

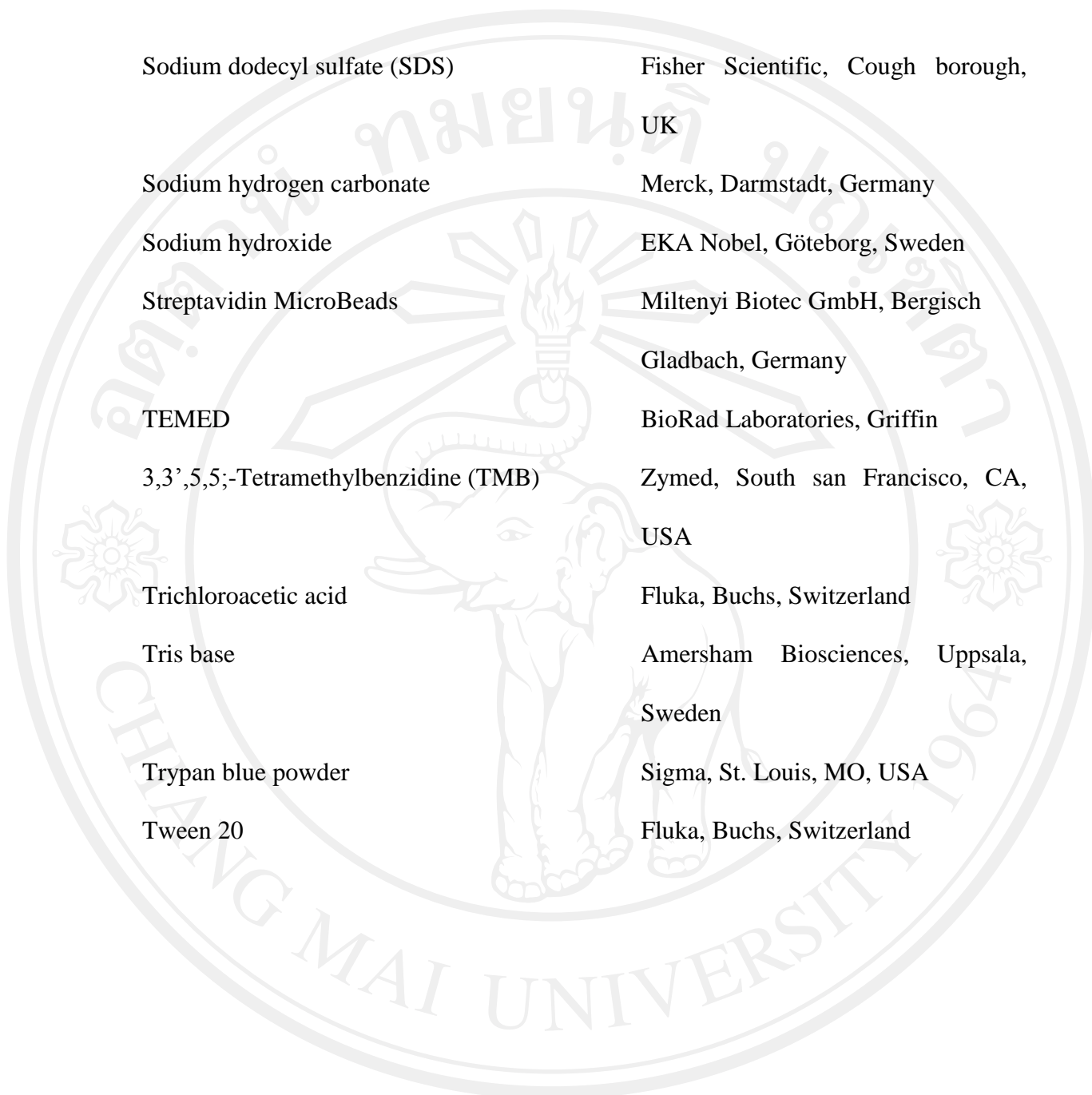
APPENDIX

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

Chemicals/Materials	Source
Acrylamide/Bis 40% Solution	Amresco, Ohio, USA
Ammonium chloride	Sigma, St. Louis, MO, USA
Ammonium persulfate	Fluka, Buchs, Switzerland
10X BM condimed HI	Roche, Mannheim, Germany
BD FACS™ lysing solution	Becton Dickinson, Franklin Lakes, NJ, USA
Bovine serum albumin	Sigma, St. Louis, MO, USA
Boric acid	Sigma, St. Louis, MO, USA
Carbon tetrachloride	May & Baker Dagenham, Ikeja Lagos, Nigeria
Cellulose acetate membrane	Helena Laboratories, Beaumont, TX, USA
Coomassie brilliant blue R-250	Bio-Rad, Hercules, CA, USA
DEAE Sepharose	Amersham Biosciences, Uppsala, Sweden
Diethyl ether	Merck, Darmstadt, Germany
Dimethyl sulfoxide (DMSO)	Sigma, St. Louis, MO, USA
Di-sodium hydrogen orthophosphate anhydrous	Fisher Scientific, Cough borough, UK

Disodium salt	England
Ethanol	Merck, Darmstadt, Germany
Ethylenediamine tetra acetic acid (EDTA)	BDH Laboratory Supplies, TD, England
Fetal bovine serum	Gibco, Gran Island, N.Y., USA
Fungizone (Amphotericin B)	Bristol-Myer Squibb, Cincinnati, OH, USA
Gentamycin	Atlantic Labs, Selangor, Malaysia
Gential violet/ Crystal violet	Sigma, St. Louis, MO, USA
Glacial acetic acid	Merck, Darmstadt, Germany
Glycerol	Merck, Darmstadt, Germany
Goat anti-mouse IgG MicroBeads	Miltenyi Biotec GmbH, Bergisch Gladbach, Germany
HRP-conjugated rabbit anti-mouse Igs antibodies	Dako, Glostrup, Denmark
Hydrochloric acid	Merck, Darmstadt, Germany
50X Hypoxanthine Aminopterin Thymidine (HAT)	Gibco, Gran Island, N.Y., USA
100X Hypoxanthine Thymidine (HT)	Gibco, Gran Island, N.Y., USA
Isocove's Modified Dulbecco's Medium (IMDM)	Gibco, Gran Island, N.Y., USA
Isopropanol	Merck, Darmstadt, Germany
2-mercaptoethanol (2-ME)	Sigma, St. Louis, MO, USA
Methanol	Merck, Darmstadt, Germany

N-Acetylmuramyl-L-alanyl-D-isoglutamine Hydrate (Muramyl dipeptide; MDP)	Sigma, St. Louis, MO, USA
PageBlue Protein Staining Solution	Fermentas, MA, USA
Paraformaldehyde	Fluka, Buchs, Switzerland
Polyoxyethylenes orbitan monolaurate (Tween 20)	Sigma, St. Louis, MO, USA
Polyclonal swine anti rabbit immunoglobulins-FITC	Dako, Glostrup, Denmark
Potassium chloride	Merck, Darmstadt, Germany
Potassium cyanide	Reidel-DE Haen AG Seelze- Handnover, Seelze, Germany
Potassium dihydrogen phosphate	Merck, Darmstadt, Germany
Potassium hydrogen carbonate	Fluka, Buchs, Switzerland
Rabbit anti-mouse immunoglobulins	Dako, Glostrup, Denmark
Rat anti-mouse IgM MicroBeads	Miltenyi Biotec GmbH, Bergisch Gladbach, Germany
RPMI 1640 medium	Gibco, Gran Island, N.Y., USA
Sodium azide	Reidel-DE Haen AG Seelze- Handnover, Seelze, Germany
Sodium bicarbonate	Merck, Darmstadt, Germany
Sodium carbonate anhydrous	Merck, Darmstadt, Germany
Sodium chloride	Merck, Darmstadt, Germany
Sodium dihydrogen phosphate	Merck, Darmstadt, Germany



Sodium dodecyl sulfate (SDS)	Fisher Scientific, Cough borough, UK
Sodium hydrogen carbonate	Merck, Darmstadt, Germany
Sodium hydroxide	EKA Nobel, Göteborg, Sweden
Streptavidin MicroBeads	Miltenyi Biotec GmbH, Bergisch Gladbach, Germany
TEMED	BioRad Laboratories, Griffin
3,3',5,5'-Tetramethylbenzidine (TMB)	Zymed, South san Francisco, CA, USA
Trichloroacetic acid	Fluka, Buchs, Switzerland
Tris base	Amersham Biosciences, Uppsala, Sweden
Trypan blue powder	Sigma, St. Louis, MO, USA
Tween 20	Fluka, Buchs, Switzerland

Appendix B: List of instruments used in this study

Instruments	Source
Autoclave	Huxey, Taiwan
Autopipette	Gilson, France
Centrifuge	Kendo Laboratory, Germany
CO ₂ incubator	Thermo electron corporation, USA
Electrophoresis and Electrotransfer unit	Amersham,,USA
ELISA reader	Tecan, Austria
GeneQuant pro	Amersham Bioscience, England
Flow cytometer-FACSCalibur	Beckton Dickinson, USA
Inverted microscope	Olympus, Japan
Laminar Flow	NUAIRE, USA
Light microscope	Olympus, Japan
Microcentrifuge	Sorvall, Germany
Multichanel autopipette	Gilson, France
pH meter	Sartorius, USA
Refrigerated centrifuge	Sorvall, Germany
Rotator	Technomara, Switzerland
Synergy4	Biotek, USA
Water bath	Memmert, Germany

Appendix C: Reagents and buffers preparation**1. Reagents for human blood cell and cell lines culture****1.1 Incomplete IMDM medium**

IMDM powder	1 pack
NaHCO ₃	3.024 g
Gentamycin (40 mg/ml)	1 ml
Dissolved in ddH ₂ O 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°C	

1.2 Complete IMDM medium

Incomplete IMDM medium	90 ml
Heat inactivated fetal bovine serum (FBS)	10 ml
Checked sterility before used	

1.3 Incomplete RPMI 1640 medium

RPMI 1640 powder	1 pack
NaHCO ₃	2 g
Gentamycin (40 mg/ml)	1 ml
HEPES	3.57 g

Dissolved in ddH₂O and adjust volume to 1000 ml

Filtrated through 0.2 µm Millipore membrane filter

Added Fungizone (5 mg/ml) 500 μ l

Stored at 4°C

1.4 Complete RPMI1640 medium

RPMI 1640 medium 90 ml

Heat inactivated fetal bovine serum (FBS) 10 ml

1.5 0.6% 2-mercaptoethanol (2-ME)

Incomplete IMDM 5 ml

2-mercaptoethanol 30 μ l

Filtrated through 0.2 μ m Millipore membrane filter

Aliquot 50 μ l/tube, stored at -20°C

1.6 1xHAT medium

Incomplete IMDM 78 ml

Heat inactivated FBS 10 ml

10X BM condimed HI 10 ml

0.6% 2-ME 30 μ l

50X HAT 2 ml

Stored at 4°C

1.7 1xHT medium

Incomplete IMDM 119 ml

Heat inactivated FBS 15 ml

BM-condimed HI	15 ml
0.6% 2-ME	30 μ l
100X HT	1 ml
Stored at 4°C	

1.8 Hypotonic solution (0.083% NH_4Cl) for RBC lysing

NH_4Cl	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 0.2 μm Millipore membrane filter	
Stored at 4°C	

1.9 Turk's solution

Glacial acetic acid	3 ml
1% gential violet	1 ml

Adjust volume to 100 ml with dH_2O

Filtrated by Whatman filter paper no. 1 and stored at room temperature.

1.10 Trypan blue (0.2%)

Trypan blue powder	0.2 g
PBS pH 7.2	100 ml

Filtrated by Whatman filter paper No. 1 and stored at room temperature.

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

Incomplete IMDM	65 ml
Heat inactivated FBS	25 ml
DMSO (Hybrimax)	10 ml
Mix well, stored at 4°C	

2. Reagent for SDS-PAGE

2.1 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	

2.2 4X Stacking gel buffer (0.5M Tris HCl pH 6.8)

Tris base	6.0 g
Deionized distilled water (ddH ₂ O)	80 ml
Adjusted pH to 6.8 by concentrate HCl	
Adjusted final volume to 100 ml	
Stored at 4°C	

2.3 10X non-reducing buffer (NRB)

ddH ₂ O	1.25 ml
1 M Tris-HCl pH 6.8	0.625 ml
Glycerol	1 ml
10% SDS	2 ml
1% Bromphenol blue	125 µl
Aliquot 300 µl/tube, kept at -20 °C	

2.4 5X reducing buffer (RB)

10X NRB	250 µl
2-ME	25 µl
ddH ₂ O	225 µl
Aliquot 100 µl/tube, kept at -20 °C	

2.5 Running buffer

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

Mixed well, prepare before use

2.7 Slab gel

	separating gel			4% stacking gel
	12.5%	10%	7.5%	
Distilled water	4.2 ml	4.85 ml	5.475 ml	3.175 ml
Acry/Bis 40% Solution	3.1 ml	2.5 ml	1.875 ml	500 μ l
4X Separating gel buffer	2.5 ml	2.5 ml	2.5 ml	-
4X Stacking gel buffer	-	-	-	1.25 ml
10% SDS (in distilled water)	100 μ l	100 μ l	100 μ l	50 μ l
10% APS (in distilled water)	50 μ l	50 μ l	50 μ l	25 μ l
TEMED	10 μ l	10 μ l	10 μ l	5 μ l

2.8 10% APS

Ammonium persulfate	0.1 g
Distilled water	1 ml

Mix well, aliquot and stored at -20°C

3. Reagents for indirect immunofluorescence staining

3.1 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

3.2 1%BSA-0.02%NaN₃ in PBS

Bovine serum albumin fraction V 10 g

PBS pH 7.2 1000 ml

10% NaN₃ in PBS 200 µl

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

3.3 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

4. Reagents for ELISA

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

Na₂CO₃ 1.06 g

NaHCO₃ 1.26 g

Distilled water 200 ml

Mixed and adjusted pH to 9.6 with concentrated HCl

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

4.2 0.05% Tween-PBS

PBS pH 7.2	500 ml
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Tween 20	250 μ l
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Mixed and stored at room temperature

4.3 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

4.4 Stop reaction solution (1N HCl)

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

5. Reagents for cellulose acetate electrophoresis**5.1 10X Tri-Borate-EDTA (TBE) buffer pH 8.6 (0.85 M Tris 0.0015 M EDTA****0.055 M Boric acid)**

Tris base	121 g
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EDTA (disodium salt)	11 g
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Boric acid	15 g
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ddH ₂ O	800 ml
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Adjusted the pH to 8.6 with saturated Boric acid

Adjusted the volume to 1000 ml with ddH₂O and stored at room temperature

5.2 Working TBE buffer pH 8.6

10X TBE buffer pH 8.6 100 ml

ddH₂O 900 ml

Mix thoroughly and kept at room temperature

6. Reagents for purification of hemoglobins

6.1 10X Tris-HCl buffer pH 9.0

Tris base 60.57 g

dH₂O 800 ml

Adjusted the pH to 9.0 with 4 N HCl

Adjusted the volume to 1000 ml with dH₂O and stored at room temperature.

6.2 Working Tris-HCl-KCN (THK) buffer pH 9.0

10X Tris-HCl buffer pH 9.0 100 ml

ddH₂O 900 ml

KCN 0.1 g

Mix thoroughly and filtrated by 0.2 μm Millipore membrane filter, kept at room temperature

6.3 Working THK buffer pH 6.5

10X Tris-HCl buffer pH 9.0 100 ml

ddH₂O 800 ml

KCN 0.1 g

Adjusted the pH to 6.5 with 4 N HCl

Adjusted the volume to 1000 ml with ddH₂O

Mix thoroughly and filtrated through 0.2 μ m Millipore membrane filter, kept at room temperature

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2001	Certificate of senior high school, La Salle Chotiravi Nakhonsawan School, Nakhon Sawan
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Publication:

1. **Napaporn Apiratmateekul**, Ponrat Phunpae, Watchara Kasinrerker. A modified hybridoma technique for production of monoclonal antibodies having desired isotypes. *Cytotechnology* 2009; 60: 45-51

2. จดสิทธิบัตร เรื่อง กรรมวิธีการผลิตโมโนโคลนอลแอนติบอดีที่กำหนดไอโซไซป์ที่ต้องการ

โดย: วัชระ กสิณฤกษ์ และ นภาพร อภิรัฐเมธีกุล

ขึ้นทะเบียนสิทธิบัตร ประเทศไทย คำขอรับสิทธิบัตร เลขที่ 0901005713 วันที่ 18 ธันวาคม

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Poster presentation:

1. **Napaporn Apiratmateekul** and Watchara Kasinrerak. Production of Conditioned Media for Generation of Hybridomas for Monoclonal Antibody Production. Annual Academic Meeting. Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand. 28-30 November 2007.
2. **Napaporn Apiratmateekul** and Watchara Kasinrerak. Novel Hybridoma Technique for Directed Production of Monoclonal Antibody IgM and IgG Isotypes. Commission on Higher Education Congress I: University Staff Development Consortium: CHE-USDC congress I. Commission on Higher Education. Ambassador City Jomtien, Chonburi, Thailand. 5-7 September 2008.
3. **Napaporn Apiratmateekul** and Watchara Kasinrerak. Novel Hybridoma Technique for Directed Production of Monoclonal Antibody IgM and IgG Isotypes. The 2nd Graduated Students Academic Day. Faculty of Associated Medical Sciences, Chiang Mai University Chiangmai, Thailand. 12 November 2008.
4. **Napaporn Apiratmateekul** and Watchara Kasinrerak. Novel Hybridoma Technique for Directed Production of Monoclonal Antibody IgM and IgG

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Production of Polyclonal and Monoclonal Antibody by *In Vitro* Immunization.
The First International Biomedical Sciences Conference "BMS Research
Driving Collaborative ONE ASEAN". Faculty of Associated Medical
Sciences, Khonkaen University. Pullman Khon Kaen Raja Orchid Hotel, Khon
Kaen, Thailand. 8-10 February 2012.

Award:

The best Poster presentation Award. Poster title: Production of Polyclonal and
Monoclonal Antibody by *In Vitro* Immunization. The First International
Biomedical Science conference "BMS Research Driving Collaborative ONE
ASEAN". February 8-10, 2012.