TABLE OF CONTENTS

	अधायक ,	
		Page
ACKNOWLEDGEMEN	NT S	iii
ABATRACT (ENGLISI		iv
ABSTRACT (THAI)		vi
LIST OF TABLES		xii
LIST OF FIGURES		xiii
ABBREVIATIONS AN	D SYMBOLS	XV
CHAPTER I INTRODU	JCTION	
1.1 Statement	and significance of the problem	1
1.2 Literature	review	5
1.2.1 Re	combinant protein expression	
in A	Escherichia coli	5
1.2.2 Dis	sulfide bond in <i>Escherichia coli</i>	8
1.2.3 Inc	lusion body and how to prevent	
opyright Wits	formation hang Mai Univers	11
1.2.4 Fus	sion technology and its roles in	
rec	ombinant protein expression	15
1.2.5 Bio	otin carboxyl carrier protein	19
1.2.6 The	e human CD147	25

27

1.2.7 Immune response

		1.2.8 Mechanism of adaptive ir	nmune response	29
		1.2.9 Immunological adjuvants		32
	1.3	Objectives		36
СНАГ	PTER I	MATERIALS AND METHOD	os	
	2.1	Chemicals and equipments		37
	2.2	Preparation of plasmid vector end	coding CD147Ex-BCCP	37
		2.2.1 CD147Ex gene amplifica	tion by PCR	37
		2.2.2 Restriction enzyme diges	tion of amplified	
		CD147Ex		38
		2.2.3 Preparation of pAK400Cl	B plasmid cloning sites	38
		2.2.4 Construction of plasmid of	containing	
		CD147Ex-BCCP gene		38
	2.3	Introduction of plasmid DNA to	the bacterial cells	39
		2.3.1 Bacterial cell transformat	ion	39
		2.3.2 Purification of plasmid D	NA by alkaline	
		lysis method		39
		2.3.3 Characterization of recom	nbinant clones	40
	2.4	Expression of biotinylated CD14	7Ex-BCCP fusion protein	41
	2.5	Bacterial total protein extraction		41
	2.6	Detection of biotinylated CD147	Ex-BCCP fusion protein	
		by immunological techniques		42

42

2.6.1 Indirect ELISA

		2.6.2 SDS-PAGE and Western immunoblotting	43
	2.7	Purification of biotinylated CD147Ex-BCCP fusion protein	
		by streptavidin-coated magnetic beads	43
	2.8	Detection of CD147Ex-BCCP on streptavidin magnetic beads	
		by indirect immunofluorescence	44
	2.9	Animal and immunization	45
	2.10	Determination of activity and specificity of polyclonal	
		antibody in mice sera	45
		2.10.1 Optimization of CD147-hIgG and CD2-hIgG	
		concentration	45
		2.10.2 Indirect ELISA	46
		2.10.3 Indirect immunofluorescence	46
		2.10.4 SDS-PAGE and Western immunoblotting	47
CHA	PTER I	III RESULTS	
	3.1	Construction of plasmid expression vector encoding	
		CD147Ex-BCCP fusion protein	49
	3.2	Production of biotinylated CD147Ex-BCCP fusion protein	53
		3.2.1 Detection of biotinylated CD147Ex-BCCP fusion	
		protein by indirect ELISA	53
		3.2.2 Analysis of CD147Ex-BCCP fusion protein by	
		Western immunoblotting	54

3.3 Purifi	cation of biotinylated fusion protein by streptavidin-	
coate	d magnetic beads	57
3.4 Produ	action of anti-CD147 polyclonal antibodies in mice	59
3.4.1	Optimization of CD147-hIgG and CD2-hIgG	
	concentration	59
3.4.2	Determination of anti-CD147 antibody responses	
	in mice by indirect ELISA	59
3.4.3	Determination of the reactivity of mouse anti-CD147	
	antibodies by indirect immunofluorescence	63
3.4.4	Determination of the reactivity of mouse anti-CD147	
	antibodies by Western immunoblotting	63
CHAPTER IV DIS	CUSSION	67
CHAPTER V CON	CLUSION	76
REFERENCES		79
APPENDICES		
- APPEN		94
	DIX B Chiang Mai Univers	
CURRICULUM VI	TAE ts reserve	105

LIST OF TABLES

Page	Table
	1.1 Characteristics, advantages and disadvantages of some commonly
17	used fusion tags
34	1.2 Classification of immunologic adjuvants
3	

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright © by Chiang Mai University All rights reserved

LIST OF FIGURES

Figur	e	Page
1.1	Cytoplasmic disulfide bond reducing system in E. coli	10
1.2	Oxidative pathways for disulfide bond formation in periplasm	12
1.3	Enzymatic biotinylation reaction carried out by E. coli biotin	
	protein ligase (BirA)	22
1.4	Structure of the biotin domain of the <i>E coli</i> BCCP	23
1.5	Schematic represents the structure of CD147	26
3.1	Agarose gel electrophoresis of insert sequence and plasmid vector	
	prior to ligation	50
3.2	Analysis of plasmid vector purified from E. coli Origami B	
	harboring pAK400CB-CD147Ex	51
3.3	Schematic illustration of the pAK400CB-CD147Ex vector	52
3.4	Detection of biotinylated CD147Ex-BCCP fusion protein in bacterial	
	extracts by indirect ELISA	55
3.5	Analysis of biotinylated CD147Ex-BCCP fusion protein by	
	Western immunoblotting technique	56
3.6	Flow cytometric analysis of CD147Ex-BCCP-immobilized	
	streptavidin magnetic beads	58
3.7	Titration of culture supernatant containing CD147-hIgG	
	and CD2-hIgG by indirect ELISA	61

3.8	Anti-CD147 antibody response of mice after immunizing with	
	CD147Ex-BCCP beads determined by indirect ELISA	62
3.9	Immunofluorescence profiles of the reactivity of anti-CD147	
	polyclonal antibodies in mouse sera	65
3.10	Analysis of mouse anti-CD147 polyclonal antibodies by	
	Western immunoblotting technique	66

ABBREVIATIONS AND SYMBOLS

°C degree Celsius

μg microgram

μl microliter

μM micromolarity

Ab, Abs antibody, antibodies

ACC acetyl-CoA carboxylase

APC antigen presenting cell

BCCP biotin carboxyl carrier protein

base pair

BSA bovine serum albumin

CD cluster of differentiation

DNA deoxyribonucleic acid

E. coli Escherichia coli

LISA enzyme-linked immunosorbent

assay

FACS fluorescence-activated cell sorter

FITC fluorescein isothiocyanate

g gravity

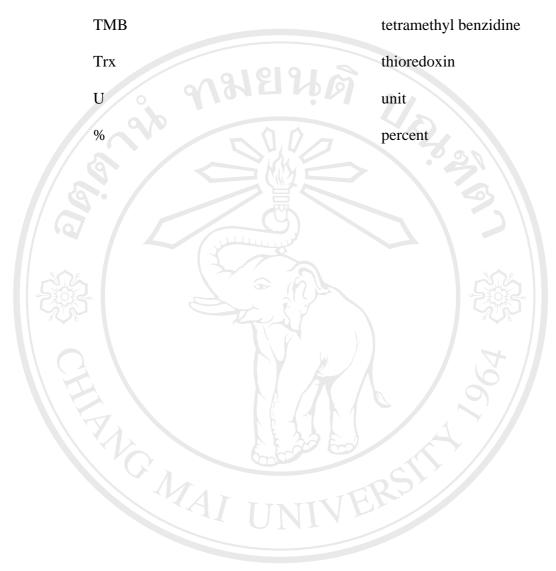
Grx glutaredoxin

GSH reduced glutathione

oxidized glutathione

GSSG

glutathione S-transferase **GST** hydrochloric acid **HCl** His₆ hexa-histidine HRP horseradish peroxidase immunoglobulin Ig IgG immunoglobulin G isopropyl-β-D-**IPTG** thiogalactopyranoside monoclonal antibody mAb maltose binding protein **MBP** milligram mg milliliter ml millimolarity mMmRNA messenger ribonucleic acid nanogram ng OD optical density phophate buffer saline round per minute ribosomal ribonucleic acid rRNA **SDS-PAGE** sodium dodecyl sulfatepolyacrylamide gel electrophoresis



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright © by Chiang Mai University All rights reserved