



APPENDIX

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

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

Chemicals/Materials	Source
Absolute ethanol	Merck, Darmstadt, Germany
Acrylamide	Merck, Darmstadt, Germany
Agarose	Sigma, St. Louis, MO, USA.
Ammonium persulfate	Sigma, St. Louis, MO, USA.
Ampicillin	Sigma, St. Louis, MO, USA.
Avidin	Sigma, St. Louis, MO, USA.
Bis-acrylamide	Sigma, St. Louis, MO, USA.
10X BM condimed HI	Roche, Mannheim, Germany
Bovine serum albumin	Sigma, St. Louis, MO, USA.
Chemilumnescent reagent	Pierce Biotechnology, Rockford, IL, USA.
Developer and replenisher	Kodak, NY, USA.
Diethyl ether	Merck, Darmstadt, Germany
dNTP	Fermantas, MA, USA.
DNA ligase	Fermantas, MA, USA.
Ethidium bromide	Sigma, St. Louis, MO, USA.
Ethylenediaminetetraacetate (EDTA)	Sigma, St. Louis, MO, USA.
Ethyl alcohol	Merck, Darmstadt, Germany
Fetal calf serum	Gibco, Grand Island, N.Y., USA.
Fungizone (Amphotericin B)	Bristol-Myer Squibb, Cincinnati, OH, USA.
Glacial acetic acid	Merck, Darmstadt, Germany

Glycerol	Sigma, St. Louis, MO, USA.
Glycine	Sigma, St. Louis, MO, USA.
50X Hypoxanthine Aminopterin	Sigma, St. Louis, MO, USA.
Thymidine (HAT)	
100X Hypoxanthine Thymidine (HT)	Gibco, Grand Island, N.Y., USA.
Hydrochloric acid	Lio, Ballerup, Denmark
Isocove's Modified Dulbecco's Medium (IMDM)	Gibco, Gran Island, N.Y., USA.
Isopropyl-B-D-thiogalactopyranoside (IPTG)	Amresco, Solon, OH, USA.
Isopropanal	Merck, Darmstadt, Germany
Kanamycin	Sigma-Aldrich Co., USA.
LB broth base	Gibco, Grand Island, N.Y., USA.
MagnaBind Streptavidin Bead	Pierce, Rockford, IL, USA.
2-mercaptoethanol	Merck, Darmstadt, Germany
Methanol	Merck, Darmstadt, Germany
MOPS	Amresco, Solon, OH, USA.
Nitrocellulose membrane	PALL, East Hill, NY, USA.
Paraformaldehyde	Fluka, Buchs, Switzerland
Potassium chloride	Merck, Darmstadt, Germany
Potassium dihydrogen phosphate	Merck, Darmstadt, Germany
Prestained SDS-PAGE standards	Fermantas, MA, USA.
Primer	Invitrogen, Carmstadt, Germany
proofStart DNA polymerase	Fermantas, MA, USA.

QIAGEN Gel Extraction kit	QIAGEN, Hiden, Germany
QIAGEN PCR purification kit	QIAGEN, Hiden, Germany
QIAGEN Plasmid mini kit	QIAGEN, Hiden, Germany
Restriction enzymes and 10X reaction buffer	Fermentas, MA, USA.
Skimmed milk	Difco laboratories, Detroit, MI, USA.
Sodium azide	Merck, Darmstadt, Germany
Sodium bicarbonate	Merck, Darmstadt, Germany
Sodium dodecyl sulfate	Sigma, St. Louis, MO, USA.
Sodium hydrogen carbonate	Merck, Darmstadt, Germany
Sodium sulfate	Merck, Darmstadt, Germany
Streptavidin-HRP	Zymed, South San Francisco, CA.
T4 ligase and 10X reaction buffer	Fermentas, MA, USA.
Taq DNA polymerase and 10X reaction buffer	Fermentas, MA, USA.
Buffer	
TEMED	BioRad Laboratories, Griffin
Tetracycline	Sigma, St. Louis, MO, USA.
3,3',5,5'-Tetramethylbenzidine (TMB)	Zymed, South San Francisco, CA.
Tris-base	Sigma-Aldrich Co., USA.
Tryptone	Life Technologies, Scotland
Tween 20	Fluka, Buchs, Switzerland
Yeast extract	Life Technologies, Scotland

Appendix B: List of antibodies used in this study

Monoclonal antibody clones	Isotype	Recognized antigen
MT4	IgM	CD4
MT99/3	IgG2a	CD99
BCCP-2	IgG1	BCCP
M6-1B9	IgG3	CD147
M6-1E9	IgG2a	CD147

Appendix C: List of Microorganism used in this study

Microorganism

1. *Escherichia coli* Origami B (Novagen, Madison, WI)

Genotype: [$F^- ompT hsdS_B r_B^- m_B^- gal dcm lacYI ahpC gor522::Tn10 (Tc^R)$
 $trxB::kan pAR5615 (Ap^R)$]

2. *Escherichia coli* Nova Blue (Novagen, Madison, WI)

Genotype: [$endA1 hsdR17(r_{K12}^- m_{K12}^+) supE44 thi-1 recA1 gyrA96 relA1 lac$
 $F' [proA^+ B^+ lacI^q Z\Delta M15::Tn10]$]

3. *Escherichia coli* TG1 (Amersham Pharmacia Biotech, Roosendaal, Sweden)

Genotype: [$K12\Delta(lac-pro), supE, thi, hsd\Delta 5/F' [traD36, proAB, lacI^q,$
 $lacZ\Delta M15]$]

4. *Escherichia coli* XL-1 Blue (Stratagene, USA)

Genotype: [$recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac F' [proA^+ B^+$
 $lacI^q Z\Delta M15::Tn10]$]

Appendix D: List of instruments used in this study

Instruments	Source
2-20 µl Autopipette	Bio-Rad, USA
20-200 µl Autopipette	Bio-Rad, USA
100-1000 µl Autopipette	Bio-Rad, USA
40-350 µl multichannel autopipette	Scorex, Switzerland
Autoclave	Huxey, Taiwan
CO ₂ incubator	Thermo electron corporation, USA
Electrophoresis and Electrotransfer unit	Amersham, USA Hoffer
Flow cytometer-FACSCalibur	Beckton Dickinson, USA
High speed micro refrigerated centrifuge	Tomy, Japan
Inverted microscope	Olympus, Japan
Laminar flow	Nuaire, USA
Light microscope	Olympus, Japan
Microcentrifuge	Sorvall, Germany
Microplate reader	Sunrise tecan, Austria
PCR Mastercycler personal	Eppendorf, USA
pH meter	Precisa, Switzerland
Refrigerated centrifuge	Sorvall, Germany
Refrigerator (-20°C)	Sanyo, Thailand
Rotator	Technomara, Switzerland
Semi-dry blotting	Amersham Biosciences, Sweden
Spectrophotometer UV-1201	Shimadzu, Japan

Appendix E: Reagents and buffers preparation**1. Reagents for agarose gel electrophoresis****1.1 10x TAE buffer**

Tris-base	24.20	g
Glacial acetic acid	5.71	ml
0.5 M EDTA (pH 8.0)	10	ml
Adjusted final volume to 500 ml, stored at room temperature		

1.2 1% agarose gel

Agarose gel	1	g
TBE buffer	100	ml
Heated until dissolved		

1.3 Ethidium bromide working solution (10 mg/ml)

Ethidium bromide	1	g
Distilled water	100	ml
Kept in the dark bottle and stored at room temperature		

2. Reagents for bacterial culture**2.1 LB broth (100 ml)**

Tryptone	1	g
Yeast extract	0.5	g
Sodium chloride	1	g
Distilled water up to	100	ml

Sterilized with Autoclave at 121°C for 15 minutes and Stored at 4°C

Checked sterility before used

2.2 LB broth contain chloramphenicol, kanamycin and tetracycline

LB broth	100	ml
Chloramphenicol (50 mg/ml)	50	μl
Kanamycin (70 mg/ml)	14.29	μl
Tetracycline (10 mg/ml)	200	μl
Checked sterility before used		

2.3 Super Broth

Tryptone	30	g
Yeast extracts	20	g
Morpholinepropanesulphonic acid (MOPS)	10	g
Distilled water	1000	ml

Sterilized with autoclave at 121°C for 15 minutes and stored at 4°C

3. Reagents for plasmid mini-preparation

3.1 3 M Sodium acetate pH 7.0

Sodium acetate	40.8	g
Distilled water	100	ml
Adjusted pH to 7.0 by HCl/NaOH		

3.2 Potassium Acetate

Potassium Acetate	29.4	g
Glacial acetic acid	11.5	ml

Adjusted final volume to 100 ml with distilled water

3.3 10M NaOH

NaOH	200	g
Distilled water	500	ml

3.4 7.5 M NH₄Acetate

NH ₄ Acetate	57.8	g
Distilled water	100	ml

3.5 1M glucose buffer

D-glucose	18.2	g
Distilled water	100	ml

Sterilized with autoclave at 121°C for 15 minutes and stored at 4°C

3.6 0.5 M EDTA pH 8.0

EDTA	32.22	g
Distilled water	150	ml

Adjusted pH to 8.0 by HCl/NaOH

Adjusted final volume to 200 with distilled water

3.7 10X glucomix

1 M glucose buffer	50	ml
0.5 M EDTA pH 8.0	20	ml
1 M Tris pH 8.0	25	ml
Distilled water	5	ml

Sterilized with Autoclave at 121°C for 15 minutes and stored at 4°C

3.8 1X glucomix-lysozyme solution

10X glucomix	300	ml
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Lysozyme (50 mg/ml in DW)	300	μl
Distilled water	2.4	ml
Freshly prepared before used		

4. Reagents for indirect immunofluorescence staining

4.1 Phosphate buffer saline (PBS)

NaCl	8	g
KCl	0.2	g
Na ₂ HPO ₄	1.15	g
KH ₂ HPO 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%NaN₃ in PBS

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

Mixed well until BSA completely dissolved, stored at 4°C

4.3 1%Paraformaldehyde in PBS

Paraformaldehyde	5	g
PBS pH 7.2	500	ml

Heat at 56°C until dissolved

Filtrated with 0.2 μm millipore filter, stored at 4°C

5. Reagents for ELISA

5.1 Coating buffer (0.1M carbonate-bicarbonate buffer pH 9.6)

Na_2CO_3	1.06	g
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NaHCO_3	1.26	g
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Distilled water	200	ml
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Mixed and adjusted pH to 9.6 with concentrated HCl

Adjusted final volume to 250 ml with distilled water, stored at 4°C

5.2 0.05% Tween-PBS

PBS (pH 7.2)	500	ml
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Tween 20	250	μl
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Mixed and stored at room temperature

5.3 Blocking buffer (2% BSA-PBS)

Bovine serum albumin	2	g
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PBS (pH 7.2)	100	ml
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Freshly prepared before used

5.4 Stop reaction solution (1N HCl)

Concentrate HCl	8.3	ml
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Distilled water	91.7	ml
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Slowly drop wise HCl to distilled water, stored at room temperature

6. Reagents for SDS-PAGE

6.1 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		
Stored at 4°C		

6.2 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		
Stored at 4°C		

6.3 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	2.2	ml
2-ME	1	ml
1% Bromphenol blue	40	μl

Mixed well, aliquot and stored at -20°C

6.4 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		

6.5 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 μ m filter, stored at room temperature		

6.6 30% Monomer (30.8% acrylamide, 2.7% bis-acrylamide)

Acrylamide	60	gm
Bis-acrylamide	1.6	gm
Deionized distilled water	200	ml

Mixed thoroughly and filtrated through 0.2 μ m Millipore membrane filter,
kept in dark at 4°C

6.7 Slab gel

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

6.8 10% SDS

Sodium dodecyl sulfate	10 g
Distilled water	100 ml
Mixed well, aliquot and stored at -20°C	

6.9 10% APS

Ammonium persulfate	0.1 g
Distilled water	1 ml
Mixed well, aliquot and stored at -20°C	

7. Reagents for tissue culture**7.1 Incomplete IMDM medium**

IMDM powder	1 pack
NaHCO ₃	3.024 g
Gentamycin (40 mg/ml)	1 ml

Dissolved in ddH₂O and adjusted volume to 1000 ml

Filtrated through 0.2 µm Millipore membrane filter

Added Fungizone (5 mg/ml) 500 µl

Mixed and stored at 4 °C

Checked sterility before used

7.2 Complete IMDM medium

Incomplete IMDM medium 90 ml

Fetal calf serum 10 ml

Checked sterility before used

7.3 0.6% 2-mercaptoethanol (2-ME)

Incomplete IMDM 5 ml

2-mercaptoethanol 30 µl

Filtrated through 0.2 µm Millipore membrane filter Aliquot 50 µl/tube,

stored at -20 °C

7.4 1X HAT medium

Incomplete IMDM 78 ml

Heat inactivated FCS 10 ml

BM condimed HI 10 ml

0.6% 2-ME 30 µl

50X HAT 2 ml

Aliquot and stored at 4 °C

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7.5 1X HT medium

Incomplete IMDM	119	ml
Heat inactivated FCS	15	ml
BM condimed HI	15	ml
0.6% 2-ME	30	μ l
100X HT	1	ml
Aliquot and stored at 4 °C		

7.6 Incomplete MEM medium

MEM powder	1	pack
Deionized distilled water	900	ml
NaHCO ₃	2.2	g
Stirred until dissolved		
Gentamycin (40 mg/ml)	1	ml
Adjusted final volume to 1000 ml with distilled water		
Filtrated with 0.2 μ m Millipore filter		
Sterile fungizone (2.5 mg/ml)	500	μ l

Checked sterility before used

7.7 Hypotonic solution (0.083% NH₄Cl) for RBC lysing

NH ₄ Cl	0.829	g
KHCO ₃	0.1	g
EDTA	0.0037	g
Deionized distilled water	90	ml

Adjusted pH to 7.2 with 1N HCl Adjusted volume to 100 ml

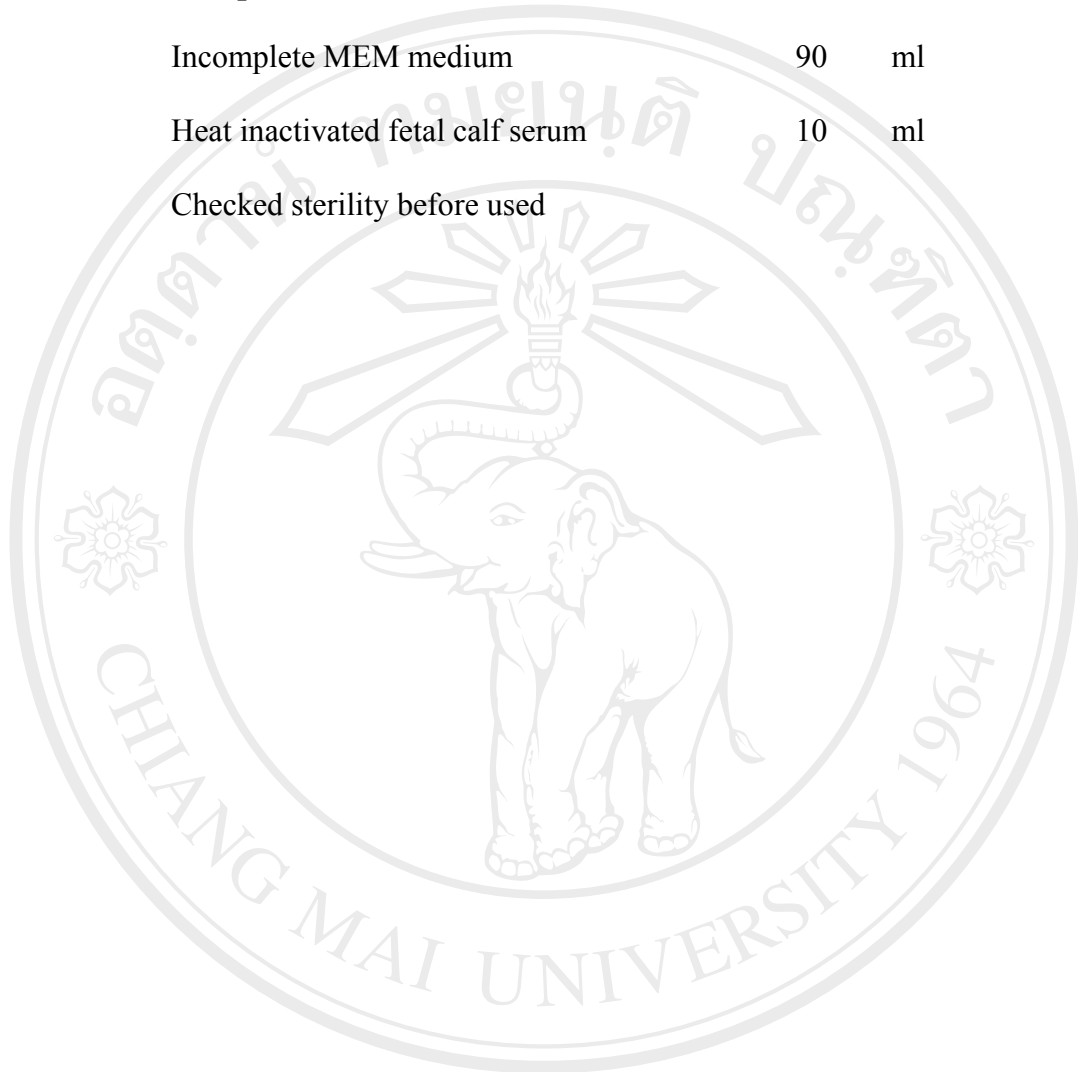
Filtrated 0.4 μm Millipore membrane filter and stored at 4 °C

7.8 Complete MEM medium

Incomplete MEM medium 90 ml

Heat inactivated fetal calf serum 10 ml

Checked sterility before used



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