



**APPENDIX**

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

Copyright © by Chiang Mai University

All rights reserved

## APPENDIX A

### CHEMICALS AND EQUIPMENTS

#### List of the chemicals and materials used in this study

Chemicals/Materials	Source
Absolute ethanol	Merck, Darmstadt, Germany
Agarose	Sigma, St. Louis, MO, USA
BM-condimed	Roche, Mannheim, Germany
Boric acid	Research Organics, St. Cleveland, USA
Bovine serum albumin fraction V	Sigma, St. Louis, MO, USA
Brilliant Cresyl blue	Merck, Darmstadt, Germany
Chloroform	Merck, Darmstadt, Germany
Coomassie brilliant blue R-250	Bio-Rad, Hercules, CA, USA
Culture dish	Corning, NY, USA
Dimethyl sulfoxide	Sigma, St. Louis, MO, USA
Disodium hydrogen phosphate	Fisher, Leics, UK
ELISA Plates	Corning, NY, USA
Horseradish peroxidase	Pierce, Rockford, USA
Ethidium bromide	Sigma, St. Louis, MO, USA
Ethylenediaminetetraacetate (EDTA)	Sigma, St. Louis, MO, USA
Fetal calf serum	Gibco, Gran Island, N.Y., USA
Glycerol	Sigma, St. Louis, MO, USA

Glycine	Sigma, St. Louis, MO, USA
Hitrap protein G	Amersham , Uppsala, Sweden
NP-40	Pierce, Rockford, IL, USA
PCR buffers	Fermantas, MA, USA
Peroxidase-conjugated rabbit anti-mouse-	DAKO, Denmark
Immunoglobulins	
Phenol	Amresco, Ohio, USA
Potassium chloride	Merck, Darmstadt, Germany
Potassium dihydrogen phosphate	Merck, Darmstadt, Germany
Skimmed milk	Oxoid, Hampshire, England
Sodium azide	Merck, Darmstadt, Germany
Sodium chloride	Merck, Darmstadt, Germany
Sodium dodecyl sulfate	Sigma, St. Louis, MO, USA
Sodium hydrogen carbonate	Sigma, St. Louis, MO, USA
Sodium hydrogen phosphate	Sigma, St. Louis, MO, USA
Sodium hydroxide	Eka, Nobel, Sweden
3,3,5,5-Tetramethylbenzidine (TMB)	Zymed, South san Francisco, CA, USA
Tris	Sigma, St. Louis, MO, USA
Tween 20	Sigma, St. Louis, MO, USA
Vivaspin tube	Satorius AG, Goettingen, Germany

## APPENDIX B

### INSTRUMENTS

#### List of instruments used in this study

<b>Instruments</b>	<b>Source</b>
AKTA prime	Amersham, Sweden
Centrifuge	Kendo Laboratory, Germany
ELISA reader	Tecan, Austria
Hematology analyzer	Sysmex, Japan
HPLC	Primus Corporation, Kansas City, USA
Incubator	Thermo electron corporation, USA
Laminar Flow	NUAIRE, USA
Microcentrifuge	Kendro, Germany
pH meter	Precisa, Switzerland
Refrigerator (-20°)	Whirlpool, Thailand
Spectrophotometer UV-1201	Shimadzu Co., Japan
Thermal cycle	MJ research, INC, USA
UV Transilluminator	Hoefer Scientific Instrument, USA
Water bath	Memmert, Germany

## APPENDIX C

### REAGENTS AND BUFFERS PREPARATION

#### 1. Reagent for purification of mAb (IgG) by AKTA prime

##### 1.1 Binding buffer (20 mM Sodium phosphate buffer, pH7.0)

1M Na<sub>2</sub>HPO<sub>4</sub> 11.6 ml.

1 M NaH<sub>2</sub>PO<sub>4</sub> 8.4 ml.

Sterile distilled water 800 ml.

Adjust pH to 7.0

Adjust volume to 1000 ml.

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

##### 1.2 Elution buffer (0.1 M glycine - HCl, pH 2.7)

Glycine 3.753 g.

Sterile distilled water 350 ml.

Adjust pH to 2.7 with HCl

Adjust volume to 500 ml.

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

##### 1.3 Neutralizing buffer (1 M Tris - HCl, pH 9.0)

Tris 12.114 g.

Sterile distilled water 60 ml.

Adjust pH to 9.0 with HCl

Adjust volume to 100 ml.

Filter with 0.2  $\mu\text{m}$  millipore filter, Store at 4° C

## 2. Reagent for ELISA

### 2.1 Phosphate buffer saline (PBS pH 7.2)

NaCl	8.000 g
KCl	0.200 g
Na <sub>2</sub> HPO <sub>4</sub>	1.150 g
KH <sub>2</sub> PO <sub>4</sub>	0.200 g
Distilled water	800 ml

Adjust pH to 7.2 by adding 1N HCl or 1N NaOH

Adjust volume to 1000 ml

Filter with 0.2  $\mu\text{m}$  millipore filter, store at room temperature

### 2.2 Coating buffer (0.1 M Carbonate/Bicarbonate pH9.6)

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, Storage at 4° C

### 2.3 Blocking buffer (2% Skimmed milk)

Skimmed milk	2 g.
PBS	100 ml.

Mix well, prepare before use

### 2.4 Stopping solution (1 N HCl)

HCl	82.8 ml.
Sterile distilled water	917.2 ml.

Slowly dropwise HCl to distilled water, Store at room temperature

### 2.5 0.05% Tween-PBS

PBS pH 7.2 500 ml.

Tween 250  $\mu$ l.

Mix well, Store at room temperature

### 3. Reagent for Hb H inclusion body test

#### 3.1 1% (w/v) Brilliant Cresyl Blue in Absolute ethyl alcohol

Brilliant Cresyl Dye 1 g

Absolute ethyl alcohol 100 ml.

Filtrate before use, Store at 4° C

### 4. Reagent for PCR

#### 4.1 0.5% NP-40

NP-40 2.5 ml.

Sterile distilled water 500 ml.

Mix well, Store at 4° C

#### 4.2 1M Tris pH 8.0

Tris 121.1 g

Sterile distilled water 800 ml.

Mix well, Adjust pH to 8.0 with 4N HCl

Adjust volume to 1000 ml, Storage at room temperature

#### 4.3 5M Sodium chloride

NaCl 146.1 g

Sterile distilled water 500 ml.

Mix well, Store at room temperature

**4.4 0.5M EDTA pH 8.0**

EDTA	73.06 g
------	---------

Sterile distilled water	800 ml.
-------------------------	---------

Mix well, Adjust pH to 8.0 with 1N NaOH

Adjust volume to 1000 ml, Storage at room temperature

**4.5 3M Sodium acetate**

CH <sub>3</sub> COONa	246.09 g
-----------------------	----------

Sterile distilled water	500 ml.
-------------------------	---------

Mix well, Adjust volume to 1000 ml, Storage at room temperature

**4.6 10% Sodium dodecyl sulfate (SDS)**

SDS	5 g
-----	-----

Sterile distilled water	50 ml.
-------------------------	--------

Mix well, Store at room temperature

**4.7 Lysis buffer**

1M Tris	0.5 ml.
---------	---------

5M NaCl	0.1 ml.
---------	---------

0.5M EDTA	1 ml.
-----------	-------

Sterile distilled water	48.4 ml.
-------------------------	----------

Mix well, Store at room temperature

**4.8 ANE buffer**

3M CH <sub>3</sub> COONa	0.5 ml.
--------------------------	---------

10% SDS	7.5 ml.
---------	---------

0.5M EDTA	0.3 ml.
-----------	---------

5M NaCl	3.0 ml.
---------	---------



Sterile distilled water 18.7 ml.

Mix well, Store at room temperature

#### 4.9 TE buffer

1M Tris 5 ml.

0.5 M EDTA 10 ml.

Sterile distilled water 485 ml.

Mix well, Store at room temperature

#### 4.10 10X TBE buffer

Tris (anhydroxymethyl)  
aminomethane 108 g

Boric acid 55 g

EDTA 9.5 g

Sterile distilled water 1000 ml.

Mix well, Store at room temperature

#### 4.11 Ethidium bromide

Ethidium bromide 50  $\mu$ l

1X TBE buffer 500 ml.

Mix well, Store at room temperature

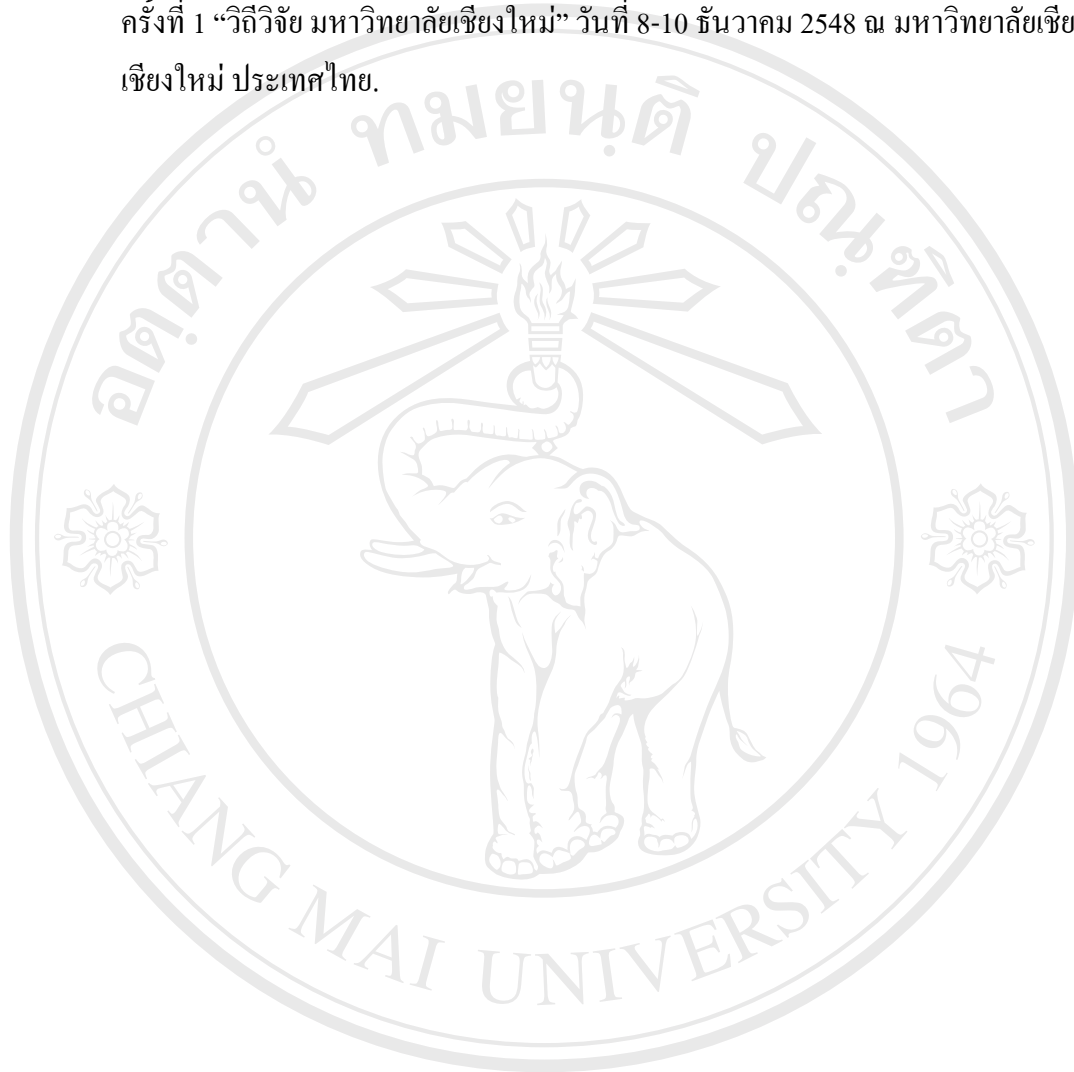
## CIRRICULUM VITAE

<b>Name</b>	Sumontida Sayachak
<b>Date of birth</b>	July 18, 1980
<b>Place of birth</b>	Phrae, Thailand
<b>Education</b>	
1999	Certificate of senior high school, Nareerat Phrae School, Phrae
2003	Bachelor Degree of Science (Medical Technology), Faculty of Associated Medical Sciences, Naresuan University

### Publication and presentations:

1. Tatu T, Prakunwisit D, **Sayachak S**. RBC Count and its Differentiation Potential between  $\alpha$ -Thalassemia (SEA type),  $\beta$ -Thalassemia and Hb E Heterozygotes. CMU. Journal (submitted).
2. Tatu T, **Sayachak S**, Chiampanichayakul S, Kasinrerak W and Piyamongkol W. Red blood cell parameters and Hb Bart's levels in simple  $\alpha$ - and double  $\alpha/\beta$ -thalassemia heterozygotes. The Thai Society of Hematology Annual Meeting, March 26-29, 2006, The Royal Golden Jubilee Building, Bangkok, Thailand.
3. **Sayachak S**, Tatu T, Kasinrerak W, Chiampanichayakul S. Simple immunoassay for screening alpha thalassemia. ประชุมวิชาการ 30 ปี คณะเทคนิคการแพทย์ วันที่ 7-9 ธันวาคม 2548 มหาวิทยาลัยเชียงใหม่ ณ โรงแรมดวงตะวัน เชียงใหม่ ประเทศไทย.

4. **Sayachak S, Chiampanichayakul S, Tatu T, Kasinrek W.** Sandwich enzyme – linked immunosorbent assay for screening of  $\alpha$ -thalassemia 1 carriers. วันวิชาการ ครั้งที่ 1 “วิถีวิจัย มหาวิทยาลัยเชียงใหม่” วันที่ 8-10 ธันวาคม 2548 ณ มหาวิทยาลัยเชียงใหม่ เชียงใหม่ ประเทศไทย.



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่  
Copyright © by Chiang Mai University  
All rights reserved