APPENDIX A

LIST OF CHEMICALS AND MATERIALS USED IN THIS STUDY

| CHEMICALS | SOURCES | |
|--|---|--|
| Absolute ethanol | Liquid distillery organization, Thailand | |
| Acetic acid | E. Merck, Germany | |
| Agarose | Invitrogen, USA | |
| Antimalarial drugs - Pyrimethamine - Chloroquine - Mefloquine - Dihydroartemisinin | Sigma-Aldrich, USA | |
| D-glucose (Dextrose) | Sigma-Aldrich, USA | |
| D-Sorbitol | Sigma-Aldrich, USA | |
| Dimethyl sulfoxide (DMSO) | Fisher Science, UK | |
| Disodium hydrogen phosphate (Na ₂ HPO ₄) | Sigma-Aldrich,USA | |
| gDNA extraction mini Kit (1/2) Blood/Culture cell | Geneaid | |
| Gentamicin sulfate 80 mg/2 ml | T.P. Drug Laboratories | |
| Giemsa's stain | E. Merck, Germany | |
| Glycerol | E. Merck, Germany | |
| HEPES | Sigma-Aldrich, USA | |
| Hydrochloric acid | E. Merck, Germany | |
| Hypoxanthine | Sigma-Aldrich, USA | |

| Magnesium chloride | E. Merck, Germany |
|---|-------------------------------|
| Methanol | E. Merck, Germany |
| PCR DNA polymerase (Phusion high-fidelity DNA polymerase) | New England Biolabs Inc., USA |
| Potassium chloride | E. Merck, Germany |
| Potassium dihydrogen phosphate (KH ₂ PO ₄) | Sigma-Aldrich, USA |
| QIAquick PCR and gel purification mini Kit | QIAgen |
| RPMI 1640 medium | GIBCO, USA |
| Saponin | Sigma-Aldrich, USA |
| Sodium bicarbonate | E. Merck, Germany |
| Sodium chloride | E. Merck, Germany |
| Sodium dihydrogen phosphate | E. Merck, Germany |
| Sodium hydrogen carbonate | Sigma-Aldrich, USA |
| SYBR green I nucleic acid gel strain | Invitrogen, USA |
| Tris-base | E. Merck, Germany |
| Triton X-100 | Sigma-Aldrich, USA |

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APPENDIX B

LIST OF INSTRUMENTS USED IN THIS STUDY

| INSTRUMENTS and EQUIPMENTS | COMPANY |
|--|------------------|
| 96 black well plate | SPL Life Science |
| 1.5 ml Microcentrifuge tube | Biologix |
| Automatic pipette | GIBCO |
| Balance for tube | Precisa |
| Balance for reagent | Sartorius |
| Bench top pH/mV/C meter, B200 | Modek |
| Block heater, SBH130D | Stuart |
| Cellulose acetate membrane filter, pore size 0.2 | |
| TC, Globert | Sartorious AG |
| ALTINITY | ERS |
| Centrifuge model 5810R | Eppendorf |
| Centrifuge tube (15 ml and 50 ml) | Corning |
| Ceramic top hotplate stirrer | Stuart |
| Chemical Duty Vacuum/Pressure Pump 220v | Millipore |
| Cyogenic vial | Corning V e d |
| Duran bottle (250 ml, 500 ml and 1000 ml) | Schott Duran |
| Electronic multichannel pipette | Eppendorf |
| Fluorescence Microscope, BX51 | Olympus |
| Fluorescence microplate reader | SpectraMax |
| Gel documentation model Chemidoc XRS+ | BIORAD |

| Glass pipette | Precicolour |
|--|-----------------------|
| Glass slide | Sail brand |
| Gloves | Sri Trang gloves |
| Incubator | Heraeus |
| Laminar flow | NUAIRE Biological |
| Liquid Nitrogen Tank, CryoSystem 2000 | MVE |
| Microplate shaker | Eppendorf |
| Mini Microcentrifuge | Gyrozen |
| PCR model C1000 | BIORAD |
| PCR model 22331 Hamburg | Eppendorf |
| Petri- dish, plastic | SPL Life Science |
| Pipette tips (30 µl, 200 µl and 1000 µl) | Bioscience |
| Pipette tips (filter; 30 µl, 200 µl and 1000 µl) | Corning |
| Plastic pipette (5 ml and 10 ml) | SPL Life Science |
| Power supply and tray for gel electrophoresis | Cosmo Bio |
| Refrigerator 4°C | SHARP |
| Refrigerator -20°C | SHARP |
| Refrigerator -80°C | Forma Scientific |
| Refrigerated Microcentrifuge, 5417R | Eppendorf |
| Reservoirs 230SD 1 channel | SPL Life Science |
| Spectrophotometer, ND-1000 | NanoDrop |
| Speed vacuum Microcentrifuge, SPD1010 | Thermo Savant |
| Syringe 10 ml | Scientific industries |
| Vortex | mixer Genie II |
| Water bath | Memmert |
| Whatman filter no.1 | Whatman |
| UV Transilluminater, UV 2000 | BIORAD |

APPENDIX C

REAGENTS FOR VASCULAR ENDOTHELIAL CELLS ISOLATION

Incomplete culture medium

| RPMI 1640 powder with L-glutamine without NSHCO ₃ | 10.4 | g |
|--|------|----|
| HEPES (Sigma) | 5.94 | g |
| Glucose (Sigma) | 2.0 | g |
| Gentamycin (80 mg/ml) | 1.0 | ml |
| Hypoxanthine (Sigma) | 0.05 | g |

Dissolved in distilled water (final volume 1,000 ml) and adjusted pH to 7.4. This solution mixture was stored at 4-8 °C.

Complete culture medium

| Sterile 5% NaHCO ₃ | 4.2 | ml |
|--|-----|----|
| Pooled human serum (inactivated at 56 $^{\circ}C$ for 30 min) | 10 | ml |
| Incomplete culture medium add up to | 100 | ml |

This solution mixture was stored at 4-8 °C.

Complete culture medium for <u>long-term culture</u> of *P. falciparum* isolates from patients

| Sterile 5% NaHCO ₃ | 4.2 | ml |
|---|-----|----|
| AB positive human serum (inactivated at 56 $^{\circ}C$ for 30 min) | 10 | ml |
| (AB blood group) | | |
| Incomplete culture medium add up to | 100 | ml |
| This solution mixture was stored at 4-8 °C. | | |
| 200 5900 - 23 gr | | |
| Washed 50% RBC for subcultivation | | |

Blood type O Rh- or Rh+ RBC in CPDA

Wash 2 times in incomplete culture medium to remove the buffy coat

Add equal volume of RBC with complete culture medium

5% Sorbitol (Synchronizing solution)

D-Sorbitol (Sigma) 5

g

g

5

Dissolved in distilled water (final volume 100 ml)

Filter the solution though 0.8 μ m and 0.2 μ m and stored at 4-8 °C.

rights reserve

5% NaHCO₃

NaHCO₃

Dissolved in distilled water (final volume 100 ml)

Filter the solution though 0.8 μ m and 0.2 μ m and stored at 4-8 °C.

3.5% NaCl (Thawing solution)

| NaCl | 3.5 | g |
|------|-----|---|
| | | |

28

3

0.65

ml

ml

g

Dissolved in distilled water (final volume 100 ml)

Filter the solution though 0.8 μ m and 0.2 μ m and stored at 4-8 °C.

Freezing solution

Glycerol (Sigma)

Sorbitol (Sigma)

NaCl

Dissolved in distilled water (final volume 100 ml)

Filter the solution though 0.8 μ m and 0.2 μ m and stored at 4-8 °C.

1.5 M Tris-HCl, pH 8.8

| Tris-base | MA | I IINTV | ERP | | 181.7 | g |
|-------------------------|-----------------------|----------------|-----------|--------------|-----------|---------|
| Dissolved in | distilled water (f | ïnal volume 1, | 000 ml) | and adjusted | l pH to 8 | .8 with |
| concentrated HCl. T | his solution mixt | ure was stored | at 4-8 °C | รียงใ | หม่ | |
| Copyr Giemsa's stain | right [©] by | Chiang | Mai | Univer | sity | |
| Sigma-Aldric | ch | LS I | es | erv | eu | |

Lysis buffer (500 ml)

| Tris-HCl (20 mM; pH 7.5) | 6.7 ml o | f 1.5 M Tris-HCl (pH 8.8) |
|-------------------------------|----------|---------------------------|
| EDTA (5 mM) | 2.5 ml o | f 1 M EDTA |
| Saponin (0.008%; wt/vol) | 0.04 | g |
| Triton X-100 (0.08%; vol/vol) | 400 | μl |

Dissolved in distilled water (final volume 500 ml) and adjusted pH to 7.4. Prepared in advance and stored at room temperature.

| riepared in advance and s | | 2 |
|----------------------------------|---------------------------------|-------------|
| 6.7 mM Phosphate buffer | r Saline (PBS) for Giemsa stain | ing, pH 7.1 |
| Na ₂ HPO ₄ | 0.41 | g |
| KH2PO4·H2O | 0.65 | g Sig |
| Dissolve in deionized | d water (final volume 1 L) | TOP |
| E | VAL | 18 |
| Phosphate buffer saline (| PBS) for cell culture, pH 7.4 | All |
| Na ₂ HPO ₄ | MAI UNIVERS | g |
| KH ₂ PO ₄ | 0.2 | g |

NaCl and the second sec

Dissolved in distilled water (final volume 1,000 ml) and adjusted pH to 7.4. This solution mixture was stored at 4-8 $^{\circ}$ C.

CIRRICULUM VITAE

| Name | Patcharaporn Saengratwatchara |
|--|--|
| Date of Birth | December 15, 1988 |
| Educations: | |
| March, 2010 | Bachelor of Science (Medical Technology), Faculty of |
| la l | Associated Medical Science, Chiang Mai University. |
| March, 2006 | Certificate of senior high school, Montfort College (Secondary Section); M.C., Chiang Mai. |
| March, 2003 | Certificate of junior high school, Regina Coeli College; R.C., Chiang Mai. |
| Scholarship: | NW Z |
| June, 2011-2013 | Thailand Graduate Institute of Science and Technology (TGIST), National Science and Technology Development Agency (NSTDA), Thailand. |
| Award: | TAI UNIVER |
| January, 2011 | Best Award of Medical Technology (MT) student; voted by MT students, Associated Medical Sciences, |
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Others:

April, 2011 Secretary of Chiang Mai University Graduation, Committee' 46th, Graduation Ceremony.

June 2010 - April 2011

Chairman of CMU Young Blood project which was cooperated between Department of Medical Technology, Faculty of Associated Medical Science and Blood Bank, Suan Dok Hospital.

June, 2009 - 2010 Secretary of AMS Student Association 2009, Faculty of Associated Medical Sciences, CMU.



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