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APPENDIX I

CHEMICAL AGENTS AND INSTRUMENTS

A. Chemical substances

Acetic acid (E.Merck, W.Germany)

Acrylamide (Sigma, U.S.A.)

Agarose (Kallestad, MN, U.S.A.)

Amphotericin B (Squibb & Sons, Inc, U.S.A.)

Ammonium persulfate (Sigma, U.S.A.)

Bovine serum albumin (Sigma, U.S.A.)

Bromphenol blue (Sigma, U.S.A.)

Coommassie brilliant blue (Sigma, U.S.A.)

Crystal violet (Sigma, U.S.A.)

4-Chloro-1-napthol (Sigma, U.S.A.)

Disodium hydrogen phosphate (Na_2HPO_4) (E.Merck, W.Germany)

Evans Blue (Sigma, U.S.A.)

EDTA (Ethylenediaminetetraacetic acid) (BDH, England)

Evan blue (Sigma, U.S.A.)

Fetal bovine serum (Flow, Australia)

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

Glyceral ($CH_2OHCHOHCH_2OH$)(BDH, England)

Glycine (Sigma, U.S.A.)

HEPES (N-2-hydroxyethlpiperazine-N-2-ethanesulfonic) (Sigma, U.S.A.)

Hydrochloric acid (HCl)(E.Merck, W.Germany)

India Ink (Pelikan, W.Germany)

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

Minimum Essential Medium (GIBCO, Grand Island, NY, U.S.A.)

2-mercaptoethanol (Sigma, U.S.A.)

Nitrocellulose paper (Bio-RAD, U.S.A.)

Non fat dry milk (Carnation, U.S.A.)

N,N-methylene bisacrylamide (Sigma, U.S.A.)

N,N,N,N-tetramethylthylenediamme (TEMED)(Sigma, U.S.A.)

PPO (2,5-Diphynenylloxoazole)(Sigma, U.S.A.)

POPOP (p-bis (2-(5-phynyloxazolyl)-benzene:NEN)(Sigma, U.S.A.)

Potassium Chloride (KCL)(E.Merck, W.Germany)

Potassium phophate monobasic (KH_2PO_4) (E.Merck, W.Germany)

Penicillin (Dumex, Bangkok, Thailand)

Sodium bicarbonate ($NaHCO_3$)(BDH, England)

Sodium chloride (NaCl)(E.Merck, W.Germany)

Sodium dodecyl sulphate (SDS)(Sigma, U.S.A.)

Sodium phosphate dibasic (Na_2HPO_4)(E.Merck, W.Germany)

Streptomycin (Thai meiji,Bangkok, Thailand)

Tritiated thymidine (Amersham, England)

Tryphan blue (BDH, England)

Tris (Hydroxymethyl aminomethane, Tris:$C_4H_{11}NO_3$) (E.merck, W.Germany)

Trypsin (E.merck, W.Germany)

Tween 20 (Sigma, U.S.A.)

Toluene (E.merck, W.Germany)

B. Antiserum

Peroxidase conjugated swine immunoglobulins to rabbit immunoglobulin (DAKO, Denmark)

Rabbit immunoglobulin to herpes simplex virus type 2 (MS strain)(DAKO, Denmark)

C. Glassware

Beaker (Pyrex, Corning, NY, U.S.A.)

Cylinder (Witeg, W.Germany)

Glass tube (Pyrex, Corning, NY, U.S.A.)

Tissue culture bottle (Kotoboki, Tokyo, Japan)

D. Other

Microcentrifuge tube (Treff AG, Schweiz, Switzerland)

Scintillation vial (Kimbel, IL, U.S.A.)

Tissue culture multi-well plate (Linbro,Flow, U.S.A.)

E. Instruments

Automatic pipet (EFLAB OY, Helsinki, Finland)

Analytical balance (Mettler PC 440, Zurich, Switzerland)

B-counter (LS 100 C,Beckman, U.S.A.)

Bio-freezer (Forma Scientific, U.S.A.)

Centrifuge (IEC CENTRA-7R, Needham Hb.,MA 02194, U.S.A.)

Eppendorf microfuge model 5412 (Beckman Instrument, Inc., U.S.A.)

Hamminton syring 10 uL.

Incubator (Memmert, W.Germany)

Mixer Vortex-Genie (Scientific Industries, NY, U.S.A.)

PH-meter, PHM 83 (Radiometer, Copenhagen, Denmark)

Power supply (LKB 2197, Sweden)

Sonicator, Soniprep 150 (MSE, United, Kingdom)

Spectrophotometer, Coleman Junior II, model 6135, (IL, U.S.A.)

Slab gel dryer (LKB 2003, Sweden)

Trans-blot Electrophoretic Transfer cell (Bio-Rad, U.S.A.)

Vertical electrophoresis unit (Kw, Pack, Medical laboratory Instrument, Thailand)

Water bath, Julabo TWA 12 (Seelbach, W.Germany)

APPENDIX II

REAGENTS AND PREPARATIONS

1. Reagents for cell culture

1.1 Fetal bovine serum (FBS)

The 100 mL of fetal bovine serum was heat inactivated 56 C min and stored at -20 °C.

1.2 HEPES buffer 1 M, pH 73

HEPES	238.3 gm.

Make up to 1,000 mL with deionized distilled water and adjust pH to 7.3 with 5 N NaOH.

The solution was sterilized by membrane filtration, and stored at 4 °C

1.3 7.5 % Sodium Bicarbonate

$NaHCO_3$	75 gm.
Deionized distilled water to	1,000 mL

The solution was sterilized by autoclave at 15 lb pressure for 15 min and stored at 4 °C

1.4 Minimum essential medium (MEM)

The medium was prepared by adding 1 package of powder MEM to 1 liter of deionized distilled water and gently stirred until it was dissolved. Then, the medium

was sterilized immediately by membrane filtration and stored at 4 °C

1.5 Culture medium

1.5.1 Growth media (GM)

MEM 10x	10	mL
Heat-inactivated FBS	10	mL
Penicillin, 20,000 U/mL	0.5	mL
Streptomycin, 20,000 mg/mL	0.5	mL
Amphotericin B, 200 mg/mL	1.0	mL
HEPES, 1 M	2.0	mL
7.5 % $NaHCO_3$	2.0	ml
Deionized distilled water to	100	mL

The medium stored at 4 °C until used.

1.5.2 Maintenance medium (MM)

MEM 10x	10	mL
Heat inactivated FBS	2	mL
Penicillin, 20,000 U/mL	0.5	mL
Streptomycin, 20,000 mg/mL	0.5	mL
Amphotericin B, 200 mg/mL	1	mL
HEPES, 1 M	2	mL
7.5 % $NaHCO_3$	2	mL
Deionized distilled water to	100	mL

The medium stored at 4 °C until used.

1.5.3 MM without serum

MEM 10x	10	mL
Penicillin, 20,000 U/mL	0.5	mL
Streptomycin, 20,000 mg/mL	0.5	mL
Amphotericin B, 200 mg/mL	1	mL
HEPES, 1 M	2	mL
7.5 % $NaHCO_3$	2	mL
Deionized distilled water to	100	mL

The medium stored at 4 °C until used.

1.6 Trypsin-versene solution (T.V-solution)

Trypsin	0.5	gm
EDTA	2.0	gm
NaCl	9.0	gm
Deionized distilled water	1000	mL

1.7 Phosphate buffer saline (PBS), 0.15 M, pH 7.4

NaCl	8.0	gm
KCl	0.2	gm
Na_2HPO_4 (anhydrous)	1.15	gm
KH_2PO_4	0.2	gm
Deionized distilled water	1000	mL

The solution was sterilized by autoclave at 15 lb pressure for 15 min and stored at 4 °C

2. Reagents for ^{3}[H]-thymidine incorporation technique

2.1 Tritiated thymidine solution (^{3}HTdR)

^{3}HTdR, sterile aqueous solution 1 mci/mL, was diluted with 4.9 mL of MM of make the final concentration to 20 uci/mL. The 50 uL of 20 uci/mL thymidine were droped in a well of cell culture to give the final concentration of thymidine to 0.5 uci/well.

2.2 Scintillation fulid

PPO	5	gm
POPOP	0.2	gm
Toluene	2.5	liter

Stored at room temperature

3. Reagents for SDS-PAGE

3.1 Stock Solution

3.1.1 Stock acrylamide

Acrylamide	30	gm
N, N-methylene-bis-acrylamide	0.8	gm
Distilled water	100	mL

The solution stored at 4 °C

3.1.2 Sodium lauryl sulfate (SDS), 10 %

SDS	10	gm
Distilled water	100	mL

The solution stored at room temperature

3.1.3 TEMED

The solution stable as undilute solution and stored at 4°C in the dark.

3.1.4 Ammonium persulfate, 10 %

Ammonium persulfate	0.1	gm
Distilled water	1	mL

The reagent was prepared just before use.

3.1.5 Bromphenol blue, 0.1 %

Bromphenol blue	0.1	gm
Distilled water	100	mL

The reagent stored at 4°C.

3.1.6 Glycine, 2.0 M

Glycine	15.015	gm
Distilled water	100	mL

The reagent stored in refrigerate and dark.

3.1.7 Tris-HCl, 3.0 M, pH 8.8

The solution was prepared by dissolved 36.342 gm of Tris in 70 ml 1 N HCl, adjusted to pH 8.8, then the volume was made up to 100 mL with 1 N HCl.

3.1.8 Tris-HCl, 3.0 M, pH 6.8

The solution was prepared as 3.1.7 but adjusted to pH 6.8.

3.2 Resolving gel, 8%

30 % stock acrylamide	8	mL
3.0 M Tris-HCl pH 8.8	3.75	mL
10 % SDS	0.3	mL
Distilled water	17.65	mL
10 % Ammonium persulfate	225	uL
TEMED	7.5	uL

The solution was prepared just before use.

3.3 Stacking gel, 3 %

30 % stock acrylamide	1	mL
3.0 M Tris-HCl pH 8.8	0.42	mL
10 % SDS	0.1	mL
Distilled water	8.4	mL
10 % Ammonium persulfate	75	uL
TEMED	2.5	uL

The solution was prepared just before use.

3.4 Electrode buffer, pH 8.3

3.0 M Tris-HCl pH 8.8	8.3	mL
2.0 M Stock glycine	96.0	mL
10 % SDS	10.0	mL
Distilled water	886.0	mL

The solution was prepared just before use.

3.5 Sample buffer

3.0 M Tris-HCl pH 8.8	8.3	uL

10 % SDS	0.8	uL
Glycerol	0.4	uL
2-Mercaptoethanol	0.2	uL
0.1 % Bromphenol blue	80	uL
Distilled water	50	uL

3.6 Coommassie brilliant blue R-250, 0.25 %

Coommassie brilliant blue R-250	2.5	gm
Methanol	454	mL
Acetic acid	92	mL
Distilled water	454	mL

Dissolve the dye in methanol first, then add actic acid and distilled water. Remove insoluble material by filtration through Whatman No.1 The dye solution can be stored for months at room temperature but any precipitate formed should be removed before use.

3.7 Destaining solution of 0.25 % Coommassie brilliant blue

Acetic acid	150	mL
Methanol	100	mL
Distilled water	750	mL

3.8 Fixative solution

Methanol	40	mL
Glycerol	5	mL
Distilled water	55	mL

4. Reagent for Western blotting technique (Towbin buffer), pH 8.3

Dissolve 3.03 gm Tris and 14.4 gm glycine in 500 mL deionized distilled water and add 200 mL of methanol, Then the solution was adjusted volume to 1 liter with deionized distilled water.

5. Reagents for immunostaining

5.1 Rabbit immunoglobulins to herpes simplex virus type-2, MS strain

5.2 Peroxidase conjugated swine immunoglobulin to rabbit immunoglobulins

5.3 PBS-Tween 20, 0.1% (PBS/T)

Dissolve 1 mL of Tween 20 in 100 mL PBS

5.4 Non fat dry milk, 5%

Dissolve 5 gm Non fat dry milk in 100 mL PBS and warm up to 37 °C until all consituents were dissolved. The solution was prepared befor use.

5.5 Tris-HCl, 1.0 M, pH 7.4

Dissolve 121.1 gm of Tris in 800 mL distilled water, adjust to pH 7.4 with HCl and make up to 1,000 mL with distilled water.

5.6 Substrate solution

The solution was prepared by dissolving 3 mg of 4-chloro-1-napthol in 1 mL methanol, then added 5 mL of 1.0 M Tris-HCL pH 7.4 and 2 mL of 30% H_2O_2 just before use.

6. Reagents for staining proteins on nitrocellulose membrane

6.1 PBS-Tween 20, 0.3%

Dissolve 3 mL of Tween 20 in 100 mL PBS.

6.2 India ink solution

The 100 uL of Pilikan fount india drawing ink was disolved in 100 mL 0.3% PBS-Tween 20. Remove the insoluble material by filtration. This solution must be prepared just before us.

VITA

Flying Lieutenant Titima Panpunnung was born on May 7, 1961 in Chiengrai, Thailand. She graduated with a Bachelor of Science (Medical Technology) from Faculty of Medicine, Chulalongkorn University in 1983. Presently, she is a Medical Technologist in the Department of Pathology at Bhumibol Adulydej Hospital.