteu phenol reagent: dilulted: 1 with distilled water to the final concentration of 1 N.

2.1.6 Bovine serum albumin stock solution (200 µg/ml): dissolved 20 mg of bovin serum albumin with distilled water and the solution was made up to 100 ml in volumetric flask.

2.2 Method

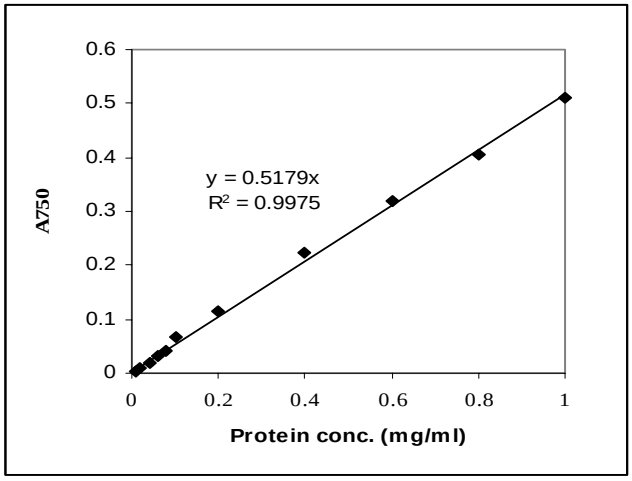
2.2.1 50 µl of sample was mixed with 1.5 ml Reagent D and incubated at room temperature for 10 min.

2.2.2 After 10 min incubation, 150 µl of 1 N Folin- ciocalteu phenol reagent was added to the sample and vortexed immediately and further incubated at room temperature for 30 min.

2.2.3 Absorbance was measured by spectrophotometer at 750 nm.

2.3 Standard curve of protein

Standard bovin serum albumin solution was prepared in the concentration of 10, 20, 40, 60, 80, 100, 200, 400, 600, 800 and 1,000 µg/ml. The reactions were carried out with the same method as described in 2.2. The standard curve of protein was plotted absorbance against standard bovin serum albumin concentration as Appendix Figure A2.



Appendix Figure A2 Standard curve of protein using Lowry method

3. Electrophoresis

3.1 Reagents

3.1.1 Acrylamide/Bis (30 % T, 2.67 % C): 14.6 g Acrylamide and 0.4 g N'N'-bis-methylene-acrylamide were dissolved in 50 ml of distilled water (keep at 4º C).

3.1.2 1.5 M Tris-HCl, pH 8.8: 18.15 g Tris base was dissolved in 50 ml of distilled water, adjusted pH to 8.8 with 6 N HCl before made volume to 100 ml (keep at 4º C).

3.1.3 0.5 M Tris-HCl, pH 6.8: 6 g Tris base was dissolved in 50 ml of distilled water, adjusted pH to 6.8 with 6 N HCl before made volume to 100 ml (keep at 4º C).

3.1.4 10 % SDS: 10 g SDS was dissolved in 50 ml of distilled water and made volume to 100 ml (keep at room temperature).

3.1.5 5X electrode (Running) buffer, pH 8.3: 6 g Tris base, 43.2 g Glycine and 3 g SDS were dissolved in 600 ml of distilled water (keep at 4º C). One-fifth dilution before used.

3.1.6 Sample buffer: (keep at room temperature) was prepared as list in Appendix Table A1.

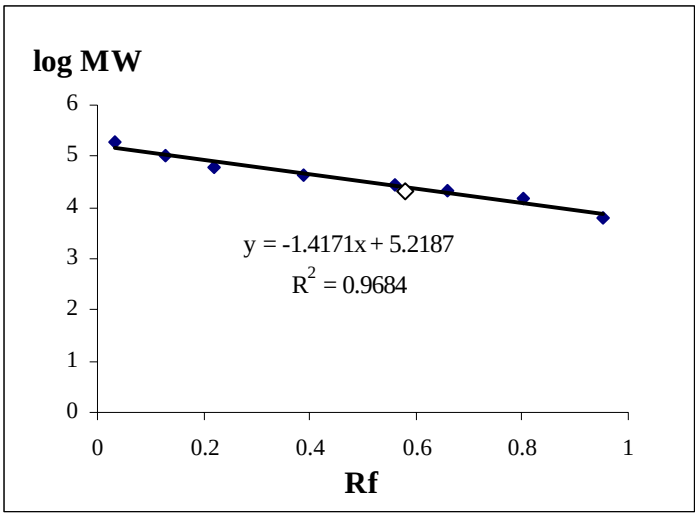
Appendix Table A1 Sample buffer preparation

Component	SDS-PAGE	PAGE
Deionized water	1.9 ml	2.9 ml
0.5 M Tris-HCl, pH 6.8	0.5 ml	0.5 ml
Glyceral	0.4 ml	0.4 ml
10 % SDS	0.8 ml	-
2-Mercaptoethanol	0.2 ml	-
1 % (w/v) Bromophenol blue	0.2 ml	0.2 ml

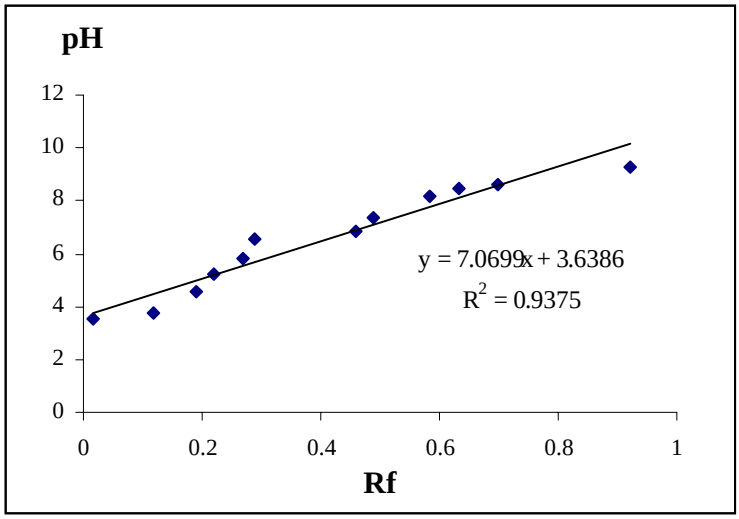
3.2 Separating gel and stacking gel preparation as shown in Appendix Table A2

Appendix Table A2 Acrylamide gel electrophoresis preparation

Component	SDS - PAGE		Native - PAGE	
	7.5 %	4 %	7.5 %	4 %
Deionized water	9.7 ml	6.1 ml	9.9 ml	6.2 ml
0.5 M Tris-HCl, pH 6.8	5.0 ml	2.5 ml	5.0 ml	2.5 ml
10 % SDS	200 µl	100 µl	-	-
Acrylamide/ Bis	5.0 ml	1.33 ml	5.0 ml	1.33 ml
TEMED	10 µl	5 µl	10 µl	5 µl
10 % Ammonium persulfate	200 µl	100 µl	200 µl	100 µl



Appendix Figure A3 Molecular weight determination using prestained marker on 10 % gel SDS-PAGE



Appendix Figure A4 pH Determination using Broad pI calibration kit on precast gel containing phamalyte 3.0 – 10.0

4. <u>Lineweaver – Burk plots</u>

To determine Michealis-Menten constant (K_m), which is an unique character of an individaul enzyme, the reactions of purified J1 and partial purified J2 were seperately performed with various concentration of barley β-glucan from 0.2 - 1 % (w/v) and followed up every 5 min at the time interval of 0-30 min. The activities were ploted against time as shown in Appendix Figure A5 and 7. Each slope was determined as each reaction rate as listed in Appendix Table A3 and 4.

K_m and V_{max} were determined from Lineweaver – Burk plots by using inverse values of substrate concentration (1/[S]) and reaction rate (1/[V]) as shown in Appendix Figure A6 and 8.