

## APPENDIX

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

Chemicals/Materials	Source
2-mercaptoethanol	Merck, Darmstadt, Germany
Ammonium persulfate	Sigma, St. Louis, MO, USA
Bovine serum albumin	Sigma, St. Louis, MO, USA
Bromphenol blue	Merck, Darmstadt, Germany
Coomassie brilliant blue R-250	Bio-Rad, Hercules, CA, USA
Dimethyl sulfoxide	Sigma, St. Louis, MO, USA
FACS lysing solution	Becton Dickinson, San Jose, CA, USA
Ficoll-Hypaque solution	Sigma, St. Louis, MO, USA
FITC	Sigma, St. Louis, MO, USA
FITC-conjugated sheep F(ab') <sub>2</sub> anti-mouse Igs	Silenus, Boronia, Victoria, Australia
Glacial acetic acid	Merck, Darmstadt, Germany
Glycerol	Merck, Darmstadt, Germany
Isotyping-ELISA kit	Sigma, St. Louis, MO, USA
Methanol	Merck, Darmstadt, Germany
<i>o</i> -phenylenediamine	Sigma, St. Louis, MO, USA
Paraformaldehyde	Fluka, Buchs, Switzerland

Potassium chloride	Merck, Darmstadt, Germany
Potassium dihydrogen phosphate	Merck, Darmstadt, Germany
Prestained SDS-PAGE standards	Bio-rad, Hercules, CA, USA
Protein G sepharose	Sigma, St. Louis, MO, USA
Rabbit anti-chicken IgG conjugated HRP	Zymed, San Francisco, CA, USA
Sodium azide	Merck, Darmstadt, Germany
Sodium chloride	Merck, Darmstadt, Germany
Sodium dodecyl sulfate	Sigma, St. Louis, MO, USA
Sodium hydrogen carbonate	Merck, Darmstadt, Germany
Sodium hydrogen phosphate	Merck, Darmstadt, Germany
Sodium sulfate	Merck, Darmstadt, Germany
TEMED	BioRad Laboratories, Griffin
Tween 20	Fluka, Buchs, Switzerland
Vivaspin tube	Satorius AG, Goettingen, Germany

**Appendix B:** List of antibodies used in this study

<b>Monoclonal antibodies</b>	<b>Isotype</b>
M6-1D4	IgM
M6-1E9	IgG2a
M6-1B9	IgG3
MT14/2	IgG2b
OKT-3	IgG1
VIMD5	IgM
MEM-97	IgG1

**Appendix C: List of instruments used in this study**

<b>Instrument-Model</b>	<b>Source</b>
Electrophoresis and Electrotransfer unit	Amersham, USA
ELISA reader	Bio Tek instruments, USA
High speed micro refrigerated centrifuge	Tomy, USA
Microcentrifuge	Kendro, USA
Microdialyzer system	Pierce, USA
pH meter	Precisa, Switzerland
Refrigerator (-20°C)	Sanyo, Thailand
Spectrophotometer UV-1201	Shimadzu Co., Japan
Ultracentrifuge	Beckman, USA
Flow cytometer-FACSCalibur	Beckton Dickinson, USA

**Appendix D: Reagents and buffers preparation****1. Reagents for mouse immunoglobulins purification****1.1 20 mM Sodium phosphate (pH 7.0)**

1M Na <sub>2</sub> HPO <sub>4</sub> · 12 H <sub>2</sub> O	5.8 ml
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1M NaH <sub>2</sub> PO <sub>4</sub>	4.2 ml
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Distilled water	400 ml
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Adjust pH to 7.0 by HCl or NaOH

Adjust volume to 500 ml

Filter with 0.2 μm millipore filter, store at room temperature

**1.2 Elute buffer (0.1M citric acid pH 3.0)**

Citric acid-1-hydrate	2.1 g
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Distilled water	70 ml
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Adjust pH to 3.0 by 5 N NaOH

Adjust volume to 100 ml

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

**1.3 Neutralizing buffer (2N Tris-HCl pH 8.0)**

Tris-base	24.22 g
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Distilled water	60 ml
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Adjust pH to pH 8.0 by concentrate HCl

Adjust volume to 100 ml, store at 4°C

**1.4 Storage buffer (0.05% NaN<sub>3</sub>-PBS pH 7.4)**

Na <sub>2</sub> HPO <sub>4</sub> · 12 H <sub>2</sub> O	1.15 g
KH <sub>2</sub> PO <sub>4</sub>	0.100 g
NaCl	0.877 g
10% NaN <sub>3</sub> in distilled water	500 µl
Distilled water	80 ml
Adjust pH to 7.4 by 5N NaOH	
Adjust volume to 100 ml, store at 4°C	

**2. Reagents for indirect immunofluorescence staining****2.1 Phosphate buffer saline (PBS)**

NaCl	8 g
KCl	0.2 g
Na <sub>2</sub> HPO <sub>4</sub>	1.15 g
KH <sub>2</sub> HPO <sub>4</sub>	0.2 g
Distilled water	900 ml

Adjust pH to 7.2 by 5N NaOH

Adjust volume to 1000 ml, store at room temperature

**2.2 1% BSA-0.02% NaN<sub>3</sub> in PBS**

Bovine serum albumin fraction V	10 g
PBS pH 7.2	1000 ml
10% NaN <sub>3</sub> in PBS	200 µl

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. Reagents for ELISA

### 3.1 Phosphate citrate buffer pH 5.0

Citric acid monohydrate 2.57 g

Na<sub>2</sub>HPO<sub>4</sub> 3.65 g

Distilled water 300 ml

Mix well, Adjust pH to 5.0 with 1N NaOH

Adjust volume to 500 ml, store at 4°C

### 3.2 Coating buffer (0.1M carbonate-bicarbonate buffer pH 9.6)

Na<sub>2</sub>CO<sub>3</sub> 1.06 g

NaHCO<sub>3</sub> 1.26 g

Distilled water 200 ml

Mix well, adjust pH to 9.6 with concentrated HCl

Adjust volume to 250 ml, store at 4°C

### 3.3 0.05% Tween-PBS

PBS pH 7.2 500 ml

Tween 20 250  $\mu$ l

Mix well, store at room temperature

### 3.4 Blocking buffer (2% BSA-PBS)

Bovine serum albumin 2 g

PBS pH 7.2 100 ml

Mix well, prepare before use

### 3.5 Stop reaction solution (4N H<sub>2</sub>SO<sub>4</sub>)

Concentrate HCl 20 ml

Distilled water 160 ml

Slowly dropwise HCl to distilled water, store at room temperature

## 4. Reagent for SDS-PAGE

### 4.1 (4x)1.5M Tris HCl pH 8.8

Tris base 18.15 g

Deionized distilled water 80 ml

Adjust pH to 8.8 by concentrate HCl

Adjust volume to 100 ml

Store at 4°C

### 4.2 (4x) 0.5M Tris HCl pH 6.8

Tris base 6 g

Deionized distilled water 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

Distilled water 1 ml

Mix well, aliquot and store at -20°C

#### 4.4 10% Sodium dodecyl sulfate (SDS)

Sodium dodecyl sulfate 10 g

Distilled water 100 ml

Mix well, aliquot and store at -20°C

#### 4.5 2x non-reducing buffer

0.25M Tris HCl pH6.8 5 ml

87% glycerol 2 ml

10% SDS 2 ml

Distilled water 700  $\mu$ l

Bromphenol blue 0.002 g

Mix well, aliquot and store at -20°C

#### 4.6 2x reducing buffer

0.5M 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 Slab gel

##### 12.5% separating gel      4% stacking gel

Distilled water 3.2 ml 1.5 ml

Monomer 4.2 ml 332.5  $\mu$ l

(4x) 1.5 M Tris HCl pH 8.8 2.5 ml -

(4x) 0.5 M Tris HCl pH 6.8 - 625  $\mu$ l

10% SDS 100  $\mu$ l 25  $\mu$ l

10% APS 50  $\mu$ l 12.5  $\mu$ l

TEMED 10  $\mu$ l 5  $\mu$ l

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**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 I**

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

**4.11 Destaining solution II**

Methanol	25 ml
Acetic acid	35 ml

Adjust volume with distilled water to 500 ml

Store at room temperature