APPENDICES

Appendix A

List of antibodies and cell line used in this study

Monoclonal antibodies	MT4 isotype IgM
Cell line:	Sup T1 human T cell line

Appendix B

List of the chemicals and materials used in this study

Chemicals/Materials	Source
Absolute methanol	J.T.Baker, Philipsburg, NJ, USA
Bovine serum albumin fraction V	Sigma, St. Louis, MO, USA
Disodium hydrogen phosphate	Fisher, Leics, UK
FACS™ lysing solution	Becton Dickinson, San Jose, CA, USA
Fetal calf serum	Biochrcom, Leonorenstr, Germany
Formaldehyde solution min. 37%	Merck, Darmstadt, Germany
Paraformaldehyde	Fluka, Buchs, Switzerland
Potassium chloride	Merck, Darmstadt, Germany
Potassium dihydrogen phosphate	Merck, Darmstadt, Germany
Potassium hydrogen carbonate	Asia Pacific, Australia
Potassium hydroxide pellets	May&Baker, Dagenham, England
Simultest™ CD45/CD14	Becton Dickinson, San Jose, CA, USA
Simultest™ CD3/CD4	Becton Dickinson, San Jose, CA, USA
Simultest™ CD3/CD8	Becton Dickinson, San Jose, CA, USA
Sodium azide	Merck, Darmstadt, Germany
Sodium chloride	Merck, Darmstadt, Germany
Sodium hydrogen carbonate	Merck, Darmstadt, Germany
Sodium hydroxide	Eka, Nobel, Sweden

Appendix C

List of instruments used in this study

Instrument-Model	Source
Analytical balance; PB303-S	Mettler Toledo, Switzerland
pH meter; AG CH-8953	Precisa, Switzerland
High speed micro refrigerated centrifuge; MRX-150	Tomy, USA
Spectrophotometer; UV-1201	Shimadzu, Japan
Refrigerator (4°C)	Toshiba, Thailand
Centrifuge; RT6000D	Sorvall, USA
Waterbath; NB9-102	Thermoline, Australia
Light microscope; CHA	Olympus, Japan
Flow cytometer; FACSCalibur	Becton Dickinson, USA
Refrigerator (-20°C)	Sanyo, Thailand
Biological safety cabinet class II; Nu-400-400E	Nuaire, USA
CO_2 incubator; TC2323	Sheldon, USA
Microcentrifuge; Biofuge pico	Kendro, USA
Inverted microscope; CX40	Olympus, Japan
Fluorescence microscope; BX-40	Olympus, Japan
Autoclave; HA-3D	Hirayama, Japan

Appendix D

Reagents and buffers preparation

1. Reagents for preparation of mAb

1.1 10% DMSO-PBS

Dimethyl sulfoxide	10 ml
PBS pH 7.2	90 ml

Mix well, filtrated by 0.2 μm millipore filter

Store at room temperature

1.2 10% FCS-MEM

Foetal calf serum (FCS)	10 ml
MEM	90 ml

Prepare in biosafety cabinet by sterile technique

Mix well and store at 4 °C

2. Reagent for immunofluorescence staining

2.1 Phosphate buffer saline (PBS pH 7.2)

NaCl	8.000 g
KCl	0.200 g
Na_2HPO_4	1.150 g
KH_2PO_4	0.200 g

Distilled water	800 ml

Adjust pH to 7.2 by adding 1N HCl or 1N NaOH

Adjust volume to 1000 ml

Filter with 0.2 µm millipore filter, store at room temperature

2.2 1% BSA-0.02% NaN_3 in PBS

Bovine serum albumin fraction V	10g
PBS pH 7.2	1000 ml
10% NaN_3 in PBS	200 ml

Mix well until BSA completely dissolved, store at 4° C

2.3 1% Paraformaldehyde in PBS

Paraformaldehyde	5 g
PBS pH 7.2	500 ml

Heat at 56° C until dissolved

Filter with 0.2 µm Millipore filter, store at 4° C

3. Reagent for purification of mAb by AKTA prime

3.1 Binding buffer for AKTA prime

(20 mM sodium phosphate buffer, 0.5 M potassium sulphate, pH 7.5)

1 M Na_2HPO_4	5.8 ml
1 M NaH_2HPO_4	4.2 ml
$(NH4)_2 SO_4$ (MW. 132.14)	52.856 g
ddH_2O	350 ml

Adjust pH to 7.5, volume to 500 ml by volumetric flask

Filtrated by filter pore size of 0.2 μm

3.2 Elution buffer AKTA prime

(20 mM sodium phosphate buffer, pH 7.5)

1 M Na_2HPO_4	11.6 ml
1 M NaH_2HPO_4	8.4 ml
ddH_2O	800 ml

Adjust pH to 7.5, volume to 1000 ml by volumetric flask

Filtrated by filter pore size of 0.2 μm

3.3 Cleaning buffer AKTA prime

(20 mM sodium phosphate buffer, pH 7.5 with 30% isopropanol)

1 M Na_2HPO_4	11.6 ml
1 M NaH_2HPO_4	8.4 ml
Isopropanol	150 ml
ddH_2O	200 ml

Adjust pH to 7.5, volume to 500 ml by volumetric flask

Filtrated by filter pore size of 0.2 μm

4. Reagent for SDS-PAGE

4.1 (4x) 1.5 M Tris HCl pH 8.8

Tris base	18.15 g
ddH_2O	80 ml

Adjust pH to 8.8 by concentrate HCl

Adjust volume to 100 ml, store at 4° C

4.2 (4x) 0.5 M Tris HCl pH 6.8

Tris base	6 g
ddH_2O	80 ml

Adjust pH to 6.8 by concentrate HCl

Adjust volume to 100 ml, store at 4° C

4.3 10% Ammonium persulfate (APS)

Ammonium persulfate	0.1 g
ddH_2O	1 ml

Mix well, aliquot and store at -20° C

4.4 10% Sodium dodecyl sulfate (SDS)

Sodium dodecyl sulfate	10 g
Distilled water	1000 ml

Mix well, aliquot and store at -20° C

4.5 (2x) non-reducing buffer

0.25 M Tris HCl pH 6.8	5 ml
87% glycerol	2 ml
10% SDS	2 ml
Distilled water	700 ml
Bromphenol blue	0.002 g

Mix well, aliquot and store at -20° C

4.6 (2x) reducing buffer

0.5 M Tris HCl pH 6.8	2.5 ml
87% glycerol	2.3 ml

10% SDS	2 ml
Distilled water	2.2 ml
2-mercaptoethanol	1 ml
Bromphenol blue	0.002 g

Mix well, aliquot and store at -20° C

4.7 Running buffer

Tris base	3.028 g
Glycine	14.413 g
Sodium dodecyl sulfate	1 g
Distilled water	1000 ml

Mix well, prepare before use

4.8 Gel preparation

	12.5% separating gel	**4% stacking gel**
Distilled water	3.2 ml	1.5 ml
Monomer	4.2 ml	332.5 µl
(4x) 1.5 M Tris HCl pH 8.8	2.5 ml	-
(4x) 0.5 M Tris HCl pH 6.8	-	625 µl
10% SDS	100 µl	25 µl
10% APS	50 µl	12.5 µl
TEMED	10 µl	5 µl

4.9 Staining solution

Coomasie brilliant blue R	0.125 g
Methanol	200 ml

Acetic acid	35 ml
Adjust volume with distilled water to	500 ml
Store at room temperature	

4.10 Destaining solution 1

Methanol	200 ml
Acetic acid	35 ml
Adjust volume with distilled water to	500 ml
Store at room temperature	

4.11 Destaining solution 2

Methanol	25 ml
Acetic acid	35 ml
Adjust volume with distilled water to	500 ml
Store at room temperature	

5. Reagent for bead coating

5.1 Coating buffer (0.1M phosphate buffer pH 7.2-8.0

Solution 1	9.5 ml
Solution 2	40.5 ml
ddH_2O to	1000 ml

Solution 1 (10x)

1M $NaH_2PO_4 \cdot H_2O$	1.38 g
ddH_2O to	10 ml

Solution 2 (10x)

1M Na_2HPO_4 . $2H_2O$	7.5 g
ddH_2O to	42 ml

5.2 2% BSA-0.02% NaN_3 in PBS

Bovine serum albumin fraction V	10g
PBS pH 7.2	500 ml
10% NaN_3 in PBS	100 ml

Mix well until BSA completely dissolved, store at 4° C

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1994-98	Faculty of Associated Medical Sciences, Khon Kaen University, Thailand
1998, March	Bachelor of Sciences (Medical Technology)
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Nouanthong P, Chiampanichayakul S, Sirisanthana T, and Kasinrerk W. Development Method and Reagent to Enumerate CD4+ T lymphocyte in Whole Blood by Non-Flow Cytometry. The 4th National Symposium on Graduate Research. Chiang Mai, Thailand. August 2004.

PUBLICATION:

Nouanthong P., Chiampanichayakul S., Sirisanthana T., and Kasinrerk W. Development of Method and Reagent to enumerate CD4+ T lymphocyte in whole blood by Non-Flow Cytometric method. Chiang Mai Medical Bulletin Vol. 43 No. 3 (Suppl) September 2004 (Abstract).

TRAINING AWARD:

1993-98	Thai Government scholarship for studying basic sciences-Bachelor degree, Khon Kaen University, Thailand
2000	Australia National Reference Laboratory fellowship for attending Workshop in Infectious Disease Quality Assurance Program, Thailand
2002	Japanese Red Cross fellowship for training on Safe Blood Transfusion Services, Japan
2004	International Cell Research Organization- UNESCO Fellowship for training in Molecular Biology& Diseases, National Institute of Hygiene& Epidemiology, Vietnam
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