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APPENDIX I

CHEMICAL AGENTS AND INSTRUMENTS

A. Chemical substances.

Amphotericin B (Squibb, NJ, USA)

Avidin-biotinylated horseradish peroxidase complex  
(DAKOPATTS, Glostrup, Denmark)

Copper sulfate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) (E. Merck, Darmstadt,  
W. Germany)

W. Germany)

3,3' - Diaminobenzidine (3,3',4,4' - Tetraaminobiphenyl)

(Sigma, MO, USA)

Disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ ) (E. Merck, Darmstadt,  
W. Germany)

W. Germany)

Ethanol Absolute ( $\text{C}_2\text{H}_5\text{OH}$ ) (E. Merck, Darmstadt, W. Germany)

Ficoll 400 (Pharmacia, Uppsala, Sweden)

Formaldehyde (E. Merck, Darmstadt, W. Germany)

Gentamicin (Atlantic laboratories, Thailand)

Gill's hematoxylin #1 (BDH, England)

Glutaraldehyde grade II 25% aqueous solution (Sigma, MO, USA)

Heparin (Leo, Ballerup, Denmark)

HEPES (N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulfonic  
acid]) (Sigma, MO, USA)



Hydrogen peroxide ( $H_2O_2$ )(E.Merck,Damstadt,W.Germany)

Hypaque 50% Sodium (Winthrop, NY, USA)

Lauryl sulfate (Sodium dodecyl sulfate)(Sigma,MO,USA)

Lysine -DL-Monohydrochloride (Sigma,MO,USA)

Magnesium sulfate ( $MgSO_4 \cdot 7H_2O$ )(E.Merck,Damstadt,W.Germany)

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

Paraformaldehyde (Fisher Scientific,NJ,USA)

Permunt (Fisher Scientific,NJ,USA)

RPMI 1640 medium (Gibco,Scotland)

Sodium azide ( $NaN_3$ )(E.Merck,Damstadt,W.Germany)

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

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

Sodium dihydrogen phosphate ( $NaH_2PO_4$ )(E.Merck,Damstadt,

W.Germany)

Sodium m- Periodate (Sigma,MO,USA)

Xylene ( $C_6H_4(CH_3)_2$ )(E.Merck,Damstadt,W.Germany)

#### B. Antiserum and serum

Fetal calf serum (FCS)(Gibco,Scotland)

Horse anti-mouse IgG, biotinylated (Vector,CA,USA)

Horse serum , normal

Mouse anti-human CD 56 (Becton Dickinson,MD,USA)

Mouse anti-human CD 57 (Becton Dickinson,MD,USA)

Mouse serum, normal

## C. Glasswares

Beaker (Pyrex, Corning, NY, USA)  
Coplin staining jar (Pyrex, Corning, NY, USA)  
Cover glasses (Menzel-Glaser, W. Germany)  
Cylinder (Witeg, W. Germany)  
Disposable syringe (Nipro, Tokyo, Japan)  
Erlenmeyer flask (Pyrex, Corning, NY, USA)  
Glass tube (Pyrex, Corning, NY, USA)  
Microscopic glass slide (Zhejiang, China)

## D. Instruments

Analytical balance (Precisa, Switzerland)  
Automatic pipette (Gilson, Lyon, France)  
Biohazard (Gelman Science Pty, NSW, Australia)  
Cytospin (Shandon Southern Instrument, PA, USA)  
Gamma counter (Beckman, USA)  
Incubator (Precision Scientific Inc, IL, USA)  
Microscope (Scientific Instrument, NY, USA)  
Mixer vortex (Scientific Industries, NY, USA)  
pH meter (Orion Research, MA, USA)  
Refrigerated centrifuge (IEC, MA, USA)  
Refrigerator (Tanin, Thailand)

## APPENDIX II

### REAGENTS AND PREPARATIONS

#### 1. Reagents for fixation.

##### 1.1 Periodate-Lysine-Paraformaldehyde-Glutaraldehyde (PLPG)

Buffered lysine 60 ml

Paraformaldehyde 4% 10 ml

Glutaraldehyde 25% 10 ml

Stir for 10 minutes

Sodium-m-periodate 0.16 g

Stir until dissolved (freshly prepared before use)

##### 1.2 Buffered lysine

Lysine HCL 9.2 g

Na HPO (anhydrous) 3.55 g

2 4

Distilled water to 500 ml

Adjust the pH to 7.4 , and store at 4 C



## 1.3 4% Paraformaldehyde

Paraformaldehyde 20 g

Distilled water to 500 ml

Heat 60 C until partially dissolved and add 10 N NaOH dropwise and wait 10 minutes between drop until solution clear.

## 2. Reagent for immunoperoxidase staining, ABC complex technique

## 2.1 Phosphate buffered saline (PBS) 0.01 M, pH 7.4

Na HPO 2.85 g  
2 4

NaH PO .12 H O 7.0 g  
2 4 2

NaCl 9.25 g

Distilled water to 1000 ml

Adjust the pH to 7.4 with 1 N NaOH

## 2.2 Hydrogen peroxide 1 % in methanol

Add 30 % H O 1 ml into methanol 29 ml  
2 2

## 2.3 Avidin-biotin-peroxidase complex (ABC)

Commercially available ABC is prepared by mixing 10 ul of "A"(avidin) and 10 ul of "B"(biotin-peroxidase) in PBS, pH 7.4, 1 ml for 30 minutes at room temperature before use.

## 3. Reagents for counterstaining.

## 3.1 Copper sulfate 0.5 % in 0.15 M NaCl

CuSO <sub>4</sub>	5.0 g
NaCl	8.766 g
Distilled water to	1000 ml

## 3.2 Double Distilled Deionized water substitute

MgSO <sub>4</sub> · 7H <sub>2</sub> O	2.0 g
NaHCO <sub>3</sub>	20 g
Double Distilled Deionized water to	1000 ml

## 4. Reagent for 4 hr microcytotoxicity assay

Sodium dodecyl sulfate (SDS) 1 % in RPMI 1640

SDS 0.1 g

Add RPMI 1640 medium pH 7.4 to 10 ml

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## CURRICULUM VITAE

Miss Taweeporn Phanphanich was born on March 31, 1965, Prachinburi, Thailand. She graduated with the Bachelor degree of Science (first class honor) in Medical Technology from Chulalongkorn University in 1986. Her position is scientist of Research and Development unit, Science Division, Thai Red Cross Society.



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