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APPENDICES

APPENDIX I

CHEMICAL AGENTS AND INSTRUMENTS

A. Chemical substances

Chromium-51 (Amersham, UK)
Clorox
Cyclosporin A (Sigma, UK)
DMSO (Sigma, UK)
Fetal Bovine Serum (Bio Whittaker, Maryland, USA)
Glutamine (Sigma, UK)
IL2 (Genzyme, USA)
IL7 (Genzyme, USA)
Isoprep (Robbins Scientific, Norway)
PBS (Sigma, UK)
Penicillin (General Drugs House, Thailand)
Peptide gag A (NIH AIDS, USA)
Recombinant vaccinia virus clade A (NIH AIDS, USA)
Recombinant vaccinia virus clade B (I.N.S.E.R.M, France)
RPMI medium1640 (GIBCO, USA)
Streptomycin (General Drugs House, Thailand)
Triton-X 100 (Sigma, UK)
Trypan blue (Sigma, UK)

B. Instruments

24-well flat plate (Costar, USA)
96-well U plate (Costar, USA)
Automatic pipette (Gilson, France)
Centrifuge
Conical tube 50, 15 ml (Falcon, USA)
Counting chamber
Cryotube (Sarstedt, Germany)
EDTA tube (Becton-Dickinson, USA)
Flask 25,75 cm^3 (Nunc, Denmark)
Freezer – 70°C
Gamma counter
Geiger counter (Ludlum, USA)

Glove
Heparin tube (Becton-Dickinson, USA)
incubator (Forma Scientific, USA)
Lead shield
Microcentrifuge (Eppendrof, USA)
Microtube 250 µl
Mixer-Vortex-Genic (Scienfic industries, USA)
Pipette tip
Serological pipette 25, 10. 5, 2,1 ml (Costar, USA)
Water bath (Shel-lab, USA)

APPENDIX II

REAGENTS AND PREPARATIONS

1. Reagent for PBMCs preparation
   1.1 Ficoll-Hypaque solution (ready to used)
   This solution has specific gravity about 1.077 g/l
   1.2 RPMI medium 1640 (ready to used)
   1.3 Penicillin 10,000 Units/ml
      1.3.1 Stock penicillin 100,000 Units/ml
      Penicillin G 1,000,000 Units per ampoule was reconstituted with sterile DW 10 ml and mixed
      1.3.2 Working penicillin 10,000 Units/ml

Stock penicillin 100,000 Units/ml	1	ml
RPMI1640	9	ml

   1.4 Streptomycin 10,000 µg/ml
      1.4.1 Stock Streptomycin 100,000 µg/ml
      Streptomycin 1 gm was reconstituted with sterile DW 10 ml and mixed
      1.4.2 Working Streptomycin 10,000 µg/ml

Stock Streptomycin 100,000 µg/ml	1	ml
RPMI1640	9	ml

   1.5 Phosphate Buffer Saline (PBS) pH 7.4
      1.5.1 Solution A

$NaH_2HPO_4.H_2O$	27.6	gm

      1.5.2 Solution B

$NaH_2HPO_4.12H_2O$	71.63	gm
DW	1000	ml

      1.5.3 PBS pH 7.4

Solution A	16.5	ml
Solution B	33.5	ml
NaCl	8.5	ml
DW to	1000	ml

      Adjusted pH to 7.4 and sterile by autoclave

2. Reagent for culture

2.1 R10		
RPMI1640 + 100 units/ml Streptomycin + 100 units/ml Penicillin	90	ml
Fetal Bovine Serum (FBS)	10	ml
2.2 R20		
RPMI1640 + 100 units/ml Streptomycin + 100 units/ml Penicillin	80	ml
Fetal Bovine Serum (FBS)	20	ml

3. Cyclosporin A 1 μg/ml

3.1 Stock CSA 1 mg/ml		
CSA 50 mg /ml	100	μl
Normal Saline Sterile	4900	μl
3.2 Stock CSA 10^{-4} g/ml		
Stock CSA 1 mg/ml	100	μl
R20	900	μl
3.3 CSA 1 μg/ml		
Stock CSA 10^{-4} g/ml	100	μl
R20	900	μl

4. Peptide preparation

4.1 Stock peptide 1 mg/ml		
Peptide	1	mg
1% DMSO in PBS	1	ml
4.2 Peptide 100 μg/ml		
Stock peptide 1 mg/ml	100	μl
Sterile PBS	900	μl

APPENDIX III

SEQUENCE OF GAG A PEPTIDE

1. Abbreviation for amino acids

A	alanine	L	leucine
R	arginine	K	lysine
N	asparagine	M	methionine
D	aspartic acid	F	phenylalanine
C	cysteine	P	proline
Q	qlutamine	S	serine
E	glutamic acid	T	threonine
G	glycine	W	trytophan
H	histidine	Y	tyrosine
I	isoleucine	V	valine

2. HIV-1 Gag A peptide (92UG037) from Uganda

Cat #	Sequence	position
3772	MGARASVLSGGKLDAWEKIR	1-20
3773	GKLDAWEKIRLRPGGKKKYR	11-30
3774	LRPGGKKKYRLKHLVWASRE	21-40
3775	LKHLVWASRELERFALNPSL	31-50
3776	LERFALNPSLLETTEGCQQI	41-60
3777	LETTEGCQQIMEQLQSALRT	51-70
3778	MEQLQSALRTGTEELRSLYN	61-80
3779	GTEELRSLYNTVATLYCVHQ	71-90
3780	TVATLYCVHQRIEVKDTKEA	81-100
3781	RIEVKDTKEALDKIEEIQKK	91-110
3782	LDKIEEIQKKSKQKTQQAAA	101-120
3783	SKQKTQQAAADTGSSSKVSQ	111-130
3784	DTGSSSKVSQNYPIVQNAQG	121-140
3785	NYPIVQNAQGQMIHQSLSPR	131-150
3786	GQMIHQSLSPRTLNAWVKVIE	140-160
3787	TLNAWVKVIEEKALSPEVIP	151-170
3788	EKALSPEVIPMFSALSEGAT	161-180
3789	MFSALSEGATPQDLNMMLNI	171-190

Cat #	Sequence	position
3790	PQDLNMMLNIVGGHQAAMQM	181-200
3742	VGGHQAAMQMLKDTINEEAA	191-210
3743	LKDTINEEAAEWDRLHPVHA	201-220
3791	EWDRLHPVHAGPVAPGQMRE	211-230
3792	GPVAPGQMREPRGSDIAGTT	221-240
3793	PRGSDIAGTTSTPQEQIAWM	231-250
3794	STPQEQIAWMTGNPPIPVGD	241-260
3795	TGNPPIPVGDIYKRWMILGL	251-270
3796	IYKRWMILGLNKIVRMYSPV	261-280
3750	NKIVRMYSPVSILDIKQGPK	271-290
3751	SILDIKQGPKEPFRDYVDRF	281-300
3752	EPFRDYVDRFFKTLRAEQAT	291-310
3797	FKTLRAEQATQEVKGWMTET	301-320
3798	QEVKGWMTETLLIQNANPDC	311-330
3799	LLIQNANPDCKSILRALGAG	321-340
3800	CKSILRALGAGATLEEMMTA	331-349
3801	ATLEEMMTACQGVGGPGHKA	341-360
3802	CQGVGGPGHKARVLAEAMSQV	350-370
3803	RVLAEAMSQVQHTNIMMQR	361-379
3804	VQHTNIMMQRGNFKGQKRI	370-388
3805	GNFKGQKRIKCFNCGKEGHL	380-399
3806	KCFNCGKEGHLAKNCRAPRK	389-408
3807	LAKNCRAPRKKGCWKCGREG	399-418
3808	KGCWKCGREGHQMKDCTERQ	409-428
3765	HQMKDCTERQANFLGKIWPS	419-438
3809	ANFLGKIWPSSKGRPGNFPQ	429-448
3810	SKGRPGNFPQSRPEPTAP	439-456
3811	SRPEPTAPPAAEIFGMREE	449-467
3812	PAAEIFGMREEIVSPPKQEQN	457-477
3813	IVSPPKQEQNDRDQNPPSVSL	468-488
3814	DRDQNPPSVSLKSLFGNDLLSQ	478-499

3. Pooled Peptide Gag A

Pooled peptide	Individual peptides
#1	3772, 3773, 3774, 3775, 3776
#2	3777, 3778, 3779, 3780, 3781
#3	3782, 3783, 3784, 3785, 3786
#4	3787, 3788, 3789, 3790, 3791
#5	3792, 3793, 3794, 3795, 3796
#6	3797, 3798, 3799, 3800, 3801
#7	3802, 3803, 3804, 3805, 3806
#8	3807, 3808, 3809, 3810, 3811
#9	3812, 3813, 3814, 3742, 3743
#10	3750, 3751, 3752, 3765

APPENDIX IV

COMPARISON OF GAG SEQUENCE OF CM240 AND 92UG037

Gag alignment of CM240 (upper line) and 92UG037 (lower line) by program Multiple Alignment Legand showed that the homologous is shown identity of 87.58% (437/499) and similar residues of 10.02% (50/499) between the 2 clades

Gag alignment of CM240 (upper line) and 92UG037 (lower line)

Identity 87.58% (437/499) Similar residues=10.02%(50/499)

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                          18-35
1     MGARASVLSGGKLDAWEKIRLRPGGRKKYRLKHLVWASRELERFALNPSFLETAEGCQQI
      ||||||||||||||||||||||||:||||||||||||||||||||:|||.||||||
1     MGARASVLSGGKLDAWEKIRLRPGGKKKYRLKHLVWASRELERFALNPSLLETTEGCQQI

61    IEQLQSTLKTGLEELKSLFNTVATLWCVHQRIEVKDTKEALDKIEEVQNKSQRKTQQAAA
      :|||||.|:|| |||:||:||||||:||||||||||||||||||:|.||..||||||||
61    MEQLQSALRTGTEELRSLYNTVATLYCVHQRIEVKDTKEALDKIEEIQKKSKQKTQQAAA

          124-160
121   GTGSSSKVSQNYPIVQNAQGQMAHQPLSPRTLNAWVKVVEEKGFNPEVIPMFSALSEGAT
      :|||||||||||||||||||| ||.||||||||||||:|||::.||||||||||||||||
121   DTGSSSKVSQNYPIVQNAQGQMIHQSLSPRTLNAWVKVIEEKALSPEVIPMFSALSEGAT

181   PQDLNMMLNIVGGHQAAMQMLKETINEEPAEWDRVHPVHAGPIPPGQMREPRGSDIAGTT
      ||||||||||||||||||||:|||||:|||||:|||||||::||||||||||||||||
181   PQDLNMMLNIVGGHQAAMQMLKDTINEEAAEWDRLHPVHAGPVAPGQMREPRGSDIAGTT
                    253-283
241   STLQEQIGWMTNNPPIPVGDIYKRWIILGLNKIVRMYSPVSILDIRQGPKEPFRDYVDRF
      || ||||:|||.||||||||||||:||||||||||||||||:||||||||||||||||
241   STPQEQIAWMTGNPPIPVGDIYKRWMILGLNKIVRMYSPVSILDIKQGPKEPFRDYVDRF

301   YKTLRAEQATQEVKNWMTETLLVQNANPDCKSILKALGTGATLEEMMTACQGVGGPSHKA
      :||||||||||||.|||||||:||||||||||:|||.|||||||||||||||||:|||
301   FKTLRAEQATQEVKGWMTETLLIQNANPDCKSILRALGAGATLEEMMTACQGVGGPGHKA

361   RVLAEAMSHAQHATIMMQRGNFKGQKRIKCFNCGREGHLARNCRAPRKQGCWKCGKEGHQ
      ||||||||:.||..|||||||||||||||||||:|||||:|||||||.||||||:||||
361   RVLAEAMSQVQHTNIMMQRGNFKGQKRIKCFNCGKEGHLAKNCRAPRKKGCWKCGREGHQ

421   MKDCTERQANFLGKIWPSNKGRPGNFPQSRPEPTAPPAENWGMGEEITGEEITSLPKQEQ
      ||||||||||||||||||.||||||||||||||||||.:         |||.| |||||
421   MKDCTERQANFLGKIWPSSKGRPGNFPQSRPEPTAPPAAEIFGMR....EEIVSPPKQEQ

481   KDKEHPPPLVSLKSLFGNDPLSQ
      .|::: || ||||||||||| |||
477   NDRDQNPPSVSLKSLFGNDLLSQ
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BIOGRAPHY

Miss Supranee Buranapraditkun was born on May 17, 1972 in Bangkok, Thailand. She graduated with the Bachelor degree of Science in Medical Technology from the Faculty of Medicine, Chulalongkorn University in 1995. Since 1995 to present, she has been working at the Division of Allergy and Clinical Immunology, Department of Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok.