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APPENDIX I

CHEMICALS AGENTS AND INSTRUMENTS

A. CHEMICAL SUBSTANCES

Acrylamide gel. (Sigma, USA)

Ammonium persulfate ($(NH_4)_2S_2O_8$) (Sigma, USA)

Bovine serum albumin (Sigma, USA)

4-Chloro-1-naphthol (Sigma, USA)

Coomassie brilliant blue R-250 (BDH, England)

Coppersulfate ($CuSO_4.5H_2O$) (Reidel Hamnover, W.Germany)

Disodium hydrogen phosphate ($Na_2HPO_4.12H_2O$) (E. Merck, Darmstadt, W. Germany)

Disodium tartate ($C_4H_4Na_2O_6.2H_2O$) (E. Merck, Darmstadt, W. Germany)

Ethylenediaminetetra-acetic acid (EDTA, disodium salt), (BDH, England)

Fletcher's medium base (Difco, Michigans, USA)

Folin-Ciocalteau phenol reagent (Sigma, USA)

Glacial acetic acid (CH_3COOH) (E. Merck, Darmstadt, W. Germany)

Glycerine ($CH_2OHCHOHCH_2OH$) (Carlo Erba)

Glycine (Sigma, USA)

Hydrochloric acid (HCL) (E. Merck, Dermstadt, W. Germany)

Leptospira EMJH base (Difco, Michigans, USA)

Leptospira EMJH enrichment (Difco, Michigans, USA)

2-mercaptoethanol (Sigma, USA)

Methanol (CH_3OH) (E. Merck, Darmstadt, W. Germany)

N, N' Methylene-bis acrylamide (Sigma, USA)

Nitrocellulose paper (Bio-RAD, California, USA)

Non-fat dry milk (Carnation, USA)

Pilikan-fount india drawing ink (Pelikan, W. Germany)

Potassium chloride (KCl) (E. Merck, Darmstadt, W. Germany)

Potassium dihydrogen phosphate (KH_2PO_4) (E. Merck, Darmstadt, W. Germany)

Peroxidase conjugated swine immunoglobulins to rabbit Igs (Dako, Denmark)

Sodium chloride (NaCl) (BDH, England)

Sodium hydroxide (NaOH) (BDH, England)

Sodium lauryl sulfate (Sigma, USA)

Standard molecular weight markers (MW-SDS-200 kit) (Sigma, USA)

TEMED (N, N, N', N' - Tetramethylenediamine) (Sigma, USA)

Trisma-base (Sigma, USA)

Tween 20 (Sigma, USA)

B. GLASSWARES

Beaker (Pyrex, Corning, N.Y., USA)

Cylinder (Witeg, W. Germany)

Erlenmeyer flask (Pyrex, Corning, N.Y., USA)

Glass tube (Pyrex, Corning, N.Y., USA)

Screw-cap erlenmayer flask (Pyrex, Corning, N.Y., USA)

Serological pipette (Pyrex, Corning, N.Y., USA)

C. INSTRUMENTS

Analytical balance (Precisa, Switzerland)

Dark-field microscope (Olympus, Japan)

Eppendorf microfuge model 5412 (Beckman Instrument, Inc., USA)

Gel Dryer (LKB 2003, Sweden)

Hamilton syringe (Switzerland)

Incubator (Memmert, W. Germany)

Magnetic strirrer

Mixer Vortex-Genie (Scientific industries, N.Y., USA)

PHM 83 Auto cal pH meter (Radiometer, Copenhagen)

Pipette washer (Scientific Apparatus, Philadelphia, PA, USA)

Power supply (LKB 2197, Sweden)

Refrigerated high speed centrifuge (J2-21 centrifuge, Beckman Instrument, Inc., USA)

Spectrophotometer (Coleman Junior II, Coleman instrument, Maywood, Illinois, USA)

Soniprep 150 Ultrasonic Disintegator (MSE Scientific Instrument, Manor Royal, England)

Thermostatic circulator (LKB 2219 Multitemp II, Sweden)

Trans-Blot cell (Bio-RAD, USA)

Vertical electrophoresis (LKB Apparatus 2001, Sweden)

APPENDIX II

MEDIA, REAGENTS AND PREPARATIONS

A. MEDIA

1. Fletcher's medium (Semisolid medium)

Fletcher's medium powder	2.5	gm.
Deionized D. W.	900	ml.

The medium was boiled until dissolved completely and the pH adjusted to 7.9. It was strilized by autoclaving at 121°c for 15 min. cooled to 50-60 °c and 100 ml of inactivated sterile normal rabbit serum was added. The medium was dispended in steriled screw-cap tubes and stored in a refrigerator.

2. Leptospira EMJH medium

Leptospira EMJH BASE	2.3	gm.
Deionized D. W.	900	ml.

The pH of this base was adjusted to 7.5 and autoclaved 121 °c for 15 min. When the temperature of EMJH has decreased, 100 ml of Leptospira EMJH enrichment media was added. Store the medium in a refrigerator.

B. REAGENTS

1. Reagent for preparation of sonic extracted antigen.

1.1 PBS, 0.15 M, pH 7.2

NaCl	8.0	gm.
KCl	0.2	gm
$Na_2HPO_4.12H_2O$)	1.125	gm.
KH_2PO_4	0.2	gm.
Deionized D. W.	1,000	ml.

2. Reagents for protein estimation.

2.1 Solution A (5% Na_2CO_3 in 0.5 N NaOH)

Na_2CO_3	5	gm.
0.5 N NaOH	100	ml.

2.2 Solution B (1% $CuSO_4.5H_2O$)

$CuSO_4.5H_2O$	100	gm.
Deionized D. W.	100	ml.

2.3 Solution C (2% $C_4H_4Na_2O_6.2H_2O$)

$C_4H_4Na_2O_6.2H_2O$	2	gm.
Deionized D. W.	100	ml.

2.4 Solution D

Solution A	20	ml.
Solution B	1	ml.
Solution C	1	ml.

Prepare freshly befor use.

2.5 Folin Ciocalteus phenol reagent.

2 N Folin-Ciocalteu phenol	5	ml.
Deionized D. W.	50	ml.

Prepare freshly before use.

3. Reagents for SDS-PAGE

3.1 Stock Acrylamide, 30%

Acrylamide	30.0	gm.
N, N' - methylene bisacrylamide	0.8	gm.
Deionized D.W.	100	ml.

Remove insoluble material by filtration and store at 4°c in a dark bottle.

3.2 Separating gel, 10%

Stock acrylamide	16.65	ml.
1.5 M. Tris-HCl pH 8.8	12.50	ml.
10% SDS	0.50	ml.
0.2 M, EDTA	0.50	ml.
Deionized D.W.	19.25	ml.
TEMED	0.025	ml.
10% ammonium per sulfate	0.25	ml.

Prepare 10% ammonium per sulfate just before use.

3.3 Stacking gel, 3%

Stock, acrylamide	1.00	ml.
0.5 M, Tris-HCl pH 6.8	2.50	ml.
10% SDS	0.1	ml.
0.2 M, EDTA	0.1	ml.
Deionized D.W.	6.25	ml.
TEMED	0.005	ml.
10% ammonium per sulfate	0.050	ml.

Prepare 10% ammonium per sulfate just before use.

3.4 Sample buffer pH 6.8 (5X)

Tris	0.3784	gm.
SDS	0.5000	gm.
Glycerol	5.0000	ml.
2-mercaptoethanol	2.5	ml.
Bromphenol blue	0.0005%	
Deionized D.W. to	10	ml.

Store in tightly sealed bottle.

3.5 Electrode buffer, pH 8.3

Tris	3.0	gm.
Glycine	14.4	gm.
SDS	1.0	gm.
Dieonized D.W. to	1,000	ml.

3.6 Standard MW Markers

Individual proteins included in the MW-SDS-200 Kit (sigma) are:

	Approx.Mol.Wt. (dalton)
Carbonic anhydrase	29,000
Albumin, egg, (Ovalbumin)	45,000
Albumin, bovine plasma (BSA)	66,000
Phosphorylase B, rabbit muscle	97,400
B-galactosidase, *Escherichia coli*	116,000
Myosin, rabbit muscle	205,000

Reconstitute contents of MW-SDS-200 kit with 1.0 ml of sample buffer. All proteins must be incubated at 37°c for 2 h in sample buffer. Aliquots may be frozen at -20°c for future use.

3.7 Coomassie brilliant blue stain 0.25%

Coomassie brilliant blue R-250	2.5	gm.
Methanol	454	ml.
Glacial acetic acid	92	ml.
D.W.	454	ml.

Dissolve the dye in methanol, then add glacial acetic acid and water. Remove insoluble material by filtration through filter paper. The dye solution can be stored for months at room temperature but any precipatate formed should be removed before use.

3.8 Destaining solution

Methanol	100	ml.
Glacial acetic acid	150	ml.
Deionized D. W.	750	ml.

3.9 Fixing solution for dry gel.

Methanol	400	ml.
Glycerol	50	ml.
Deionized D. W.	550	ml.

4. Reagents for immunoblotting.

4.1 Towbin buffer, pH 8.3

Tris	3.03	gm.
Glycine	14.4	gm.
Methanol	800	ml.

Add deionized D.W. to 1,000 ml.

4.2 PBS, 0.15 M, pH 7.4 containing 0.1% Tween 20 (Washing buffer)

NaCl	8.0	gm.
KCl	0.2	gm.
Na_2HPO_4	1.15	gm.
KH_2PO_4	0.2	gm.
Tween-20	1.0	ml.
Deionized D.W. to	1,000	ml.

4.3 Blocking buffer

Washing buffer	100	ml.
Non-fat dry milk	5	gm.

Dissolve non-fat dry milk in washing buffer and remove insoluble material by filtration through filter paper.

4.4 Sobstrate solution for immunostaining.

4-chloro-1-naphthol	0.006	gm.
Methanol	2.000	ml.
Tris 1.0 M, pH 7.4	10.000	ml.
H_2O_2 30%	0.004	ml.

Dissolve 4-chloro-1-naphthol in methanol, then add substrate buffer (Tris 1.0 M, pH 7.4) and H_2O_2 before use.

5. Reagents for staining of Standard MW. markers.

5.1 PBS, 0.15 M, pH 7.4 containing 0.3% Tween-20 (Washing buffer)

NaCl	8.0	gm.
KCl	0.2	gm.
Na_2HPO_4	1.15	gm.
KH_2PO_4	0.2	gm.
Tween-20	3.0	ml.
Deionized D.W. to	1,000	ml.

5.2 India ink solution

India ink	0.01	ml.
PBS, pH 7.4 containing 0.3% Tween-20	10	ml.

Dissolve india ink in PBS, pH 7.4 (containing 0.3% Tween-20) and remove insoluble material by filtration through filter paper.

APPENDIX III

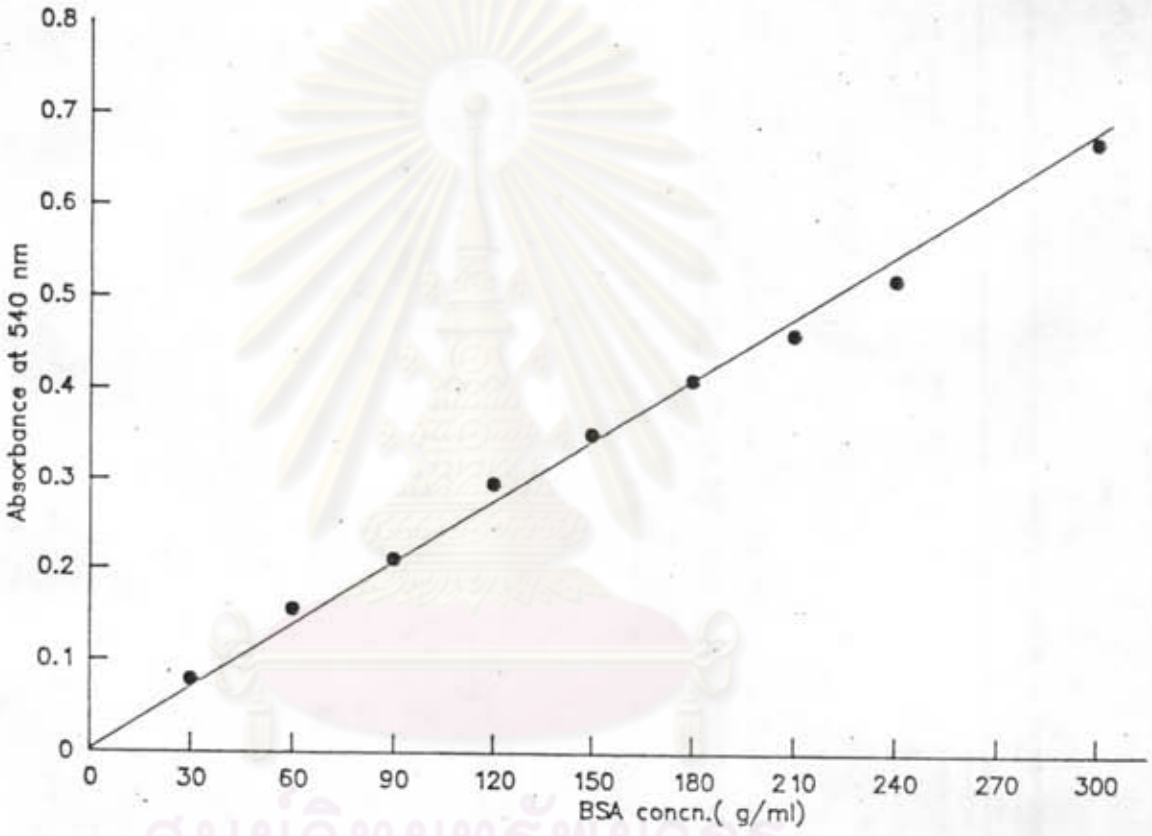

Figure 14 : The standard curve of protein concentration ranging from 30-300 μg of BSA is determined by Lowry method.

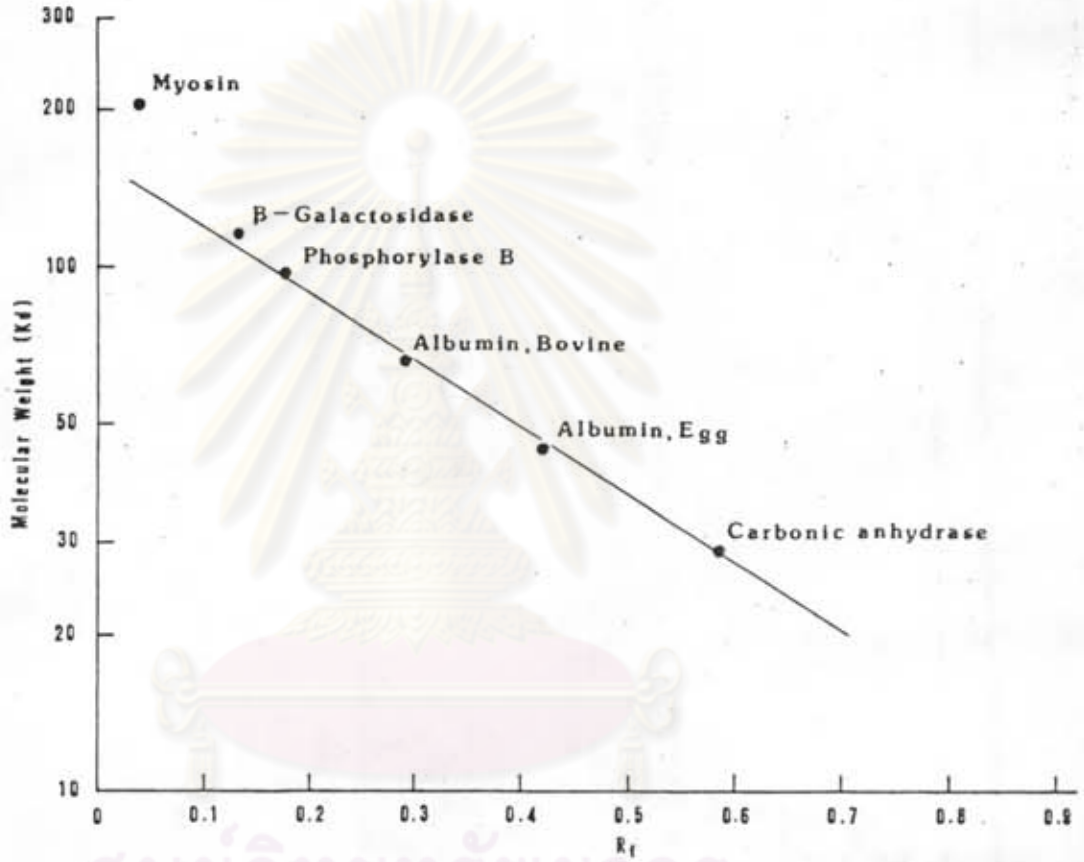

Figure 15 : The relative standard molecular weight curve.

BIOGRAPHY

Miss Chantana Mekseepralard was born on October 15, 1962 in Bangkok, Thailand. She graduated with the Bachelor degree of Science in Medical Technology from Faculty of Medical Technology, Mahidol University in 1984.