APPENDIX

Appendix A List of the chemicals and materials were used in the study

All chemical and reagents used in this study are analytical grade and are listed as follows:

Chemicals/Materials	Source
Agar noble	Difco Laboratories, Detroit, MI, USA
Agarose	FCM Bioproducts, Rockland, ME, USA
Ampicillin	Sigma, St. Louis, MO, USA
Chloroquine diphosphate	Sigma, St. Louis, MO, USA
DEAE-Dextran	Sigma, St. Louis, MO, USA
Dimethyl sulfoxide (DMSO)	Sigma, St. Louis, MO, USA
Ethylenediaminetetraacetic acid	Fluka, Buchs, Switzerland
Fetal calf serum	Gibco, Grand Island, NY, USA
Glycerol	Merck, Darmstadt,Germany
Hydrochloric acid	Merck, Darmstadt,Germany
LB broth base	Gibco, Grand Island, NY, USA
NucleoSpin® Plasmid Mini kit	Macherey-Nagel GmbH & Co., Germany
Paraformaldehyde	Fluka, Buchs, Switzerland
Potassium chloride	Merck, Darmstadt,Germany

Potassium dihydrogen phosphate	Merck, Darmstadt,Germany
Protein A sepharose	Zymed Laboratories, Inc., CA, USA
QIAGEN Plasmid mega kit	QIAGEN, Hiden, Germany
Rabbit anti-mouse immunoglobulins	Dako, Glostrup, Denmark
RPMI-1640 medium	Gibco, Grand Island, NY, USA
Sheep anti-mouse immunoglobulins conjugated FITC	Silenus, Melbourne, Australia
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 hydrogen carbonate	Merck, Darmstadt,Germany
Sodium hydrogen phosphate	Merck, Darmstadt,Germany
Tetracycline	Sigma, St. Louis, MO, USA
Tris-base	Sigma, St. Louis, MO, USA
Tween 20	Fluka, Buchs, Switzerland

Appendix B List of instruments used in the present study

Instrument-Model	**Source**
Analytical balance	Mettler Toledo, Switzerland
Electrophoresis & Electrotransfer unit	Amersham, USA
ELISA reader	Bio Tek instrument
Flow cytometer-FACSCalibur	Beckton Dickinson, USA
Fluorescent microscope	Olympus, USA
High-speed micro refrigerated centrifuge	Tommy, USA
Inverted microscope	Olympus, USA
Laminar flow	NUAIR Fembrook Lane Plymouth, MN 55447
Light microscope	Olympus, USA
Liquid nitrogen tank	International Cryogenics Inc.
Microcentrifuge	Sorvall, Germany
pH meter	Precisa
Refrigerator (-20°C)	Sanyo, Thailand
Refrigerator (-70°C)	Foma Scientific
Spectrophotometer UV-1201	Shimadzu Co., Kyoto, Japan
Ultracentrifuge	Beckman, USA
Water bath	Thermoline, Australia

Appendix C Reagents and buffers preparation

1. Reagents for CD147-hIgG purification.

1.1 20 mM Sodium phosphate (pH 7.0)

1M Na_2HPO_4	5.8 ml
1M NaH_2PO_4	4.2 ml
Distilled water	400 ml

Adjusted pH to 7.0 by HCl or NaOH

Adjusted final volume to 500 ml

Filtered with 0.2μ Millipore filter, stored at 4°C

1.2 Elution buffer (0.1 M citric acid pH 3.0)

Citric acid-1-hydrate	2.1 g
Distilled water	70 ml

Adjusted pH to 3.0 by 5N NaOH

Adjusted final volume to 100 ml

Filtered with 0.2 μ Millipore filter, stored at 4°C

1.3 Neutralizing buffer (2M Tris-HCl pH 8.0)

Tris-base	24.22 g
Distilled water	60 ml

Adjusted pH to 8.0 by concentrate HCl

Adjusted final volume to 100 ml, stored at room temperature

1.4 Storage buffer (0.05% NaN_3-PBS pH 7.4)

$Na_2HPO_4.12H_2O$	1.15 g
KH_2PO_4	0.1 g

NaCl	0.877 g

Adjusted pH to 7.4 by by 5N NaOH

Adjusted final volume to 100 ml, stored at 4°C

2. Reagents for DEAE-Dextran transfection

2.1 Incomplete MEM medium

MEM powder	9.6 g (1 package)
Distilled water	900 ml
$NaHCO_3$	2.2 g

Stirred until dissolved

Gentamycin (40 mg/ml)	1 ml

Adjust final volume to 1000 ml

Filtered with 0.2 μ Millipore filter

Sterile fungizone (2.5 mg/ml)	500 μl

Checked sterility before used

2.2 Complete MEM medium

Incomplete MEM medium	90 ml
Fetal calf serum	10 ml

Checked sterility before used

2.3 0.5 mM EDTA-PBS

PBS pH 7.2	100 ml
0.5 M EDTA pH 8.0	100 μl

Filtered with 0.2 μ Millipore filter, stored at room temperature

2.4 DEAE-Dextran stock solution (10 mg/ml)

DEAE-Dextran (M.W. 500,000)	0.1 g
PBS pH 7.2	10 ml

Filtered with 0.2 μ Millipore filter

Aliquot to vials and stored at -20°C

2.5 Chloroquine diphosphate stock solution (10 mM)

Chloroquine diphosphate	0.103 g
PBS pH 7.2	20 ml

Filtered with 0.2 μ Millipore filter

Aliquot to vials and stored at -20°C

2.6 10% DMSO-PBS

Dimethyl sulfoxide	10 ml
PBS pH 7.2	90 ml

Filtered with 0.2 μ Millipore filter, stored at room temperature

3. Reagents for direct and indirect immunofluorescence staining

3.1 Phosphate buffered 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

3.2 1% BSA-0.02% NaN_3 in PBS

Bovine serum albumin fraction V	10 g
PBS (pH 7.2)	1000 ml

Mixed well until BSA completely dissolved

Added 10% (w/v) NaN_3 to final concentration 0.02%, mixed well

Stored at 4°C

3.3 1% Paraformaldehyde

Paraformaldehyde	1 g
PBS (pH 7.2)	100 ml

Heat at 56 °C until dissolved

Adjusted pH to 7.4 by 0.1 M HCl or 0.1 M NaOH

Filtered with 0.2 μ Millipore filter, stored at 4°C

4. Reagents for bacterial culture

4.1 LB broth

LB broth base	20 g
Distilled water	1000 ml

Sterilized in Autoclave at 121°C 15 minutes

Stored at 4°C

Checked sterility before used

4.2 LB broth contain ampicillin and tetracycline

LB broth	100 ml
Ampicillin (50 mg/ml)	30 μl
Tetracycline (30 mg/ml)	33.6 μl

Checked sterility before used

4.3 LB agar contain ampicillin and tetracycline

LB broth base	10 g
Agar noble	7.5 g
Distilled water	500 ml

Sterilized in Autoclave at 121°C 15 minutes

Ampicillin (50 mg/ml)	150 µl
Tetracycline (30 mg/ml)	168 µl

Pour plate 25 ml/plate, stored at 4°C

Checked sterility before used

4.4 2x TY broth

Tryptone	1.6 g
Yeast extract	1.0 g
Sodium chloride	0.5 g
Distilled water to	100 ml

Autoclave and kept at 4 °C

5. Reagents for production of plasmid DNA

5.1 3 M Sodium acetate pH 7.0

Sodium acetate.$3H_2O$	40.8 g

Adjust pH to 7.0 with NaOH/HCl

Distilled water to	100 ml

Stored at 4 °C

5.2 Potassium acetate

Potassium acetate	29.4 g
Glacial acetic acid	11.5 ml
Distilled water to	100 ml

Stored at 4 °

5.3 10 M NaOH

NaOH	200 g
Distilled water to	500 ml

Stored at 4 °C

5.4 10% SDS

SDS	5 g
Distilled water to	50 ml

Stored at room temperature

5.5 7.5 M Ammonium acetate

Ammonium acetate	57.8 g
Distilled water to	100 ml

Stored at 4 °C

5.6 1 M glucose buffer

D-glucose	18.02 g
Distilled water to	100 ml

Stored at 4 °C

5.7 0.5 M EDTA pH 8.0

EDTA	37.22 g
Distilled water	100 ml
Adjust pH to 8.0 with conc. HCl	
Distilled water to	200 ml
Stored at 4 °C	

5.8 1 M Tris pH 8.0

Tris-Base	24.22 g
Distilled water	100 ml
Adjust pH to 8.0 with conc. HCl	
Distilled water to	200 ml
Autoclave and kept at 4 °C	

5.9 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
Autoclave and kept at 4 °C	

5.10 1X glucomix-lysozyme solution

10X GLUCOMIX	300 µl
lysozyme stock (50 mg/ml in distilled water)	300 µl
Distilled water	2.4 ml
Stored at 4 °C for 7 days	

5.11 TE buffer

Tris-base	0.121 g
EDTA	0.307 g
Distilled water	80 ml

Adjusted pH to 7.0 by 1N HCl

Adjusted final volume to 100 ml, stored at room temperature

5.12 TBE buffer

Tris-base	27 g
Boric acid	13.75 g
0.5 M EDTA (pH 8.0)	8 ml

Adjusted final volume to 500 ml, stored at room temperature

5.13 1% Agarose gel

Agarose gel	1 g
TBE buffer	100 ml

Heat until dissolved

6. Reagents for using in ELISA

6.1 0.1 M Carbonate/bicarbonate coating buffer

NA_2CO_3	1.06 g
$NaHCO_3$	1.26 g
Distilled water	200 ml

Adjusted pH to 9.6 by concentrate HCl

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

6.2 Washing buffer

PBS (pH 7.2)	500 ml
Tween 20	0.25 ml

Mix thoroughly and stored at room temperature

6.3 5% BSA-PBS

BSA	0.5 g
PBS pH 7.2	10 ml

Mixed well until BSA completely dissolved, prepare before use

6.4 Phosphate citrate buffer pH 5.0

Citric acid .H_2O	1.028 g
Na_2HPO_4	1.46 g
Distilled water	150 ml

Adjusted pH to 5.0

Adjusted final volume to 200 ml, stored at 4°C

6.5 Ortho-phenylenediamine (OPD) substrate solution

Phosphate citrate buffer pH 5.0	5 ml
Ortho-phenylenediamine	0.02 g
6% H_2O_2	10 µl

Mixed well and used immediately after preparati

6.6 Stop reaction solution (4N H_2SO_4)

Concentrate H_2SO_4	20 ml
Distilled water	160 ml

Slowly dropwise H_2SO_4 to distilled water, and stored at room temperature

CIRRICULUM VITAE

Name	:	Saibua Boonmuen
Date of birth	:	September 30, 1965
Place of birth	:	Chiang Mai, Thailand
Education		
February, 1993	:	Bachelor of Science (Medical Technology, 2nd Class Honor), Faculty of Associated medical sciences, Khon Kaen University, Khon Kaen Thailand.
August, 1993	:	Bachelor of Science (Public Health), Faculty of Public Health, Sukhothai Thamathirat University, Bangkok Thailand.

Publication

อรัญญา มโนสร้อย, สายบัว บุญหมื่น และจีรเดช มโนสร้อย บทความทางวิชาการเรื่อง "การนำสารธรรมชาติในร่างกาย: อินเทอร์ลิวคินทู (IL-2) ใช้ในการรักษาโรค" ในงานสัมนาวิชาการเทคโนโลยีชีวภาพเภสัชกรรมครั้งที่ 3 เรื่อง "การวิจัยและพัฒนาผลิตภัณฑ์ธรรมชาติเพื่อใช้เป็นยา เครื่องสำอางและผลิตภัณฑ์อาหารเสริม" วันที่ 27-29 มิถุนายน 2544 จัดโดย ศูนย์วิจัยและ

พัฒนาวัตถุดิบยาและผลิตภัณฑ์ธรรมชาติ สถาบันวิจัยและพัฒนาวิทยาศาสตร์และเทคโนโลยี มหาวิทยาลัยเชียงใหม่

Oral Presentation

Boonmuen S. Application of microplate for ABO blood group in blood. Presented in 24th Annual Meeting of the Association of Medical Technologists of Thailand, Thamarin Tana Hotel, Trung, Thailand, April 19-21, 2000. Organized by The Medical Technologist Association of Thailand.

Boonmuen S, Kasinrerk W, Tayapiwatana C. Production of polyclonal antibody by phage display carrier system. Presented in 10th Annual Meeting of AIDS and Flow Cytometry, Metropole Hotel, Phuket, Thailand, March 31- April 2, 2004. Organized by The Flow Cytometry Association of Thailand.

Poster Presentation

Manosroi A, Boonmuen S and Manosroi J. Development of techniques to increase numbers of luciferase plasmid DNA for pharmaceutical formulations in gene therapy. Presented in the 3th national seminar on pharmaceutical biotechnology "R&D of natural products for pharmaceuticals, cosmetics and functional foods" Chiang Mai, Thailand, June 27-29, 2001. Organized by the Pharmaceutical Cosmatic Raw Materials and Natural Products Research and Development Center, Institute for

Science and Technology Research and Development, Chiang Mai University, Thailand.

Manosroi A, Boonmuen S, Werner R. and Manosroi J. Stability and release study of luciferase plasmid DNA (pluc) entrapped in liposomes for gene therapy. Presented in RGJ-Ph.D Congress III, Cholburi, Thailand, April 25-27, 2002. Organized by the Thailand Research Fund.

Boonmuen S, Tayapiwatana C, Kasinrerk W. Comparision of polyclonal anti-CD147 antibodies production using DNA based and phage-displayed CD147 immunizations. Presented in the 10th ASEAN Conference in Medical Laboratory Technology (10th ACMLT), Lotus Pang Suan Kaew Hotel, Chiang Mai, Thailand, April 26-30, 2004. Organized the Asean Association of Medical Laboratory Technologist.

Award

The third awards of poster presentation presented at the 10th ASEAN Conference in Medical Laboratory Technology. Held during 26-30 April, 2004 at the 10th ASEAN Conference in Medical Laboratory Technology (10th ACMLT), Chiang Mai, Thailand.

CERTIFICATE OF HONOR

This is to honor

Boonmuen S. *et al.*

as a winner of the third awards of poster presentation entitled

"Comparison of Polyclonal Anti-CD147 Antibodies Production Using DNA Based and Phage-Displayed CD147 Immunizations"

presented at the 10th ASEAN Conference in Medical Laboratory Technology

held during 26-30 April, 2004

at Chaing Mai, Thailand

S. W

Mr.Somchai Viriyayudhakorn
Chairman, Organising Committee

R Santiyanont.

Dr.Rachana Santiyanont
Chairman, Scientific Committee

ScienceAsia
Journal of the Science Society of Thailand
วารสารของสมาคมวิทยาศาสตร์แห่งประเทศไทย
http://scienceasia.tiac.or.th ISSN 1513-1874

November 1, 2004

Dr. Watchara Kasinrerk,
Department of Clinical Immunology,
Faculty of Associated Medical Sciences,
Chiang Mai University,
Chiang Mai 50200.
E-mail: watchara@chiangmai.ac.th

Dear Dr. Watchara,

Thank you very much for submitting the manuscript entitled: Comparison of Polyclonal Anti-CD147 Antibody Production Using DNA Based and Phage-Displayed CD147 Immunizations (Code 0408-491, received 20 August 2004) by Saibua Boonmuen, Chatchai Tayapiwatana and Watchara Kasinrerk, for consideration for publication in *ScienceAsia.*

The manuscript has been read by three independent referees, who have recommended acceptance of the manuscript for publication in *ScienceAsia*, **as a short report**. Would you please ensure that your final manuscript follows the style of the journal, especially references, and send to us together with a diskette of the final manuscript. Your paper is expected to be published in ScienceAsia Vol.31 No.1. You will receive further information later.

Thank you for your interest in contributing to our journal.

Yours sincerely,

Prof. Dr. MR. Jisnuson Svasti
Editor *ScienceAsia*

N.B. 1. In the title page, please make sure that the authors' names are in full, including both first and last names.

2. In the references, list all author names and initials up to 7 authors. After that, use the word "et al" for the eighth name upwards.

3. In the text, mention of author names should use the surname of the first author plus "et al", when there are three or more authors.