

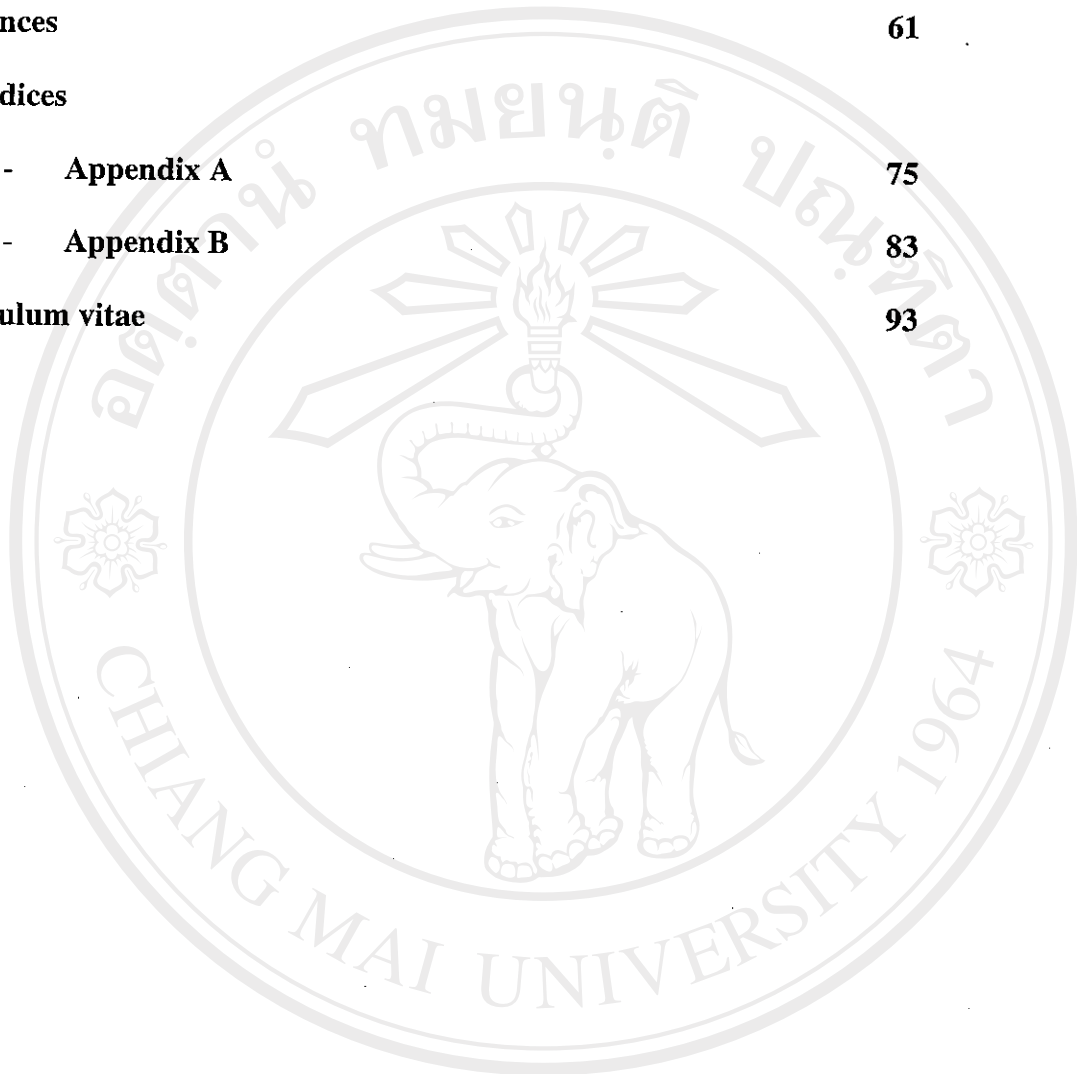
TABLE OF CONTENTS

	Page
Acknowledgement	III
Abstract (English)	IV
Abstract (Thai)	VI
List of Tables	XI
List of Illustrations	XII
Abbreviations and Symbols	XIV
Chapter 1 Introduction	
1.1 Statement and significance of the problem	1
1.2 Literature reviews	3
1.2.1 The human CD147	3
1.2.2 Protein expression in <i>Escherichia coli</i>	8
1.2.3 Protein folding in <i>Escherichia coli</i>	10
1.2.4 Phage display technique	12
1.2.5 Filamentous phage	14
1.3 Objectives	19
1.4 Scope of study	20
Chapter 2 Experimental	
2.1 Materials and equipments	21
2.1.1 Chemicals	21
2.1.2 Molecular reagents and materials	22
2.1.3 Microorganisms	23

IX

2.1.4 Equipments	23
2.2 Methods	24
2.2.1 Construction of the recombinant phagemid vector containing the extracellular domain gene of human CD147	24
2.2.2 Phage display technique for the expression of recombinant CD147Ex	26
2.2.3 Detection of the phage-displayed CD147 (CD147-Φ) by immunological technique	31
2.2.4. Epitope mapping of CD147-Φ by competitive inhibition ELISA	33
Chapter 3 Results	
3.1 Construction of phagemid carrying CD147Ex gene	35
3.2 Phage-displayed recombinant CD147Ex (CD147Ex-Φ)	42
3.3 Comparison of the expression of CD147Ex in <i>E. coli</i> XL-1 Blue and TG-1 Strains	44
3.4 Western immunoblotting	46
3.5 Epitope mapping of CD147 mAbs	48

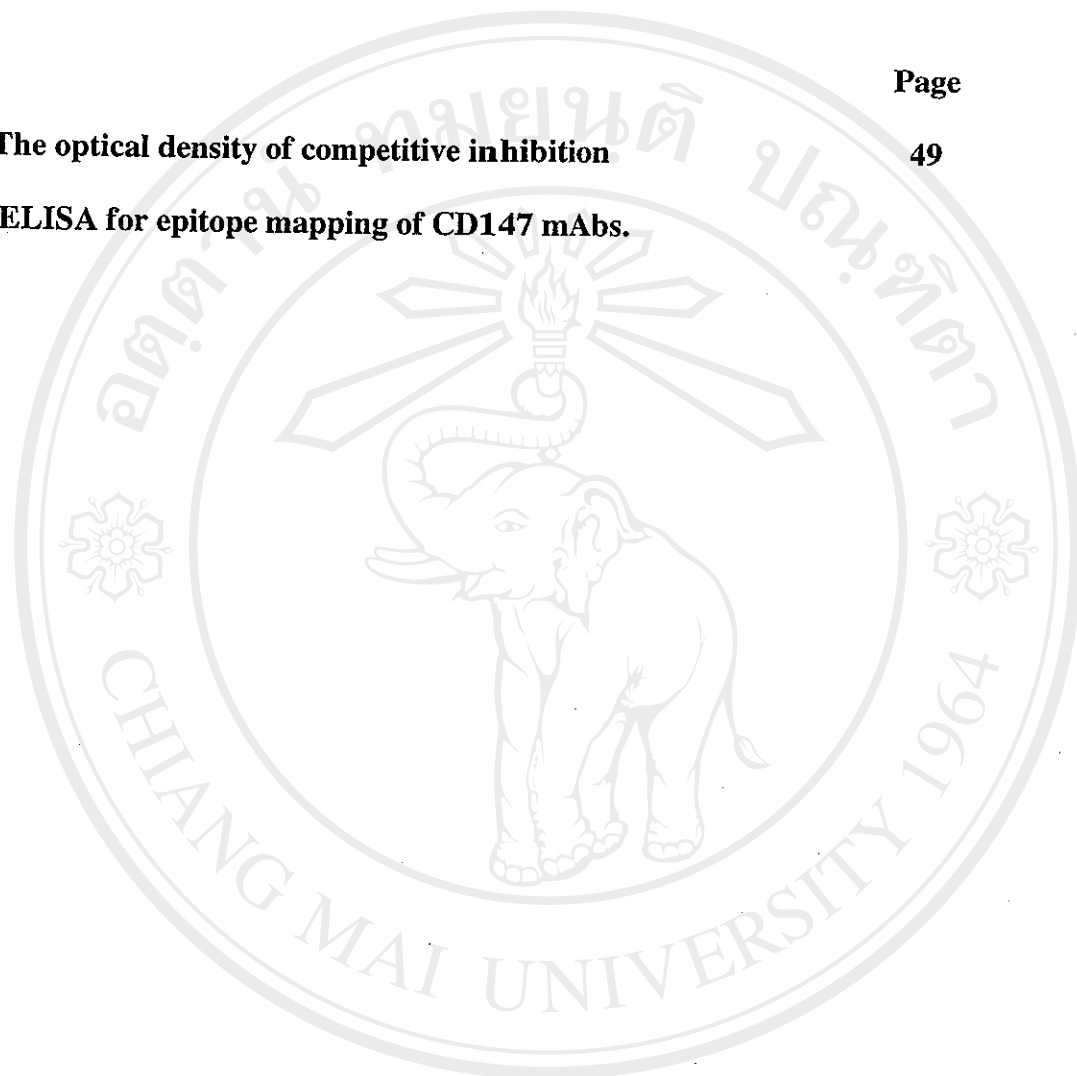
Chapter 4 Discussion	51
Chapter 5 Conclusion	59
References	61
Appendices	
- Appendix A	75
- Appendix B	83
Curriculum vitae	93



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

LIST OF TABLE

Table	Page
3.1. The optical density of competitive inhibition ELISA for epitope mapping of CD147 mAbs.	49



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
 Copyright© by Chiang Mai University
 All rights reserved

LIST OF ILLUSTRATIONS

Figure	Page
1.1. The transmembrane prediction of CD147 molecule by using SOSUI web-base software.	5
1.2. A schematic view of protein folding and exportation.	12
1.3. Schematic diagram of a filamentous phage displaying scFv molecule.	15
1.4. The schematic demonstrates the life cycle of filamentous phage in <i>E. coli</i> host.	17
1.5 Schematic diagram of pComb3HSS phagemid expressing vector.	19
2.1. Schematic diagram of competitive inhibition ELISA.	34
3.1. Analysis of the amplified CD147Ex gene fragment which are amplified from pCDM-8-CD147 using CD147ExFw and CD147ExRev primers.	37
3.2. Ethidium bromide-stained DNA gel demonstrates the purified <i>Sfi</i> I-treated pComb3HSS.	37
3.3. Gel electrophoresis of the purified pComb3H-CD147Ex by plasmid mini-prep.	38
3.4. Restriction fragment analysis of purified pComb3H-CD147Ex which was treated with <i>Sfi</i> I.	39

XIII

- 3.5. PCR reamplification of CD147Ex gene from the purified pComb3H-CD147Ex using CD147ExFw and CD147ExRev primers. 40
- 3.6. Construction of CD147Ex expression vector pComb3H-CD147. 41
- 3.7. The *E. coli* strain TG-1 after reinfected with CD147Ex- Φ at the dilution of $1:10^6$ and plated on LB-agar containing ampicillin. 43
- 3.8. Comparison of the binding efficiency of CD147- Φ derived from *E. coli* XL-1 Blue or TG-1 host to the indicated CD147 mAbs by sandwich ELISA. 45
- 3.9. Analysis of CD147- Φ by using the western immunoblotting technique. 47
- 3.10. Relation of the bioactive epitope recognized by M6-1B9, M6-1E9, M6-1F3 and M6-2F9 on CD147 molecule. 50
- 4.1. Schematic illustration of the allosteric effect of antibody-antigen interaction on CD147 molecule. 58

ABBREVIATIONS AND SYMBOLS

Ab, Abs	antibody, antibodies
bp	base pair
°C	degree Celsius
<i>E. coli</i>	<i>Escherichia coli</i>
g	gravity
h	hour
µg	microgram
µl	microliter
mAb	monoclonal antibody
mg	milli gram
min	minute
ml	milli liter
mM	milli Molar
ng	nanograms
nm	nano meter
%	percent
Φ	phage
PBS	phosphate buffer saline
PCR	polymerase chain reaction
PEG	polyethylene glycol
rpm	revolutions per minute

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

Copyright © by Chiang Mai University

All rights reserved

sec

second

t.u.

transforming unit



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved