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APPENDIX

APPENDIX I

Reagent, Materials, and Instruments

A. Media and Reagents

Absolute ethanol	(Merck, Germany)
Agarose (ultrapure)	(Merck, Germany)
Alkaline phosphatase substrate	(Bio Rad Labs., Hercules, CA)
Anti-IFN-γ mAb 1-D1K	(Mabtech, Stockholm, Sweden)
Anti-IFN-γ mAb 7-B6-1	(Mabtech, Stockholm, Sweden)
DMSO	(Sigma, UK)
dNTPs	(Invitrogen, U.S.A.)
EDTA	(Amreso, U.S.A.)
Ethydium bromide	(Amreso, U.S.A.)
Fetal Bovine Serum	(Bio Whittaker, Maryland, USA)
Glutamine	(Sigma, UK)
Isoprep	(Robbins Scientific, Norway)
PBS	(Sigma, UK)
Penicillin	(General Drugs House, Thailand)
RPMI medium 1640	(GIBCO, USA)
Streptavidin-alkaline phosphatase conjugate	(Mabtech, Stockholm, Sweden)
Trypan blue	(Sigma, UK)

B. Instruments

6-well flat plate	(Costar, USA)
24-well flat plate	(Costar, USA)
96-well polyvinylidene difluoride backed plates	(Millipore, Bedford, MA)
Automatic pipette	(Gilson, USA)
Microcentrifuge	(Fotodyne, U.S.A.)
Conical tube 50, 15 ml	(Falcon, USA)

Freeser -70 °C	(Sanyo, Japan)
Heparin tube	(Becton-Dickinson, USA)
Incubator	(Forma Scientific, USA)
Microcentrifuge	(Eppendrof, USA)
Microtube 250 µl	(Eppendrof, USA)
Mixer-Vortex-Genic	(Scientific industries, USA)
Multichannel pipette	(Drummon, USA)
Pipette tip	(Eppendrof, USA)
Serological pipette 10, 5, 2, 1 ml	(Falcon, USA)
Stereomicroscope	(Olympus, Japan)
Water bath	(Shel-lab, USA)

APPENDIX II

REAGENTS PREPARATION

Reagents for molecular analysis

1. TE buffer (Tris/EDTA)

Tris, PH 7.4	10	mM
EDTA, pH 8.0	1	mM

2. 10 mg/ml Ethidium bromide

Ethidium bromide	1.0	g
Distilled water	100	ml

Mix the solution and store in the dark at 4°C.

3. Tris-borate buffer (TBE)

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

Steriled by autoclaving

4. 1.5% Agarose gel

Agarose	0.525	g
1x TBE	35	ml

Dissolved by heating in microwave oven and occasionally mixed until no granules of agarose are visible.

5. Luria-Bertani broth

Tryptone	10	g
Yeast extract	5	g
NaCl	10	g

1 M NaOH	1	ml
Distilled water	1	L

Sterilized by autoclaving 121 °C 15 minutes

6. Luria-Bertani agar plate

Tryptone	10	g
Yeast extract	5	g
NaCl	10	g
Agar	15	g
1M NaOH	1	ml
Distilled water to	1	L

Sterilized by autoclaving 121 °C 15 minutes

To pour plates, agar was allowed to cool down to 50°C and then ampicillin 100 mg/ml was added. The agar solution was mixed well and then poured to the plate. After drying, plates were stored at 4°C until used.

6. 1 M $CaCl_2$

$CaCl_2$	11.1	g
Distilled water	100	ml

Sterilised by autoclaving 121 °C 15 minutes

7. Ca/Glyceral (15 % glyceral)

1 M $CaCl_2$	5	ml
Glyceral	15	ml
Distilled water	80	ml

Sterilised by filtration using 0.2 μm membrane

Reagents for Cell culture

1. Penicillin 100,000 Units/ml

1,000,000 unit Penicillin G	1	ampoule
Distilled water	10	ml

2. Streptomycin 100,000 µg/ml

1 gm Streptomycin	1	ampoule
Distilled water	10	ml

3. R10

RPMI 1640	900	ml
100,000 unit/ml Penicillin G	1	ml
100,000 µg/ml Streptomycin	1	ml
Fetal Bovine Serum (FBS)	100	ml

4. R20

RPMI 1640	800	ml
100,000 unit/ml Penicillin G	1	ml
100,000 µg/ml Streptomycin	1	ml
Fetal Bovine Serum (FBS)	200	ml

5. Cyclosporin A (CsA) 1 mg/ml

50 mg/ml CsA	100	µl
Normal Saline Sterile	4900	µl

6. Stock CsA 10^{-4} g/ml

1 mg/ml CsA	100	µl
R20	900	µl

7. Cycrosporin A (CsA) 1 µg/ml

10^{-4} g/ml CSA	100	µl
R20	9900	µl

BIOGRAPHY

Miss. Patcha Incomserb was born on June 2, 1972 in ChiangMai, Thailand. She previously graduated with the Bachelor degree of Biology, Faculty of Science, ChiangMai University, ChiangMai, Thailand in 1993.