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APPENDICES

ศูนย์วิทยทรัพยากร จุฬาลงกรณ์มหาวิทยาลัย

APPENDIX I

REAGENTS, MATERIALS AND INSTRUMENTS

A. Media and Reagents

Absolute ethanol	(Merck, Germany)
AccuGel™ 19:1	(National Diagnostics, U.S.A.)
Acetone	(Merck, Germany)
Agarose	(Bio Basic, U.S.A.)
Ammonium persulphate	(Pharmacia Biotech, Sweden)
Ampicilin	(Sigma, U.S.A.)
Anti-CD8-coated magnetic bead	(Dynal, U.S.A.)
Biotinylated anti INF- \square mAb	(Mabtech, Sweden)
Boric acid	(Sigma, U.S.A.)
100 bp DNA Ladder	(New England Bio Labs, U.S.A.)
DMSO	(Sigma, U.S.A.)
5-bromo-4-chlor-3-indolylphosphate/ nitroblue tetrazolium	(Bio-Rad, U.S.A.)
Bromphenol blue	(USB, U.S.A.)
dNTPs	(Promega, U.S.A.)
Ethylenediamine tetraacetic (EDTA)	(Amresco, U.S.A.)
Ethydium bromide	(Bio-Rad, U.S.A.)
Fetal bovine serum	(GIBCO BRL, U.S.A.)
IsoPrep	(Robbins Scientific, Norway)
Glycerol	(USB, U.S.A.)
<i>Hae III</i>	(New England Bio Labs, U.S.A.)
<i>Hinf I</i>	(New England Bio Labs, U.S.A.)
IL-2	(R & B, UK)
IL-7	(R & B, UK)
NaCl	(Merck, Germany)
Monoclonal antibody IFN-g	(Mabtech, Sweden)
pBR322 DNA- <i>Msp I</i> digest	(New England Bio Labs, U.S.A.)
PHA	(Sigma, U.S.A.)

Streptavidin-conjugate alkaline phosphatase (Mabtech, Sweden)	
RPMI medium1640 (with L-glutamine)	(GIBCO BRL, U.S.A.)
<i>Rsa I</i>	(New England Bio Labs, U.S.A.)
Taq DNA polymerase (with MgCl ₂ and PCR buffer) (Promega, U.S.A.)	
TEMED	(USB, U.S.A.)
Tris-base	(Sigma, U.S.A.)
Tween® 20	(USB, U.S.A.)
Tryptone	(Difco, U.S.A.)
Xylene	(Merck, U.S.A.)
Xylene cyanol	(USB, U.S.A.)
Yeast extract	(Difco, U.S.A.)

B. Materials

Centrifuge tube	(Corning, U.S.A.)
Disposable serological pipette	(Costar, U.S.A.)
ELISpot plate	(Millipore, U.S.A.)
Filter Tip	(Sorenson, U.S.A.)
Microcentrifuge tube	(Sorenson, U.S.A.)
Tissue culture plate	(IWAKI, Japan)

C. Instruments

Autoclave (model-SS-325)	(Tomy, Japan)
Chemi doc	(Bio-Rad, U.S.A.)
CO ₂ Incubator	(Thermo Forma, U.S.A.)
DNA thermocycle system	(Hybaid, U.S.A.)
Electrophoresis chamber	(CBS, U.S.A.)
Microcentrifuge	(Fotodyne, U.S.A.)
Mixer-Vertex-Genic	(Scientific industries, U.S.A.)
Power supply (Model 1000/500)	(Bio-Rad, U.S.A.)
Refrigerator	(Toshiba, Japan)
Spectrophotometer (SmartSpect™ 3000)	(Bio-Rad, U.S.A.)

Vertical electrophoresis chamber
Water bath

(CBS, U.S.A.)
(Julabo, Germany)

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APPENDIX II

REAGENTS PREPARATION

Reagent for Preparation of Standard HPV-DNA

1. Luria-Bertani broth

Tryptone	10 g
Yeast extract	5 g
NaCl	10 g
Distilled water to	1 L
Sterilized by autoclaving 121 °C 15 minutes	

2. Luria-Bertani agar plate

Tryptone	10 g
Yeast extract	5 g
NaCl	10 g
Agar	10 g
Distilled water to	1 L
Sterilized by autoclaving 121 °C 15 minutes	
To pour plates, agar was allowed to cool about 50°C and then ampicillin 100 mg/ml was added. After drying, plates were stored at 4°C until used.	

3. 0.5 M EDTA

Na ₂ EDTA·2H ₂ O	18.61 g
Distilled water to	100 ml
Adjust the pH to 8.0 with 10 M NaOH	
Sterilized by autoclaving 121 °C 15 minutes and store at room temperature	

4. 2 M Tris-Cl, pH 7.4

Tris base	24.22 g
Concentrated HCl	14 ml
Distilled water to	100 ml

Sterilized by autoclaving 121 °C 15 minutes

5. TE buffer (Tris/EDTA)

2 M Tris-Cl, pH 7.4	0.5 ml
0.5 M EDTA	20 ml
Distilled water	99.48 ml
Sterilized by autoclaving 121 °C 15 minutes and store at room temperature	

Reagent for Sequencing

1. 70 % Ethanol

Absolute Ethanol	70 ml
Sterile distilled water	30 ml
Store at -20 °C	

2. 3 M Sodium acetate

Sodium acetate 3H ₂ O	40.8 g
Deionised distilled water	100 ml
Adjusted to pH 5.2 with glacial acid	

Reagent for Electrophoresis

1. 10 X Tris-borate buffer (TBE)

Tris-base	60.50 g
Boric acid	30.85 g
Na ₂ EDTA·2H ₂ O	3.72 g
Distilled water to	1 L
Sterilize by autoclaving 121 °C 15 minutes and store at room temperature	

2. Ethidium bromide (10 mg/ml)

Ethidium bromide	1 g
Sterile distilled water	100 ml

3. Loading dye

Bromphenol blue	0.25 g
Xylene cyanol	0.25 g
Glycerol	30 g
Deionised distilled water	69.5 ml

4. 1.5% Agarose gel

Agarose	0.325 g
0.5 X TBE buffer	35 ml

5. 10% Acrylamide gel loading solution

40% Acrylamide	5 ml
Deionised distilled water	12.8 ml
10X TBE	2 ml
10% ammonium persulphate	200 µl
TEMED	16 µl

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Miss Rungkarn Suebsing was born on July 9, 1980 in Bangkok, Thailand. She previously graduated with the Bachelor degree of Science (Medical Technology), Rangsit University in 2002.



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