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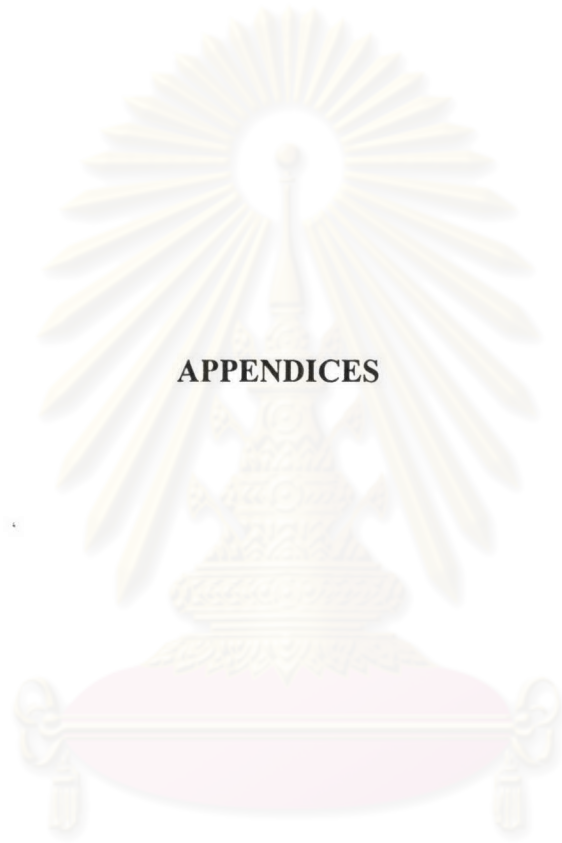
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APPENDICES

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## APPENDIX I

### REAGENTS, METERIALS AND INSTRUMENTS

#### A. MEDIA AND REAGENTS

Absolute ethanol	(Merck, Germany)
Acetone	(Merck, Germany)
Agarose (ultrapure)	(GIBCO BRL, U.S.A.)
Bromphenol blue	(Sigma, U.S.A.)
dNTPs	(Invitrogen, U.S.A.)
2-Mercaptoethanol	(Sigma, U.S.A.)
Bovine Serum Albumin	(Sigma, U.S.A.)
Ethylenediamine tetraacetic (EDTA)	(Amresco, U.S.A.)
Ethidium bromide	(Amresco, U.S.A.)
Fetal bovine serum (FBS)	(GIBCO BRL, U.S.A.)
FACS Permeabilizing solution with saponin	(Becton Dickinson, U.S.A.)
Gum tragacanth	(Sigma, U.S.A.)
L-glutamine	(Sigma, U.S.A.)
Medium 199 (M199)	(GIBCO BRL, U.S.A.)
Minimum Essential Medium	(GIBCO BRL, U.S.A.)
1 Kb Plus DNA Ladder	(Invitrogen, U.S.A.)
Paraformaldehyde	(Sigma, U.S.A.)
Penicillin	(Sigma, U.S.A.)
Periclinin chlorophyl protein-conjugated anti CD3 monoclonal antibodies (CD3/PerCP)	(Becton Dickinson, U.S.A.)
Potassium chloride (KCl)	(Merk, Germany)
Rabbit-anti HSV-1 antibody	(B114, DAKO A/S, Denmark)
Rabbit-anti HSV-2 antibody	(B116, DAKO A/S, Denmark)
RPMI 1640 ( with L-glutamine)	(GIBCO BRL, U.S.A.)
Sodium Azide (NaN <sub>3</sub> )	(Sigma, U.S.A.)

Sodium chloride (NaCl)	(Merk, Germany)
Sodium hydrogencarbonate (NaHCO <sub>3</sub> )	(Merk, Germany)
Swine-anti rabbit antibody conjugated with FITC	(DAKO A/S, Denmark)
Streptomycin	(Sigma, U.S.A.)
Taq DNA polymerase (with MgCl <sub>2</sub> and PCR buffer)	(GIBCO BRL, U.S.A.)
Tris-base	(Sigma, U.S.A.)
Trypsin	(Sigma, U.S.A.)

## B. MATERIALS

Microcentrifuge tube	(SRS, U.S.A)
Polaroid film (no. 667)	(Berlijucker, U.S.A.)
Tissue culture flask	(Nunc, Denmark)
Tissue culture plate	(Nunc, Denmark)
12x75 mm polystyrene tubes with caps	(Becton Dickinson, U.S.A.)

## C. INSTRUMENTS

Autoclave (model S-90N)	(Tomy seiko, Japan)
Chemi doc	(Bio-Rad, U.S.A.)
DNA thermocycle system	(Hybaid, England)
FACSort Flow cytometry with CELL QUEST software	(Becton Dickinson, U.S.A.)
Gelmate 2000 electrophoresis	(Toyobo, Japan)
Incubator type 80	(Memmert, Germany)
Microcentrifuge	(Fotodyne, U.S.A.)
Mixer-Vertex-Genic	(Scientific industries, U.S.A.)
Refrigerator	(Toshiba, Japan)
Water bath	(Julabo, Germany)

## APPENDIX II

### REAGENTS PREPARATION

#### REAGENTS AND MEDIA FOR CELL CULTURE

##### 1. 10% M199 medium

2X M199 with Earle's salts, with L-glutamine, without NaHCO <sub>3</sub>	50	ml
1 M HEPES	1	ml
Pen/Strep. Antibiotic (10 <sup>5</sup> unit/ml)	0.1	ml
10% NaHCO <sub>3</sub>	1	ml
L-glutamine	1	ml
FBS	10	ml
DDW	46	ml
Stored at 4 °C		

##### 2. 10% MEM

2X MEM with Earle's salts, with L-glutamine, without NaHCO <sub>3</sub>	50	ml
1 M HEPES	1	ml
Pen/Strep. Antibiotic (10 <sup>5</sup> unit/ml)	0.1	ml
10% NaHCO <sub>3</sub>	1	ml
L-glutamine	1	ml
FBS	10	ml
DDW	46	ml
Stored at 4 °C		

**3. 1X RPMI 1640**

RPMI 1640 with L-glutamine	10.4	g
NaHCO <sub>3</sub>	2	g
HEPES	2.383	g
DDW	1,000	ml

Sterilized by filtration and stored at 4 °C

**4. 10% RPMI 1640**

1X RPMI 1640 with L-glutamine	90	ml
FBS	10	ml
Pen/Strep. Antibiotic (10 <sup>5</sup> unit/ml)	0.1	ml

Stored at 4 °C

**5. 10X PBS (phosphate-buffer saline)**

NaCl	40	g
KCL	1	g
NaHPO <sub>4</sub>	5.75	g
KH <sub>2</sub> PO <sub>4</sub>	1	g
DDW	300	ml

Adjusted to pH 7.4 and adjusted volume to 500 ml with DDW and sterilized by autoclaving

**6. 1X PBS**

10X stock PBS	100	ml
DDW	900	ml

**7. 10X Trypsin**

Trypsin	0.5	g
EDTA	0.2	g
NaCl	9.0	g
DDW	100	ml

Sterilized by filtration and stored at -20 °C

**8. 1X Trypsin**

10X stock trypsin	10	ml
DDW	90	ml

Store at 4 °C

**9. Plaque overlay medium****Solution A**

10X M199 with Earle's salts, with L-glutamine, without NaHCO <sub>3</sub>	20	ml
FBS	20	ml
1 M HEPES	2	ml
Pen/Strep. Antibiotic (10 <sup>5</sup> unit/ml)	0.2	ml
10% NaHCO <sub>3</sub>	3	ml
L-glutamine	2	ml
DDW	42	ml

**Solution B**

Gum tragacanth	1.6	g
DDW	100	ml

Solution A and B were mixed at the ratio 1:1 before use.

## REAGENTS FOR FLOW CYTOMETRY

### 1. Washed buffer

BSA	5	g
NaN <sub>3</sub>	1	g
1X PBS	1,000	ml
Store at 4°C		

### 2. FACS Permeabilizing solution (1X)

10X stock FACS Permeabilizing solution

Dilute 1:10 with DDW only

Can be stored at RT for one month and at 4°C for one year

## REAGENTS FOR PCR AND ELECTROPHORESIS

### 1. 5X Tris-borate buffer (TBE)

Tris-base	54	g
Boric acid	27.5	g
5M EDTA, pH 8.0	20	ml
Sterilized by autoclaving		

### 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 FF	0.25	g
Glycerol	30	ml
DDW	69.5	ml

**4. 1% Agarose gel**

Agarose ultrapure	0.4	g
0.5X TBE buffer	40	ml



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