

APPENDIX A
LIST OF THE CHEMICALS AND INSTRUMENTS

1. Chemicals

All chemicals used as in this study were analytical grade reagents.

Chemical name	Source
100X Hypoxanthine Thymidine (HT)	Gibco, Grand Island, NY, USA
10X BM condimed HI	Roche, Mannheim, Germany
4', 6-diamidino-2-phenylindole (DAPI)	Molecular Probes, Eugene, OR, USA
50X Hyposanthine Aminopterin	Gibco, Grand Island, NY, USA
Thymidin (HAT)	
5-bromo-4-chloro-3-indoly- β -D-galactoside (X-gal)	Invitrogen, San Diego, CA
Acrylamide	Biorad, Hercules, CA, USA
Agarose (electrophoresis grade)	Sigma-Aldrich. St.Louis, MO, USA
Amersham Hybond TM -ECL	GE healthcare Bio-Sciences Co. Piscataway, NJ
Ammonium peroxodisulphate	GE healthcare Bio-Sciences Co. Piscataway, NJ
Ampiillin	Sigma-Aldrich. St.Louis, MO, USA
Bac-N-Blue TM transfection kit	Invitrogen, San Diego, CA

Chemical name	Source
BCA Protein Assay	Thermo Fisher Scientific Inc., Rockford, IL, USA
BCML (N,N-bis(carboxymethyl) lysine hydrate)	Sigma-Aldrich. St.Louis, MO, USA
Bis-acrylamide	Biorad, Hercules, CA, USA
BM condimed	Gibco, Grand Island, NY, USA
Bovine Serum Albumin (BSA)	Sigma-Aldrich. St.Louis, MO, USA
Bradford protein assay	Thermo Fisher Scientific Inc., Rockford, IL, USA
Bromphenol blue	Sigma-Aldrich. St.Louis, MO, USA
Desthiobiotin	IBA GmbH, Göttingen, Germany
DTT	AMRESCO, Salon, OH, USA
Durapore® membrane filter	Millipore, Billerica, MA
EDTA	Sigma-Aldrich. St.Louis, MO, USA
Ethanol	Merck, Darmstadt, Germany
Ethidium bromide	Sigma-Aldrich. St.Louis, MO, USA
Extravidin-HRP	Sigma-Aldrich. St.Louis, MO, USA
EZ-Link Sulfo-NHS-LC-Biotin kit	Thermo Fisher Scientific Inc., Rockford, IL, USA

Chemical name	Source
Fetal bovine serum (FBS)	Gibco, Grand Island, NY, USA
GeneJet™ PCR purification kit	Fermentas, Burlington, ON, Canada
GENETIC SYSTEM™ HIV-1 Ag EIA kit	Biorad, Hercules, CA, USA
Glacial acetic acid	BDH Laboratory Supplies, UK
Glycerol	Sigma-Aldrich. St.Louis, MO, USA
Glycine, Ultrapure	USB Corporation, Cleveland, OH, USA
Grace's insect medium	Gibco, Grand Island, NY, USA
HisTrap column	GE healthcare Bio-Sciences Co. Piscataway, NJ
HiTrap protein G HP column	GE healthcare Bio-Sciences Co. Piscataway, NJ
Hygromycin B	Hyclone, Rockford, IL, USA
Illustra TempliPhi 100 amplification kit	GE healthcare Bio-Sciences Co. Piscataway, NJ
IMDM medium	Gibco, Grand Island, NY, USA
Imidazole	Sigma-Aldrich. St.Louis, MO, USA
IPTG, dioxan-free	Fermentas, Burlington, ON, Canada
Kanamycin	Sigma-Aldrich. St.Louis, MO, USA
LB Broth Agar	Bio Basic inc., Ontario, Canada
L-glutamine	Gibco, Grand Island, NY, USA

Chemical name	Source
Methanol	Merck, Darmstadt, Germany
Micro particles, magnetic, streptavidin coated	Sigma-Aldrich. St.Louis, MO, USA
NaCl	Sigma-Aldrich. St.Louis, MO, USA
NaOH	Sigma-Aldrich. St.Louis, MO, USA
Non-fat dried milk	Difco Laboratories, Detroit, MI, USA
Nucleofector™ transfection reagent V	Lonza, Basel, Switzerland
NucleoSpin® Extract II	Macherey- Nagel, Düren, Germany
NucleoSpin® Plasmid miniprep kit	Macherey- Nagel, Düren, Germany
Paraformaldehyde	Sigma-Aldrich. St.Louis, MO, USA
Penicillin/Streptomycin	Gibco, Grand Island, NY, USA
Polyethylene Glycol 8000, Ultrapure	USB Corporation, Cleveland, OH, USA
PVDF membrane	PALL, East Hills, NY, USA
RPMI-1640 medium	Gibco, Grand Island, NY, USA
Serum-free medium, PFHM-II	Gibco, Grand Island, NY, USA
<i>Strep-tactin</i> ® coated magnetic beads	IBA GmbH, Göttingen, Germany
SureBlue™ TMB Microwell Peroxidase substrate	KPL, Gaithersburg, MD, USA

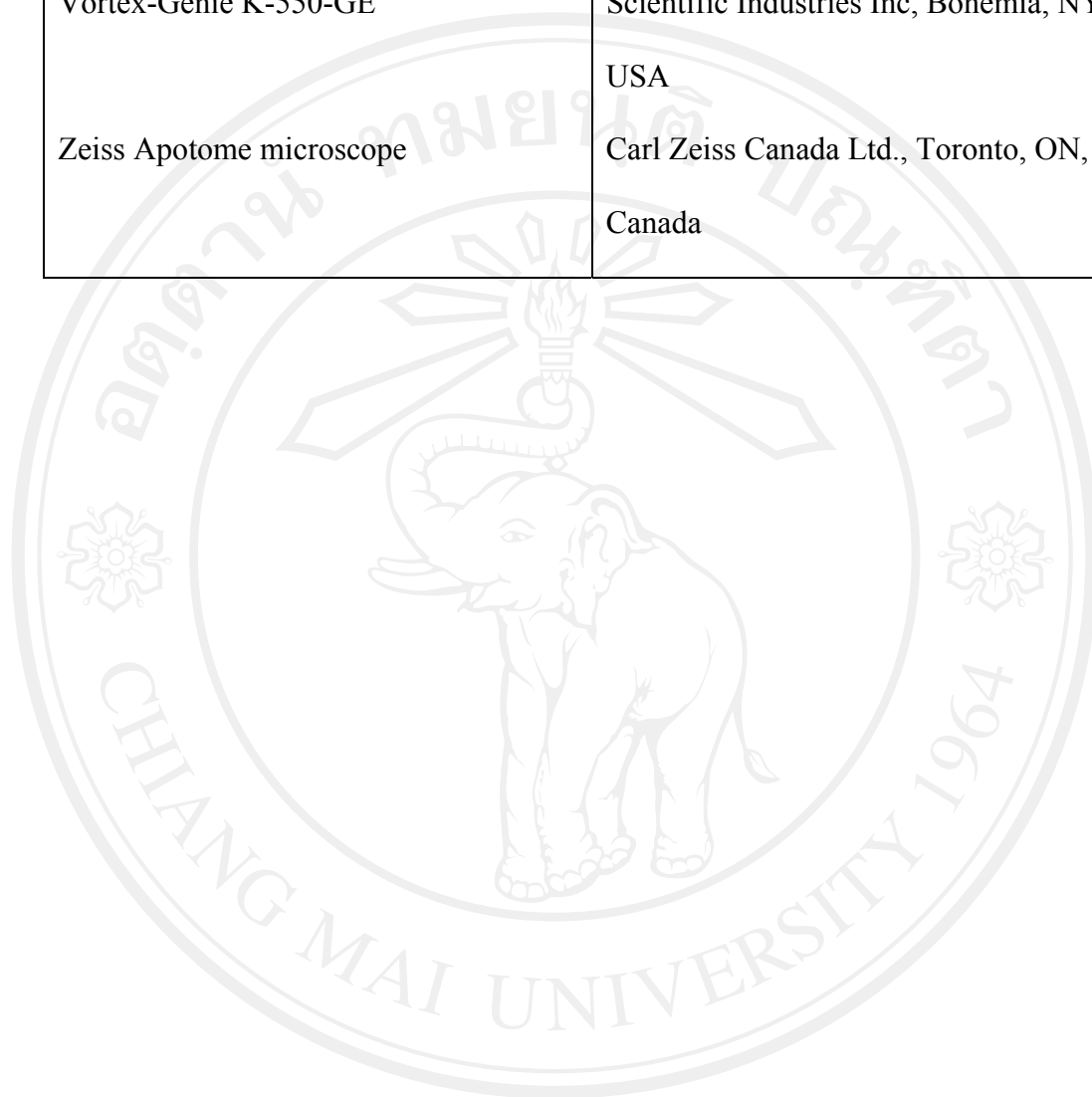
Chemical name	Source
TEMED	Biorad, Hercules, CA, USA
Tetracyclin	Sigma-Aldrich. St.Louis, MO, USA
The BM Blue POD Substrate, precipitating	Roche, Mannheim, Germany
TNM-FH medium	Invitrogen, San Diego, CA, USA
Tris [hydroxymethyl] aminomethane,	USB Corporation, Cleveland, OH, USA
Ultrapure	
Triton X-100	Sigma-Aldrich. St.Louis, MO, USA
Trypan Blue 0.4%	Sigma-Aldrich. St.Louis, MO, USA
Tryptone water	Merck, Darmstadt, Germany
Tween 20	Fluka, Buchs, Switzerland
Urea	Sigma-Aldrich. St.Louis, MO, USA
Whatman 3MM blotting paper	GE healthcare Bio-Sciences Co. Piscataway, NJ
Yeast extract	Bio Basic inc., Ontario, Canada
Zeba™ Desalt Spin Column	Thermo Fisher Scientific Inc., Rockford, IL, USA

2. Instruments

Instruments	Source
27°C incubator	Shel Lab, Cornelius, OR, USA
37 °C CO ₂ incubator EG 115 IR	Jouan GmbH, Unterhaching, Germany
37 °C incubator	JP Selecta, Barcelona, Spain
ÄKTA prime™ plus	GE healthcare Bio-Sciences Co. Piscataway, NJ, USA
Amicon Ultra centrifugal filter units	Millipore, Cork, Ireland
BECKMAN L-60 ultracentrifuge	Beckman Coulter, Fullerton, CA, USA
Electrophoretic power supply 3000Xi	BioRad, Hercules, CA, USA
Eppendorf Thermomixer®	Eppendorf, Hauppauge, NY, USA
Flow cytometry	BD FACSort™, San Diego, CA, USA
Fluorescence microscopy, OLYMPUS AX70	Olympus, Tokyo, Japan
Shaking incubator (JSSI-100C)	JS Research Inc., Gongju-city, Korea
Inverted fluorescence microscope	Nikon eclipse TE2000-S, Japan
Inverted microscope	Olympus, Japan
Laminar Flow biological safety cabinet	NUAIRE, Plymouth, MN, USA
MicroCal iTC ₂₀₀ isothermal titration calorimeter	Microcal, Piscataway, NJ, USA

Instruments	Source
Microcentrifuge	Eppendorf AG, Hamburg, Germany
Microplate	NUNC, Roskilde, Denmark
MicroPulser™	BioRad, Hercules, CA, USA
MiniVE vertical electrophoresis system	Amersham Pharmacia Biotech, Buckinghamshire, UK
MRX-150 Refrigerated microcentrifuge	Tomy Tech USA Inc., CA, USA
MTP-120 ELISA plate reader	Corona Electric, Japan
MyLab orbital shaker OS-20	BioSan Ltd., Riga, Latvia
NanoDrop 2000	ThermoScientific, Rockford, IL, USA
Nucleofector™	Amaxa, Koeln, Germany
Odyssey® infrared scanner	LI-COR Biosciences, Lincoln, NE, USA
RT 6000 D refrigerated centrifuge	Sorvall, Kendro Laboratory Products GmbH, Langenselbold, Germany
The ORIGIN software	Microcal, Piscataway, NJ, USA
Ultrasonic Processor UP100H	Hielscher, Teltow, Germany
BioRad Chemidoc XRS Gel	BioRad, Hercules, CA, USA
Documentation System	Shimadzu Scientific Instruments Inc, Kyoto, Japan
UV spectrophotometer	Shimadzu Scientific Instruments Inc, Kyoto, Japan
UV-2450/2550 spectrophotometer	Shimadzu, Columbia, MD, USA

Instruments	Source
Vortex-Genie K-550-GE	Scientific Industries Inc, Bohemia, NY, USA
Zeiss Apotome microscope	Carl Zeiss Canada Ltd., Toronto, ON, Canada



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APPENDIX B

LIST OF CELL LINES AND MICROORGANISMS

1. Cell lines

Name	Type of cell lines
Sf9	Pupal ovarian tissue of <i>Spodoptera frugiperda</i>
Sup-T1	Human T cells lymphoblastic lymphoma
P3-X63Ag8.653	Mouse myeloma cells

2. Microorganisms

2.1 *Escherichia coli* XL-1 Blue MRF'

Genotype: $\Delta(mcrA)183 \Delta(mcrCB-hsdSMR-mrr)173 \text{ endA1 supE44 thi-1 recA1 gyrA96 relA1 lac [F' proAB lacI}^q\text{ZAM15 Tn10 (Tet}^r\text{)]}$

2.2 *Escherichia coli* BL21 (DE3)

Genotype: $F^- ompT gal dcm lon hsdS_B(r_B^- m_B^-) \lambda(DE3 [lacI lacUV5-T7 gene 1 ind1 sam7 nin5])$

2.3 *Escherichia coli* M15 [pREP4]

Genotype: $Lac \text{ ara gal mtl recA}^+ \text{ uvr}^+ [\text{pREP4 lacI kana}^r]$

APPENDIX C

LIST OF ANTIBODIES AND CONJUGATED ANTIBODIES

Antibodies name	Source
Anti-CA (Clone G188)	Generated in this study
Anti-CA (Clone M88)	Generated in this study
Anti-His tag antibody	GenScript, Piscataway, NJ, USA
Anti-mouse immunoglobulins conjugated Alexa Fluor [®] 680	Invitrogen, San Diego, CA, USA
Anti-mouse immunoglobulins conjugated HRP	KPL, Gaithersburg, MD, USA
Anti-p17 (clone MH-SVM33)	Purchased from ATCC
Extravidin conjugated HRP	Sigma-Aldrich. St.Louis, MO, USA
Goat anti-rabbit immunoglobulins conjugated HRP	KPL, Gaithersburg, MD, USA
Mouse anti-M13 conjugated HRP	GE healthcare Bio-Sciences Co. Piscataway, NJ, USA
Rabbit-anti Gag	Invitrogen, San Diego, CA, USA

APPENDIX D
LIST OF ENZYMES

Enzymes	Sources
Accuprime™ Pfx DNA polymerase	Invitrogen, San Diego, CA
<i>Bam</i> HI	NEB, Pickering, Ontario, USA
<i>Bcl</i> I	NEB, Pickering, Ontario, USA
<i>Bsm</i> BI	NEB, Pickering, Ontario, USA
<i>Bsp</i> MI	NEB, Pickering, Ontario, USA
<i>Hind</i> III	NEB, Pickering, Ontario, USA
<i>Kpn</i> I	NEB, Pickering, Ontario, USA
<i>Kpn</i> I	NEB, Pickering, Ontario, USA
<i>Nde</i> I	NEB, Pickering, Ontario, USA
<i>Nhe</i> I	NEB, Pickering, Ontario, USA
<i>Not</i> I	NEB, Pickering, Ontario, USA
T4 DNA ligase enzyme	NEB, Pickering, Ontario, USA

APPENDIX E
REAGENT PREPARATIONS

1. Reagents for gel electrophoresis

1.1 10× Tris-acetate/EDTA electrophoresis buffer (TAE)

Tris-base	48.40	gm
Glacial acetic acid	11.42	ml
0.5 M EDTA, pH 8.0	20	ml

Dissolved all ingredients in deionized distilled water and filled up to 1,000 ml.
Sterilized by autoclave and kept at room temperature.

1.2 1 or 2 % Agarose gel

Agarose	1 or 2	gm
1× TAE	100	ml

Melted by microwave oven until the agarose was completely dissolved.

1.3 Ethidium bromide working solution (10 mg/ml)

Ethidium bromide	1.0	gm
Distilled water	100	ml

Dissolved and kept in dark bottle at 4 °C.

1.4 6X gel loading buffer

Bromphenol blue	0.25	%
Glycerol	30	%

Mixed thoroughly and stored at -20 °C.

2. Reagents for SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting

2.1 1.5 M Tris-HCl, pH 8.8

Tris-base	18.15	gm
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Dissolved in 75 ml deionized distilled water.

Adjusted pH to 8.8 with concentrated HCL.

Adjusted the volume to 100 ml with deionized distilled water and stored at 4 °C.

2.2 0.5 M Tris-HCl, pH 6.8

Tris-base	6.0	gm
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Dissolved in 75 ml deionized distilled water.

Adjusted pH to 6.8 with concentrated HCl.

Adjusted the volume to 100 ml with deionized distilled water and stored at 4 °C.

2.3 Running buffer

Tris-base	1.51	gm
Glycine	7.20	gm
Sodium dodesyl sulfate	0.5	gm

Dissolved in 500 ml deionized distilled water and kept at 4 °C.

2.4 Blotting buffer

Tris-base	3.03	gm
Glycine	14.41	gm
SDS	0.5	gm

Added deionized distilled water to 700 ml and mixed well.

Added 200 ml of methanol

Adjusted the volume to 1,000 ml with deionized distilled water and kept at 4 °C.

2.5 Copolymerization of 4% stacking gel (5 ml)

Stock acrylamide 30%	0.83	ml
0.5 M Tris-HCl pH 6.8	0.63	ml
10% SDS	0.05	ml
DW	3.40	ml
10% Ammonium persulfate	0.05	ml
TEMED	0.01	ml

2.6 Copolymerization of 12% stacking gel (10 ml)

Stock acrylamide 30%	4.00	ml
Gel buffer pH 8.8	2.50	ml
10% SDS	0.10	ml
DW	3.30	ml
10% Ammonium persulfate	0.10	ml
TEMED	0.01	ml

3. Medium for bacterial culture

3.1 50% glucose

D-glucose 5 gm

Added distilled water to 10 ml and boiled in boiling water.

Filtered through 0.2 μ m Millipore filter and stored at 4 °C.

3.2 LB broth

Yeast extract 5.0 gm

Tryptone 10.0 gm

NaCl 10.0 gm

Dissolved all ingredients in 1,000 ml distilled water

Sterilized by autoclave, and kept at 4 °C.

3.3 LB agar

LB agar 15 gm

Dissolved all ingredients in 1,000 ml distilled water.

Sterilized by autoclave, poured on Petri dish (plate) and stored at 4 °C.

3.4 2XYT broth

Tryptone	16 gm
Yeast extract	10 gm
NaCl	5 gm

Dissolved in 1,000 ml distilled water.

Sterilized by autoclave and kept at 4 °C.

4. Reagents for Fluorescence microscopy and Flow cytometry analysis**4.1 4% Paraformaldehyde in PBS**

Paraformaldehyde	4 gm
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PBS pH 7.2	100 ml
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Heat at 56°C until dissolved

Filtrated with 0.2 µm Millipore filter, stored at 4°C.

4.2 1% BSA-PBS-NaN₃

BSA	1 gm
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NaN ₃	0.09 gm
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Dissolved in PBS 100 ml

4.3 0.2% Triton X-100

Triton X-100	0.2 ml
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Dissolved in PBS 100 ml

5. Reagents for cell culture

5.1 RPMI 1640 medium

RPMI powder	1	pack
NaHCO ₃	2	gm
ddH ₂ O	800	ml
Penicillin (10,000 units/ml)/Streptomycin (10,000 µg/ml)	1	ml

Stirred until dissolved and adjust pH with acetic acid.

Dissolved in ddH₂O and adjust volume to 1,000 ml.

Filtrated through 0.2 µm Millipore membrane filter.

Mixed and stored at 4 °C.

5.2 Complete RPMI culture medium

RPMI 1640 medium	90	ml
Fetal bovine serum (FBS)	10	ml

Checked sterility before used.

5.3 Freezing medium (10%DMSO in 90%FCS)

Fetal calf serum	9	ml
DMSO	1	ml

Freshly preparation before use.

5.4 Trypan blue (0.2%)

Trypan blue powder	0.2	gm
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PBS pH 7.2	100	ml
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Filtrated by Whatman filter paper No. 1 and stored at room temperature.

6. Reagent for phage precipitation and phage selection**6.1 PEG/NaCl**

PEG-8000	100	gm
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NaCl	73	gm
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Dissolved in ddH₂O and adjust volume to 1,000 ml.

Mixed and Filtrated through 0.2 µm Millipore membrane filter.

Stored at 4 °C.

6.2 0.1M Glycine pH2.5

Glycine	0.375	gm
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Dissolved in ddH₂O 40 ml and adjusted pH to 2.5 with 1N HCl

Mixed and adjusted volume to 50 ml

Filtrated through 0.2 µm Millipore membrane filter and stored at 4 °C.

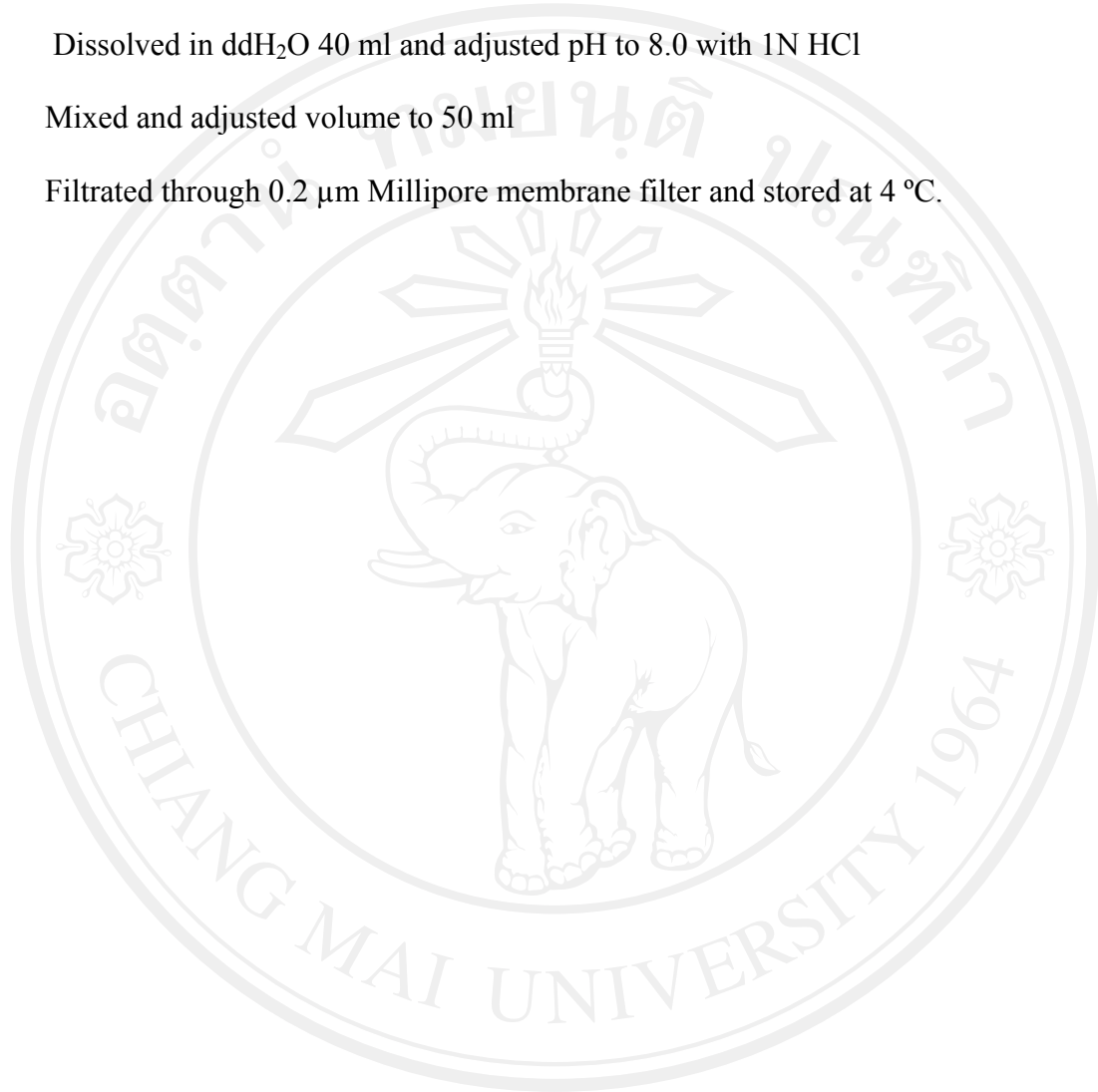
6.3 1M Tris pH8.0

Tris base 6.07 gm

Dissolved in ddH₂O 40 ml and adjusted pH to 8.0 with 1N HCl

Mixed and adjusted volume to 50 ml

Filtrated through 0.2 μ m Millipore membrane filter and stored at 4 °C.



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APPENDIX F

PRESENTATIONS AND PUBLICATIONS

List of presentation

1. Creation of proteins specifically binding to Indinavir by directed evolution. 2ème Colloque Doc/Post-Doc de l'IBBMC at Institut de Biochimie et Biophysique Moléculaire et Cellulaire, Bât 430-Université de Paris-Sud 11 ORSAY Cedex, Paris FRANCE. 26-27 March 2007 (Oral presentation).
2. The influence of translocation pathways on phage displaying ankyrin repeat protein. 3rd Colloque Doc/Post-Doc de l'IBBMC at La Rochelle, FRANCE. 16-19 November 2009 (Oral presentation).
3. The interference of viral assembly and viral maturation by artificial ankyrin repeat protein: directed evolution and intracellular applications. Journée de école doctorale "Signalisations, neurosciences, endocrinology, reproduction" at faculté de médecine Paris-SUD, Hopital Bicetre, Paris FRANCE. 27 May 2010 (Poster presentation).
4. Ankyrin repeat proteins which bind specifically to target molecules by directed evolution. RGJ seminar series LXXIV "From basic biomedical research to

sustainable development” at Chiang Mai, Thailand. 16 September 2010 (Poster presentation, outstanding poster presentation award).

List of publications

1. Sakkhachornphop, S., Jiranusornkul, S., Kodchakorn, K., **Nangola, S.**, Sirisanthana, T. and Tayapiwatana, C. (2009) Designed zinc finger protein interacting with the HIV-1 integrase recognition sequence at 2-LTR-circle junctions. *Protein Sci* 18, 2219-30.

Impact Factor 2.937

2. Lee, V.S., Tue-ngeun, P., **Nangola, S.**, Kitidee, K., Jitonnom, J., Nimmanpipug, P., Jiranusornkul, S. and Tayapiwatana, C. (2010) Pairwise decomposition of residue interaction energies of single chain Fv with HIV-1 p17 epitope variants. *Mol Immunol* 47, 982-90.

Impact Factor 3.202

3. **Nangola, S.**, Minard, P. and Tayapiwatana, C. (2010) Appraisal of translocation pathways for displaying ankyrin repeat protein on phage particles. *Protein Expr Purif* 74, 156-61.

Impact Factor 1.563

4. Kitidee, K., **Nangola, S.**, Gonzalez, G., Boulanger, P., Tayapiwatana, C. and Hong, S.S. (2010) Baculovirus display of single chain antibody (scFv) using a novel signal peptide. *BMC Biotechnol* 10, 80.

Impact Factor 2.723

CURRICULUM VITAE

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Education

1997-1999 High school, Yupparaj Wittayalai School, Chiang Mai,
Thailand.

2000-2003 Bachelor of Science (Medical Technology, Second class
Honor), Faculty of Associated Medical Sciences,
Chiang Mai University, Chiang Mai, Thailand

Employment history

April 2004 – May 2006 Research Assistance,
Assoc. Prof. Chatchai Tayapiwatana,
Faculty of Associated Medical Sciences,
Chiang Mai University, Chiang Mai, Thailand

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April 2004 – Present R&D, i+Med Laboratories (iMED),
Research center at Faculty of Associated Medical
Sciences, Chiang Mai University, Chiang Mai, Thailand

List of publications

1. Pattarawarapan, M., **Nangola, S.**, Cressey, T.R. and Tayapiwatana, C. (2007)
Development of a one-step immunochromatographic strip test for the rapid
detection of nevirapine (NVP), a commonly used antiretroviral drug for the
treatment of HIV/AIDS. *Talanta* 71, 462-70.

Impact Factor 3.290

2. Cressey, T.R., **Nangola, S.**, Tawon, Y., Pattarawarapan, M., Lallemand, M.
and Tayapiwatana, C. (2007) Immunochromatographic strip test for rapid
detection of nevirapine in plasma samples from human immunodeficiency
virus-infected patients. *Antimicrob Agents Chemother* 51, 3361-3.

Impact Factor 4.802

3. Khamta, Y., Pattarawarapan, M., **Nangola, S.** and Tayapiwatana, C. (2009)
Development of immunochromatographic assay for the on-site detection of
salbutamol. *J Immunoassay Immunochem* 30, 441-56.

Impact Factor 0.508

4. Sakkhachornphop, S., Jiranusornkul, S., Kodchakorn, K., **Nangola, S.**,
Sirisanthana, T. and Tayapiwatana, C. (2009) Designed zinc finger protein
interacting with the HIV-1 integrase recognition sequence at 2-LTR-circle
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Impact Factor 2.937

5. Lee, V.S., Tue-ngeun, P., **Nangola, S.**, Kitidee, K., Jitonnorn, J., Nimmanpipug, P., Jiranusornkul, S. and Tayapiwatana, C. (2010) Pairwise decomposition of residue interaction energies of single chain Fv with HIV-1 p17 epitope variants. *Mol Immunol* 47, 982-90.

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7. Kitidee, K., **Nangola, S.**, Gonzalez, G., Boulanger, P., Tayapiwatana, C. and Hong, S.S. (2010) Baculovirus display of single chain antibody (scFv) using a novel signal peptide. *BMC Biotechnol* 10, 80.

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