APPENDIX A

Reducing sugar determination by DNS method

Chemical reagents

DNS (Dinitrosalicylic acid)	10.0 g
Na_2SO_3	0.5 g
Na-K tartrate	182.0 g
NaOH	10.0 g
Phenol	2.0 g
Distilled water	1.0 l

DNS solution preparation:

1. Dissolve NaOH in 700 ml of distilled water
2. Add Na-K tartrate, stir until well dissolve
3. Add DNS and stir continuously
4. After all DNS is well dissolved add Na_2CO_3 and phenol, respectively
5. Adjust to final volume of 1l with volumetric flask
6. Keep DNS solution in brown glass bottle.

Reducing sugar determination procedure

1. Mix 1 ml of sample with 1 ml of DNS solution and boil for 10 min.
2. Cool down the sample by immerse the sample tube into cold water immediately, add 5 ml of water, mix well, and measure A_{540}
3. Convert A_{540} to reducing sugar concentration with standard curve

Reducing sugar determination by Somogyi and Nelson method

Chemical reagents

Copper reagent:

1. 10 % $CuSO_4 \cdot 5H_2O$ 100 ml
2. Phosphate-tartrate solution:

   Dissolve NH_2HPO_4 28 g (or $NH_2HPO_4 \cdot 12H_2O$ 70.5495 g) in 700 ml of distilled water, add Na-K tartrate (tetrahydrate) 40 g, 1N NaOH 100 ml, and Na_2SO_4 (anhydrous) 120 g, respectively. Adjust to a final volume of 900 ml, store at a room temperature 2 days. If any precipitant occurs, filtrate the solution with Whatman No.4 filter paper
3. Mix solution 1 with solution 2

Nelson's asenomolybdate color reagent:

1. Dissolve Ammoniummolybdate $[(NH_4)_6Mo_7O_{24} \cdot H_2O]$ 25 g in distilled water of 450 ml and add 21 ml of Conc. Sulfuric acid
2. Dissolve Disodium Arsenate $(Na_2HAsO_4 \cdot 7H_2O)$ 3 g in distilled water of 25 ml
3. Mix solution 1 with solution 2 and store at 37 $^{\circ}C$, for 48 h before transfer to a brown color bottle. This solution is called Nelson's color reagent.

Reducing sugar determination

1. Mix 1 ml of sample with 1 ml of Copper reagent and boil for 10 min.
2. Cool down the sample by immerse the sample tube into cold water immediately and add 1 ml of Nelson's asenomolybdate color reagent, mix well
3. Add 5 ml of water, mix well, and measure A_{520}
4. Convert A_{520} to reducing sugar concentration with standard curve

Standard curve preparation

Xylose standard solution preparation

1. Dissolve 0.100 g of xylose in 50 mM Citrate buffer pH 6.0
2. Adjust to final volume of 100 ml with the same buffer in volumetric flask
3. Prepare xylose in various concentrations by using table A1.
4. Determine the amount of reducing sugar by the method previously mentioned

Table A1 Xylose standard solution preparation for standard curve

Xylose concentration (μg/ml)	Xylose solution (μl)	50 mM Citrate buffer pH 6.0 (μl)
1000	1000	0
900	900	100
800	800	200
700	700	300
600	600	400
500	500	500
400	400	600
300	300	700
200	200	800
100	100	900
50	50	950
25	25	975
0	0	1000
Total volume	1000 μl	

APPENDIX B

Protein determination by Lowry method

Chemical reagents

Solution A: 1% (w/v) $CuSO_4 \cdot 7H_2O$

Solution B: 2% (w/v) Na-K tartrate

Solution C: 0.2 N NaOH

Solution D: 4% (w/v) Na_2CO_3

Folin-ciocalteau reagent

Reagent preparation

1. Solution E

   Mix 49 ml of solution C with 49 ml of solution D, and then add 1 ml of each solution A and solution B, respectively. Solution E must be prepared fresh daily.

2. Folin-ciocalteau solution

   Dilute folin-ciocalteau reagent 1:1 with water

Protein determination

1. Put 0.5 ml of protein sample in test tube, water as a blank, and then add solution E 2.5 ml: mix well, and leave at room temperature for 10 minutes.
2. Add diluted folin-ciocalteau solution 0.25 ml: mix immediately and leave at room temperature for 40 min
3. Measure absorbance at 750 nm

Standard curve preparation

Protein standard solution preparation

1. Dissolve 0.100 g of Bovine serum albumin Fraction V (BSA) in 50 mM Citrate buffer pH 6.0
2. Adjust to final volume of 100 ml with the same buffer in volumetric flask
3. Prepare BSA in various concentrations by using table B1
4. Determine the amount of protein by the procedure mention above

Table B1 Protein standard solution preparation for standard curve

BSA concentration (mg/ml)	BSA solution (µl)	Distilled water (µl)
1.0	1000	0
0.9	900	100
0.8	800	200
0.7	700	300
0.6	600	400
0.5	500	500
0.4	400	600
0.3	300	700
0.2	200	800
0.1	100	900
0	0	1000
Total volume	1000 µl	

APPENDIX C

Determination of protein molecular weight by SDS-PAGE

Stock solution preparation

1. Acrylamide solution:

   Dissolve 29.2 g of acrylamide in 50 ml of distilled water.

2. Bis-acrylamide solution:

   Dissolve 0.8 g of Bis-acrylamide in 50 ml of distilled water.

3. 1.5 M Tris-HCl, pH 8.8, concentrated resolving gel buffer:

   Dissolve 18.2 g of Tris base in ~80 ml of water, adjust to pH 8.8 with HCl, and adjust to a final volume of 100 ml with water. Store at 4 ^{o}C.

4. 0.5 M Tris-HCl, pH 6.8, concentrated stacking gel buffer:

   Dissolve 6.1 g of Tris base in ~80 ml of water, adjust to pH 6.8 with HCl, and adjust to a final volume of 100 ml with water.

5. 10% (w/v) Sodium dodecyl sulfate (SDS):

   Dissolve 10 g of SDS in ~60 ml of water and adjust to a final volume of 100 ml with water.

6. Loading buffer:

Water	4.8	ml
0.5 M Tris-HCl, pH 6.8	1.2	ml
10% SDS	2.0	ml
Glycerol	1.0	ml
0.5 % Bromophenol Blue (w/v water)	0.5	ml

Store at room temperature. The SDS-reducing buffer is prepared by adding 50 µl of 2-mercaptoethanol to each 0.95 ml of stock sample buffer before use

Catalyst

1. 10% APS: Dissolve 100 mg Ammonium persulfate (APS) in 1 ml of water. Prepare the APS solution fresh daily
2. N,N,N',N' tetramethylethylenediamine (TEMED): Use TEMED undiluted from the bottle. Store cool, dry, and protected from light

Electrode buffer

5 X concentration of electrode buffer consisting of 15 g Tris, 72 g glycine, and 5 g SDS per 1 l. of water. The concentrated buffer must be stored in glass container. To use, dilute with four part or water.

Stacking gel and Resolving gel

The formula for stacking gel and resolving gel preparations are shown in table C1 and C2, respectively.

Table C1 Formulation of stacking gel

	Solutions volume	
H_2O	6.10 ml	3.05 ml
0.5 M Tris-HCl, pH 6.8	2.50 ml	1.25 ml
Acrylamide	0.65 ml	0.325 ml
Bis-Acrylamide	0.65 ml	0.325 ml
10% SDS	0.10 ml	0.05 ml
10% APS	50 µl	25 µl
TEMED	10 µl	5 µl
Total volume	10 ml*	5 ml*

* Use 5 ml formulation for 1 gel preparation, 10 ml formulation of 2 gels preparation

Table C2 Formulation of resolving gel

Component	Gel concentration				
	7.5%	10%	12%	15%	18%
H_2O	4.85 ml	4.05 ml	3.35 ml	2.345 ml	1.345 ml
1.5 M Tris-HCl pH 8.8	2.5 ml	2.5 ml	2.5 ml	2.5 ml	2.5 ml
10 % SDS	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml
Acrylamide	1.25 ml	1.65 ml	2 ml	2.5 ml	3 ml
Bis-acrylamide	1.25 ml	1.65 ml	2 ml	2.5 ml	3 ml
10% APS	50 µl	50 µl	50 µl	50 µl	50 µl
TEMED	5 µl	5 µl	5 µl	5 µl	5 µl
Total volume	10 ml	10 ml	10 ml	10 ml	10 ml

Gel staining solution:

1. Coomassie gel stain solution: dissolve 1 g of Coomassie brilliant blue R-250 in the mixture of 400 ml of methanol, 100 ml of glacial acetic acid and 500 ml of distilled water.
2. Coomassie gel destain solution: a mixture of 400 ml of methanol, 100 ml acid and 500 ml of water.

Procedure

Gel casting

1. Clean glass plates and mount to the casting stand.
2. Prepare the resolving gel and pipette into the glass cassette until gel reaches the desire height
3. Add distilled water on the top of the gel and let it polymerizes for 45 min, the interface between water and gel will appear when the polymerization complete.
4. Removes distilled water with tissue paper
5. Prepare the stacking gel and pipette into the top of polymerized resolving gel.
6. Insert the comb and let the stacking gel polymerized
7. After the polymerization finish, remove comb and rinse the wells with distilled water to remove bubbles.

Sample preparation

1. Prepare loading buffer by mix 50 µl of 2-mercaptoethanol to each 0.95 loading buffer, this step can be omitted, if reduction of disulfide bonds is not desired.
2. Mix loading buffer 1 ml with 1 ml of a protein sample, to avoid distort band of protein, sample should be desalted before loading into gel.
3. Heat the diluted samples at 95 °C for 4 min by suspending the sample tubes in hot water. Do not store prepared sample.

Electrophoresis

1. Mount the gel cassette into the electrophoresis tank
2. Fill the tank with the electrode buffer
3. Load samples into wells
4. Cover the lid and plug the leads into the electrophoresis power supply
5. Switch the power supply on and adjust the voltage to 100 V

6. Let the electrophoresis run until the blue front of loading buffer reaches the end of gel
7. Turn off the power supply and disassemble gel cassette
8. Disassemble the glass plate and take the gel off.
9. Immerse the gel into staining solution for 15 min
10. De-stain the background color by immerse the stained gel into de-staining solution

Activity stain

1. Mix 1 ml of loading buffer, which no mercaptoethanol content, with 1 ml of a protein sample
2. Heat the sample at 70 °C for 10 min
3. Electrophoresis is carried out as previously described
4. After electrophoresis, to remove SDS, wash gel for 30 min twice with 100 ml of 50mM Citrate buffer pH 6.0 containing 2.5% (v/v) of Triton X 100
5. Wash gel for 30 min twice with 100 ml of 50 mM Citrate buffer pH 6.0
6. Immerse washed gel in 100 ml of 0.5%(w/v) of beech wood xylan in 50 mM Citrate buffer pH 6.0 and incubated at 55 °C for 30 min
7. The gel is stained with 100 ml of Congo red (100mg/ml) for 15 min and de-stained in 1 M NaCl until bands of activity, yellow halos on orange background, become visible.
8. Stop the de-staining by adding 2 ml of 0.5% (v/v) glacial acetic acid until the gel color turns from orange to purple.

APPENDIX D

Table D1 Final Concentration of Ammonium Sulfate: Percentage Saturation at 0 °C

Initial concentration of ammonium sulfate (percentage saturation at 0 degree C)	Percentage saturation at 0 degree C)																
	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
	Solid ammonium sulfate (grams) to be added to 1 liter of solution																
0	106	134	164	194	226	258	291	326	361	398	436	476	516	559	603	650	697
5	79	108	137	166	197	229	262	296	331	368	405	444	484	526	570	615	662
10	53	81	109	139	169	200	233	266	301	337	374	412	452	493	356	581	627
15	26	54	82	111	141	172	204	237	271	306	343	381	420	460	503	547	592
20	0	27	55	83	113	143	175	207	241	276	312	349	387	427	469	512	557
25		0	27	56	84	115	146	179	211	245	280	317	355	395	436	478	522
30			0	28	56	86	117	148	181	214	249	285	323	362	402	445	488
35				0	28	57	87	118	151	184	218	254	291	329	369	410	453
40					0	29	58	89	120	153	187	222	258	296	335	376	418
45						0	29	59	90	123	156	190	226	263	302	342	383
50							0	30	60	92	125	159	194	230	268	308	348
55								0	30	61	93	127	161	197	235	273	313
60									0	31	62	95	129	164	201	239	279
65										0	31	63	97	132	168	205	244
70											0	32	65	99	134	171	209
75												0	32	66	101	137	174
80													0	33	67	103	139
85														0	34	68	105
90															0	34	70
95																0	35
100																	0

Curriculum vitae

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