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APPENDIX

APPENDIX I

REAGENTS, MATHERIALS 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.)
Ethylenediamine tetraacetic (EDTA)	(Amreso, U.S.A.)
Ethydium bromide	(Bio-Rad, U.S.A.)
Fetal Bovine Serum	(Bio Whittaker, Maryland, USA)
Glycerol	(USB, U.S.A.)
Isoprep	(Robbins Scientific, Norway)
1 kb DNA Ladder	(Invitrogen, U.S.A.)
PBS	(Sigma, UK)
Penicillin	(General Drugs House, Thailand)
RPMI medium 1640	(GIBCO, USA)
Streptavidin-alkaline phosphatase conjugate	(Mabtech, Stockholm, Sweden)
Peptide	(Minotope, Australia)
Taq DNA polymerase (with $MgCl_2$ and PCR buffer)	(Fermentus, U.S.A.)
Tris-base	(Sigma, U.S.A.)
Trypan blue	(Sigma, UK)

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.)
6-well flat plate	(Costar, USA)
24-well flat plate	(Costar, USA)
96-well polyvinylidene difluoride backed plates	(Millipore, Bedford, MA)

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

APPENDIX II

REAGENTS PREPARATION

Reagents for CTL analysis

1. **Ficoll-Hypaque solution** (ready to use)

2. **RPMI medium 1640** (ready to use)

3. **Penicillin 100,000 Units/ml**

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

3. **Streptomycin 100,000 µg/ml**

1 gm Streptomycin	1	ampoule
Distilled water	10	ml

4. **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

5. **Peptide**

6.1 Stock peptide 1 mg/ml

Peptide	1	mg
1% DMSO in PBS	1	ml

6.2 Peptide 200 µg/ml

Stock peptide 1 mg/ml	200	µl

Sterile PBS	800	µl

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
Sterile distilled water	100	ml

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

3. **1.5% Agarose gel**

Agarose	0.325	g
0.5X TBE	35	ml

Dissolve by heating in microwave oven and occasional mix unit no granules of agarose are visible.

Reagent for Cloning

1. **Luria-Bertani (LB) broth**

Luria-Bertini (ready to use)	20	g
Distilled water	1	L

Sterilized by autoclave

2. **Luria-Bertani (LB)/Ampicillin agar plate**

Luria-Bertini	20	g
Agar	15	g
Distilled water	1	L

Sterilized by autoclave

To pour plates, allow agar to cool about 50°C was added ampicillin 100 mg/ml. After drying, store plates at 4°C until used.

3. TE buffer (Tris/EDTA)

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

4. 1 M $CaCl_2$

$CaCl_2$	11.1	g
Distilled water	100	ml

Sterilised by autoclaving 121 °C 15 minutes

5. 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

Reagent for Sequencing

1. 70% Ethanol

Absolute Ethanol	70	ml
Sterile distilled water	30	ml

2. 3 M Sodium acetate

Sodium acetate $3H_2O$	40.8	g
Deionised distilled water	100	ml

Adjusted to pH 5.2 with glacial acid

APPENDIX III

AMINO ACID

Abbreviation for amino acids

A	Ala	Alanine
C	Cys	Cysteine
D	Asp	Aspartic acid
E	Glu	Glutamic acid
F	Phe	Phenylalanine
G	Gly	Glycine
H	His	Histidine
I	Ile	Isoleucine
K	Lys	Lysine
L	Leu	Leucine
M	Met	Methionine
N	Asn	Asparagine
P	Pro	Proline
Q	Gln	Glutamine
R	Arg	Arginine
S	Ser	Serine
T	Thr	Threonine
V	Val	Valine
W	Trp	Tryptophan
Y	Tyr	Tyrosine

BIOGRAPHY

Miss Chitima Kittitananukul was born on July 15, 1979 in Nakornpathom, Thailand. She previously graduated with the Bachelor degree of Science in Biology from Srinakarinvirot University, Bangkok, Thailand in 2001.