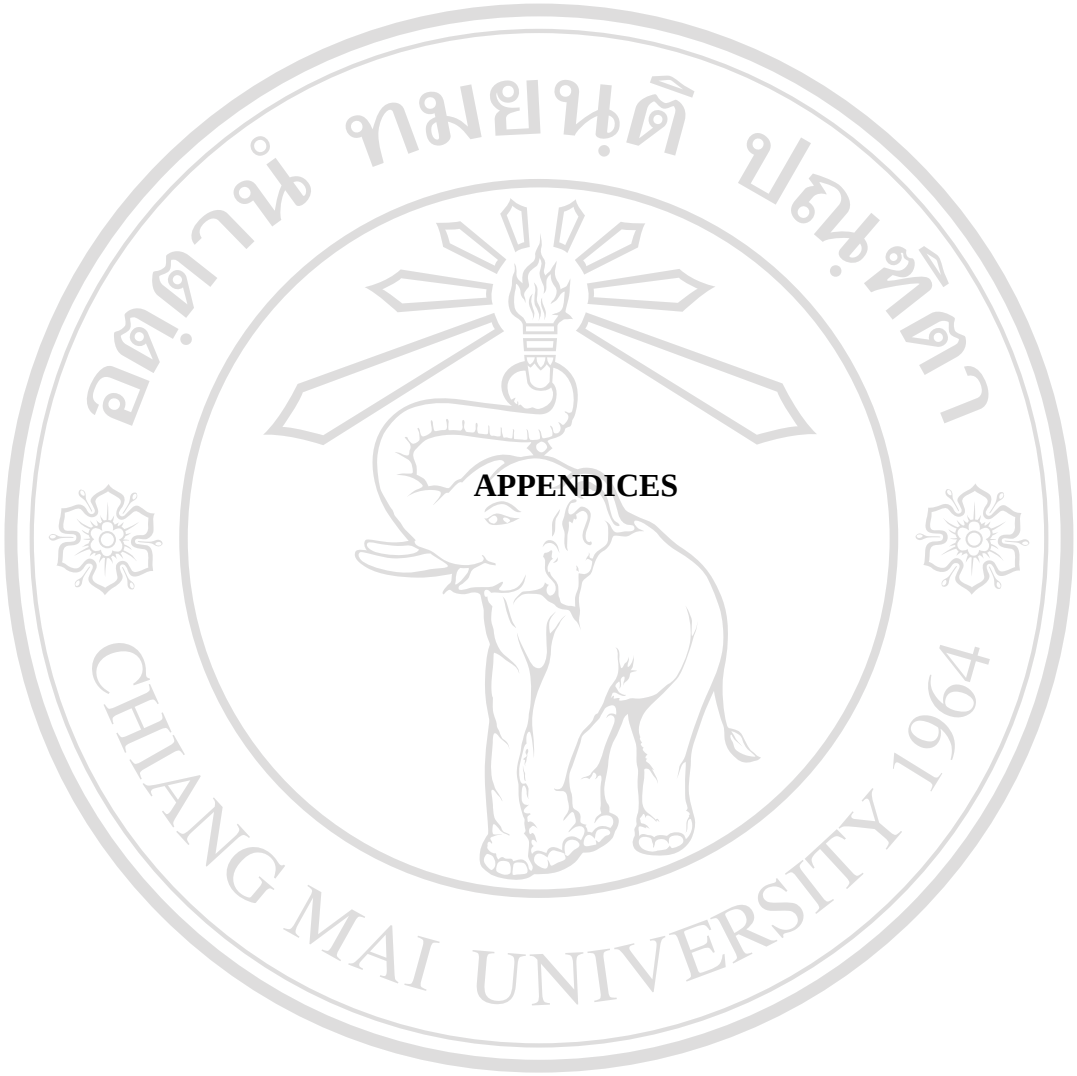
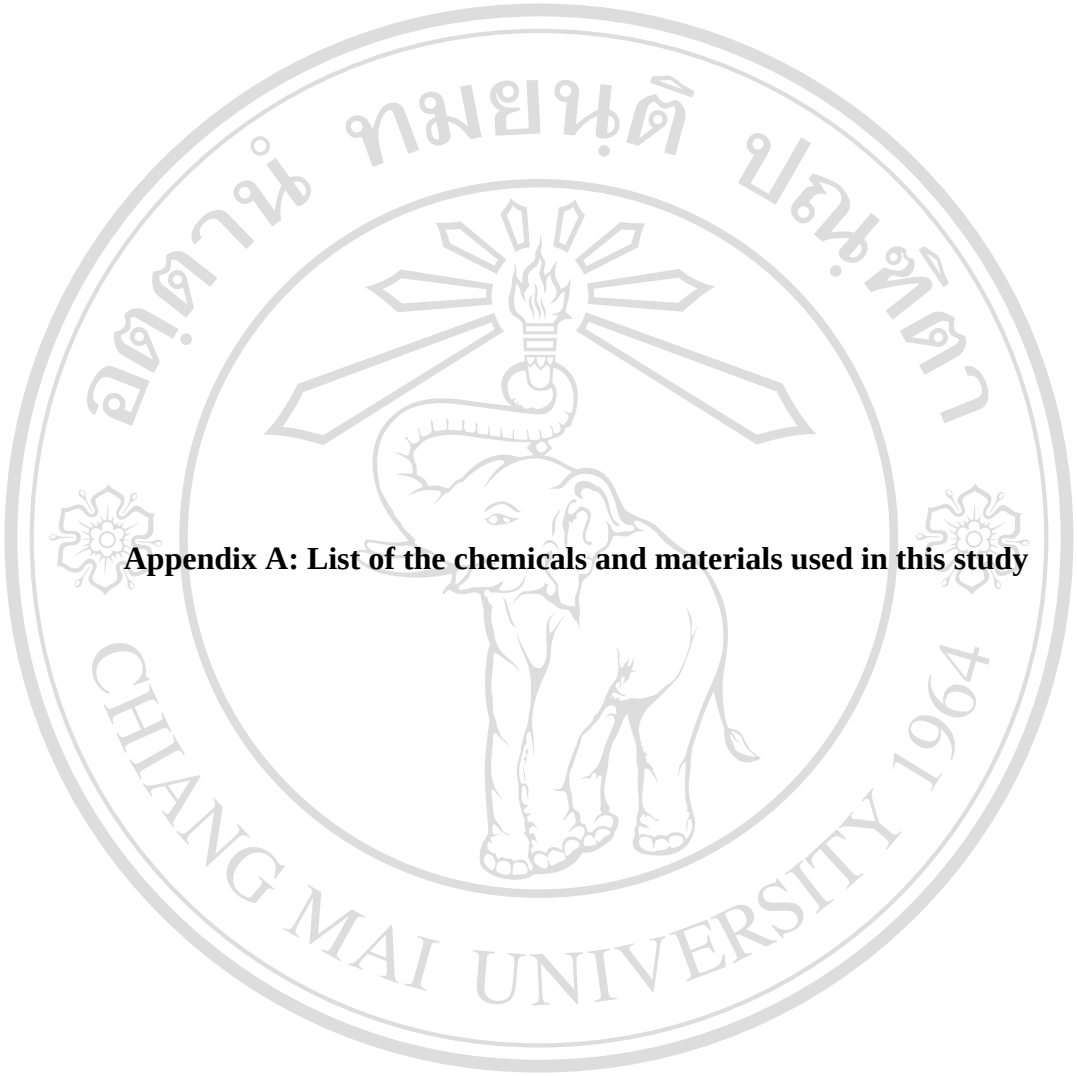


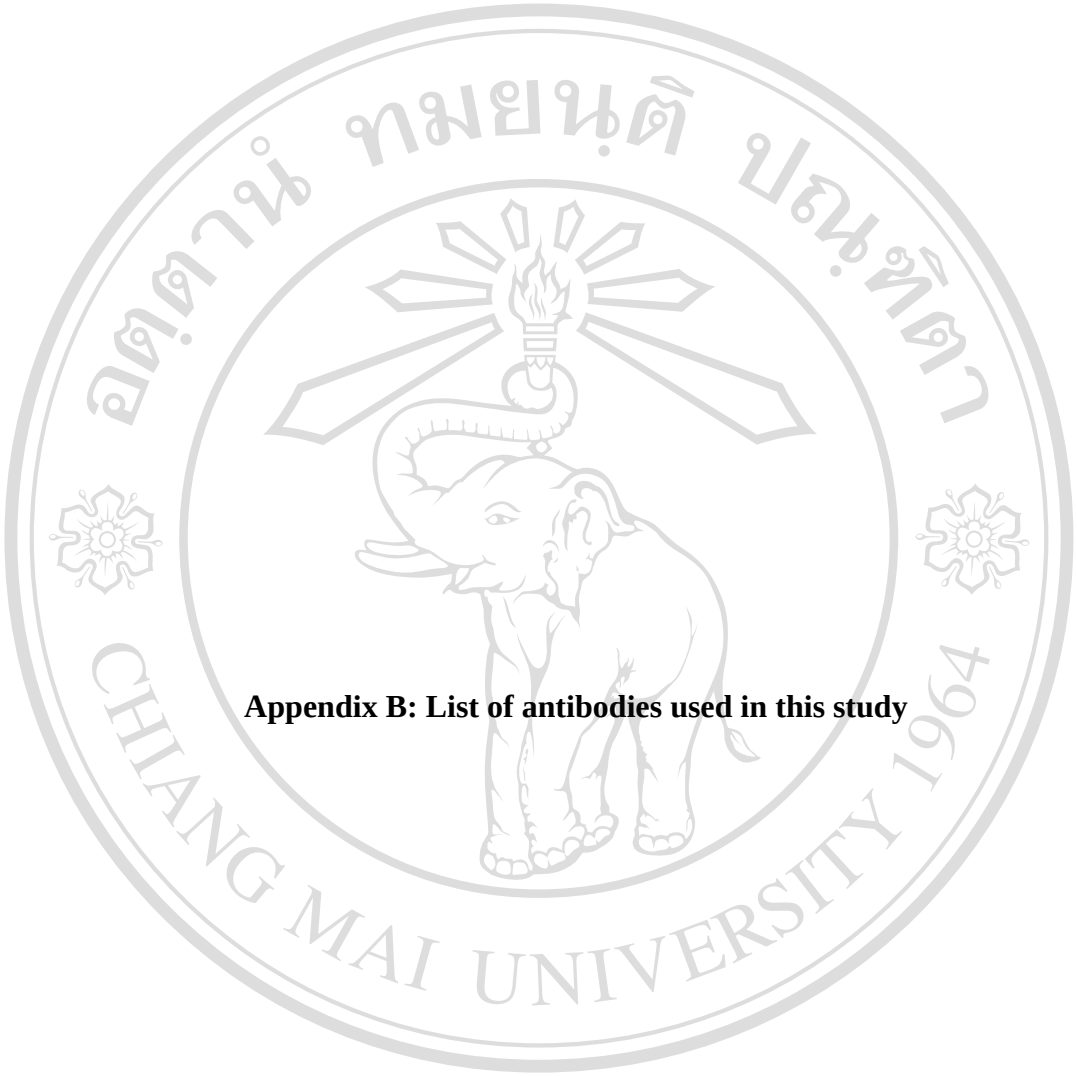
APPENDICES



Appendix A: List of the chemicals and materials used in this study

Chemicals/Materials	Source
Acetone	Merck, Darmstadt, Germany
Acrylamide	Merck, Darmstadt, Germany
Ammonium persulfate	Sigma, St. Louis, MO, USA
Amplicillin	Sigma, St. Louis, MO, USA
Aprotinin	Sigma, St. Louis, MO, USA
Bisacrylamide	Sigma, St. Louis, MO, USA
Bovine serum albumin	Sigma, St. Louis, MO, USA
Chemilumnescent reagent	Pierce, Rockford, IL, USA
Coomassie brilliant blue R-250	Bio-Rad, Hercules, CA, USA
Developer and replenisher	Kodak, NY, USA
Dimethyl sulfoxide	Sigma, St. Louis, MO, USA
Ethylenediaminetetraacetic acid	Fluka, Buchs, Switzerland
Ethyl alcohol	Merck, Darmstadt, Germany
Fetal calf serum	Gibco, Grand Island, NY, USA
Ficoll-Hypaque solution	Sigma, St. Louis, MO, USA
FITC-conjugated sheep $F(ab')_2$ anti-mouse Igs	Silenus, Boronia, Victoria, Australia
Gentamicin	Russel, London, UK
Heparin	Lio, Ballerup, Denmark
Iodoacetamide	Sigma, St. Louis, MO, USA
Iscove's modified Dulbecco's medium	Gibco, Grand Island, NY, USA
Isopropanol	Merck, Darmstadt, Germany
Isotyping-ELISA kit	Sigma, St. Louis, MO, USA

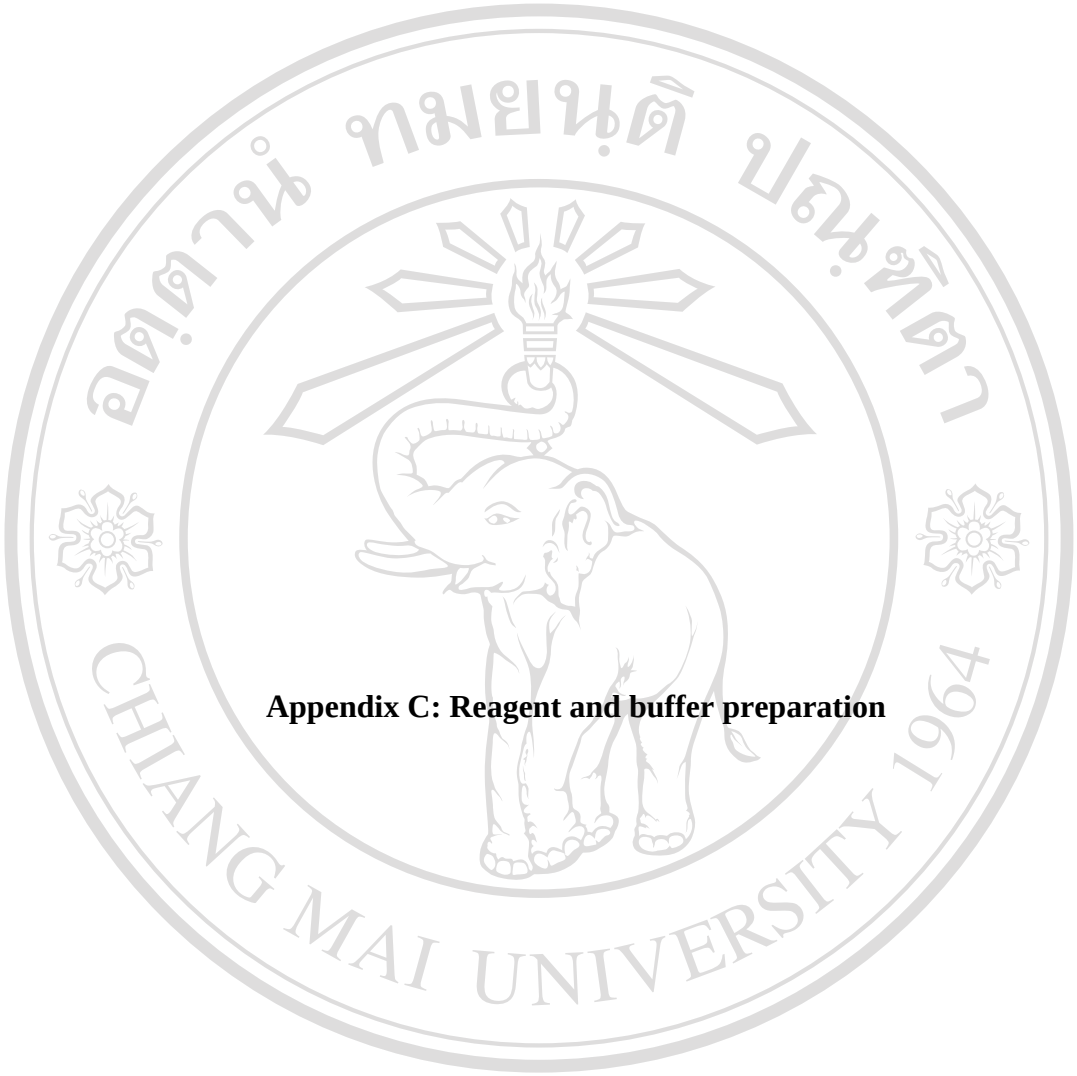
Chemicals/Materials	Source
2-mercaptoethanol	Merck, Darmstadt, Germany
Methanol	Merck, Darmstadt, Germany
Nitrocellulose membrane	PALL, East Hill, NY, USA
Nonidet P-40	Pierce, Rockford, IL, USA
Paraformaldehyde	Fluka, Buchs, Switzerland
Potassium chloride	Merck, Darmstadt, Germany
Potassium dihydrogen phosphate	Merck, Darmstadt, Germany
Prestained SDS-PAGE standards	Fermentas, MA, USA
Skimmed milk	Difco laboratories, Detroit,MI, USA
Sodium azide	Merck, Darmstadt, Germany
Sodium bicarbonate	Merck, Darmstadt, Germany
Sodium carbonate	Merck, Darmstadt, Germany
Sodium chloride	Merck, Darmstadt, Germany
Sodium dihydrogen phosphate	Merck, Darmstadt, Germany
Sodium dodecyl sulfate	Merck, Darmstadt, Germany
Sodium hydrogen carbonate	Merck, Darmstadt, Germany
Sodium hydrogen phosphate	Merck, Darmstadt, Germany
Sulfo-NHS-LC-biotin	Pierce, Rockford, IL, USA
Sreptavidin-HRP	Zymed, South san Francisco, CA
TEMED	BioRad Laboratories, Griffin
Tris-base	Sigma, St. Louis, MO, USA
Tween 20	Fluka, Buchs, Switzerland



Appendix B: List of antibodies used in this study

List of antibodies used in this study

Monoclonal antibodies	Isotype
FE-1H10	IgM
MEM55	IgG1
MEM93	IgG1
MT4	IgM
MT4/2	IgM
MT4/3	IgG2a
MT8	IgG
MT99/3	IgG2a
M6-1B9	IgG3
OKT3	IgG1
UCHL-1	IgG2a



Appendix C: Reagent and buffer preparation

1. Reagents for cell culture

1.1 Incomplete IMDM medium

IMDM powder (GibcoBRL)	1	pack
$NaHCO_3$	3.024	g
Gentamycin (40 mg/ml)	1	ml
Dissolved in ddH_20 and adjust volume to	1000	ml
Filtrated through 0.2 μm Millipore membrane filter		
Added Fungizone (5 mg/ml)	500	μl
Mixed and stored at 4^oC		

1.2 Complete IMDM medium

Incomplete IMDM medium	90	ml
Fetal calf serum	10	ml
Checked sterility before used		

1.3 RPMI 1640 medium

RPMI 1640 powder (GibcoBRL)	1	pack
$NaHCO_3$	2	g
Streptomycin (0.2 g/ml) (M & H MANUFACTURING CO.,LTD.)	500	μl
Penicillin ($1x10^6$ U/ml) (M & H MANUFACTURING CO.,LTD.)	100	μl
Dissolved in ddH_2O and adjusted volume to 1000 ml and pH to 7.2		
Filtered through 0.2 μm millipore membrane filter		
then added Fungizone (5 mg/ml) (Bristol-Myers)	500	μl

and stored at 4°C

1.4 Complete culture medium

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

1.5 Freezing medium (10%DMSO in 25%FCS-IMDM)

Incomplete IMDM	65	ml
Fetal calf serum	25	ml
DMSO (Hybrimax)	10	ml

Mixed well and stored at 4°C

2. Reagents for Immunoprecipitation

2.1 Tris lysis buffer pH 8.2 (100mM NaCl, 50mM Tris-base, 2 mM EDTA, 0.02% NaN3)

Tris base	3.03	g
NaCl	2.922	g
EDTA (M.W. 292.25)	0.292	g
NaN_3	0.1	g
Distilled water	200	ml

Adjusted pH to 8.2 by 0.1M NaOH

Adjusted final volume to 500 ml, stored at room temperature

2.2 Lysis buffer

Phenylmethylsulfonyl fluoride (PMSF)	100	µl
(100 mM in acetone)		
Iodoacetamide (0.5M in distilled water)	100	µl
Aprotinin (1 mg/ml in PBS)	100	µl
10% NP-40 (in Tris lysis buffer)	1	ml
Tris-lysis buffer pH 8.2	8.7	ml
Pepstatin A	10	µl

Mixed well, aliquot to vial and stored at -20 ºC

2.3 mM Glycine in PBS

Glycine	0.0375	g
PBS pH 7.2	500	ml

Stored at 4ºC

2.4 5 mM Biotin in PBS

Sulfo-NHS-LC-biotin	0.00278	g
PBS pH 7.2	1	ml

Freshly prepared before used

3. Reagents for SDS-PAGE

3.1 4X Separating gel buffer (1.5M Tris HCl pH 8.8)

Tris base	18.15	g
Deionized distilled water	80	ml

Adjusted pH to 8.8 by concentrate HCl

Adjusted final volume to 100 ml

Filtrated 0.2 µm Millipore membrane filter

Stored at 4ºC

3.2 4X Stacking gel buffer (0.5M Tris HCl pH 6.8)

Tris base	6.0	g
Deionized distilled water	80	ml

Adjusted pH to 6.8 by concentrate HCl

Adjusted final volume to 100 ml

Filtrated 0.2 µm Millipore membrane filter

Stored at 4ºC

3.3 2x non-reducing buffer

0.5 M Tris HCl pH 6.8	2.5	ml
87% glycerol	2.3	ml
Sodium dodecyl sulfate	0.4	g
Distilled water	5.16	ml
1% Bromphenol blue	40	µl

Mixed well, aliquot and stored at -20ºC

3.4 2x reducing buffer

0.5M Tris HCl pH 6.8	2.5	ml
87% glycerol	2.3	ml
Sodium dodecyl sulfate	0.4	g
Distilled water	4.16	ml
2-ME	1	ml
1% Bromphenol blue	40	µl

Mixed well, aliquot and stored at -20ºC

3.5 1X Running buffer

Tris base	3.028	g
Glycine	14.413	g
Sodium dodesyl sulfate	1.0	g
Distilled water	1000	ml

Mixed well, prepared before used

3.6 30% Monomer (30.8% acrylamide, 2.7% bis-acrylamide)

Acrylamide	60	g
Bis-acrylamide	1.6	g
ddH_2O	200	ml

Mixed thoroughly and filtrated through 0.2 µm

Millipore membrane filter, kept in dark at 4ºC

3.7 Slab gel

	separating gel						stacking gel	
	12.5%		10%		7.5%		4%	
Distilled water	3.2	ml	4	ml	4.85	ml	1.5	ml
30% Monomer	4.2	ml	3.3	ml	2.5	ml	332.5	µl
4X Separating gel buffer	2.5	ml	2.5	ml	2.5	ml	-	
4X Stacking gel buffer	-		-		-		625	µl
10% SDS (in distilled water)	100	µl	100	µl	100	µl	25	µl
10% APS (in distilled water)	50	µl	50	µl	50	µl	12.5	µl
TEMED	10	µl	10	µl	10	µl	5	µl

3.8 10% APS

Ammonium persulfate	0.1	g
Distilled water	1	ml

Mixed well, aliquot and stored at -20ºC

3.9 10% SDS

Sodium dodecyl sulfate	10	g
Distilled water	100	ml

Mixed well, aliquot and stored at -20ºC

3.10 1X Blotting buffer

Tris-base	1.515	g
Glycine	7.205	g
Sodium dodesyl sulfate	0.5	g
Distilled water	350	ml

Mixed well

Methanol	100	ml
Adjusted final volume to	500	ml

Filtrated with 0.2 µm filter, stored at room temperature

4. Reagents for indirect immunofluorescence staining

4.1 1XPhosphate buffer saline (PBS)

NaCl	8	g
KCl	0.2	g
Na_2HPO_4	1.15	g
KH_2PO_4	0.2	g
Distilled water	900	ml

Adjusted pH to 7.2 by 5N NaOH

Adjusted volume to 1000 ml, stored at room temperature

4.2 1%BSA-0.02%NaN_3 in PBS

Bovine serum albumin fraction V	10	g
PBS pH 7.2	1000	ml
10% NaN_3 in PBS	2000	µl

Mixed well until BSA completely dissolved, stored at 4ºC

4.3 1%Para-formaldehyde in PBS

Para-formaldehyde	5	g
PBS pH 7.2	500	ml

Heat at 56ºC until dissolved

Filtrated with 0.2 µm millipore filter, stored at 4ºC

5. Reagents for IgM purification

5.1 Binding buffer (20 mM sodium phosphate, 0.8 M $(NH_4)_2SO_4$, pH 7.5)

1 M Na_2HPO_4	5.8 ml
1 M NaH_2PO_4	4.2 ml
$(NH_4)_2SO_4$	52.856 g
ddH_2O	400 ml

Adjusted the pH to 7.5 with 5 N NaOH

Adjusted the volume to 500 ml with ddH_2O

Mixed thoroughly and filtrated through 0.2 µm Millipore membrane filter

Kept at 4 ºC, degas for 30 min before used

5.2 4X Binding buffer (100 ml)

1 M Na_2HPO_4	4.6 ml
1 M NaH_2PO_4	3.36 ml
$(NH_4)_2SO_4$	42.284 gm
ddH_2O	70 ml

Adjusted the pH to 7.5 with 5 N NaOH

Adjusted the volume to 100 ml. with ddH_2O

Mixed thoroughly and filtrated through 0.2 µm Millipore membrane filter

Kept at 4 ºC, degas for 30 min before used

5.3 Eluting buffer (20 mM sodium phosphate pH 7.5)

1 M Na_2HPO_4	11.6 ml
1 M NaH_2PO_4	8.4 ml
ddH_2O	800 ml

Adjusted the pH to 7.5 with 5 N NaOH

Adjusted the volume to 1000 ml. with ddH_2O

Mixed thoroughly and filtrated through 0. 2 µm Millipore membrane filter

Kept at 4 ºC, degas for 30 min before used

5.4 Regeneration buffer

1 M Na_2HPO_4	5.8	ml
1 M NaH_2PO_4	4.2	ml
Isopropanol	150	ml
ddH_2O	200	ml

Adjusted the pH to 7.5 with 5 N NaOH

Adjusted the volume to 500 ml. with ddH_2O

Mixed thoroughly and filtrated through 0.2 µm Millipore membrane filter

Kept at 4 ºC, degas for 30 min before used

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Presentation

Kodchakorn Mahasongkram, Napaporn Apiratmateekul, Supansa Pata, Panida Khunkeawla and Watchara Kasinrerk. Monoclonal Antibody MT3 Recognizes a New T Lymphocyte Subpopulation. The Annual Academic Meeting, Faculty of Associated Medical Sciences. Chiang Mai, Thailand. November 2007.