# TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENT	iii
ABATRACT (ENGLISH)	iv
ABSTRACT (THAI)	vi
LIST OF FIGURES	xi
ABBREVIATIONS AND SYMBOLS	xiii
CHAPTER I INTRODUCTION	
1.1 Statement and significance of the problem	1
1.2 Literature reviews	4
1.2.1 The human CD147	4
1.2.2 Protein translocation across bacterial	9
cytoplasmic membrane	
1.2.3 Protein folding in <i>E. coli</i>	21
1.2.4 Protein misfolding and Inclusion body formation	29
1.2.5 The stress response systems in <i>E. coli</i>	30
1.2.6 The filamentous bacteriophage	32
1.2.7 Phage display Technique	37
1.3 Objectives	41

#### CHAPTER II MATERIALS AND METHODS

	2.1	1 Chemicals and equipments	
	2.2	Construction of phagemid containing Tat-CD147Ex gene	
		2.2.1 Site-directed mutagenesis of pComb8-CD147Ex	42
		phagemid	
		2.2.2 TorA signal sequences amplification by PCR	43
		2.2.3 Purification of PCR product by QIAquick PCR	43
		Purification Kit	
		2.2.4 Construction of phagemid containing Tat-CD147Ex gene	44
	<ul><li>2.3 Bacterial cell transformation</li><li>2.4 Purification of phagemid by using alkaline lysis method</li></ul>		46
			46
2.5 Characterization of recombinant clones		Characterization of recombinant clones	47
	2.6 Phage displaying CD147Ex <i>via</i> gpVIII		47
		2.3.1 Preparation of phage-displayed CD147	47
		2.3.2 Harvesting phage by PEG precipitation	48
		2.3.3 Phage titration by reinfecting the <i>E. coli</i> cell	48
	2.7	Detection of the phage-displayed CD147ExgpVIII by	
		immunological techniques	49
		2.7.1 Immunoassay for phage-displayed CD147ExgpVIII	
		by Sandwich ELISA	49
		2.7.2 SDS–PAGE and Western immunoblotting	50

### **CHAPTER III RESULTS**

3.1	Construction of phagemid containing Tat-CD147Ex gene	51
	3.1.1 Site-directed mutagenesis of pComb8-CD147Ex	
	Phagemid	51
	3.1.2 TorA signal sequences amplification by PCR	53
	3.1.3 Construction of phagemid containing Tat-CD147Ex gene	53
	3.1.4 Characterization of recombinant clones	57
3.2	Comparison of phage-displayed CD147Ex expression	
	by Sandwich ELISA	63
3.3	Demonstration of anchored CD147Ex on phage particles	
	by Sandwich ELISA	65
3.4	Western immunoblotting	65
CHAPTER I	V DISCUSSION	68
CHAPTER V	CONCLUSION	75
REFERENCI	ES	77
APPENDICE	มหาวิทยาลยเชียงไห	
	PPENDIX A	98
	PPENDIX B	101
	ignts reserve	100
CURRICULI		108

### LIST OF FIGURES

Figur	Figure	
1.1	Schematic represents the structure of CD147	5
1.2	The general secretory (Sec) pathway in bacteria	10
1.3	The twin-arginine translocation (Tat) pathway	12
1.4	The tripartite structure of Sec and Tat signal peptides	14
1.5	Predicted topological organization of the E. coli Tat components	17
1.6	A model of quality control mediated by twin-arginine signal-peptide	
	binding chaperones	20
1.7	Chaperone-assisted protein folding in the cytoplasm of <i>E. coli</i> .	24
1.8	Disulfide bond formation in bacterial periplasm	28
1.9	Structure of a filamentous bacteriophage	33
1.10	Model for infection by filamentous phage	36
2.1	Schematic diagram of pTat8-CD147 phagemid construction	45
3.1	Gel electrophoresis of the Nsi I- and Xba I-digested MpComb8-	
	CD147Ex fragments	52
3.2	Analysis of PCR product of the TorA signal sequence, 161 bp,	
	amplified from pSPL04 vector using TatNsiIFw and TatXhoIRev	
	primers	54
3.3	The fragment of 844 bp (asterisk) of Xho I- and Xba I-digested pCom8-	
	CD147Ex which used for ligating with TorA signal sequence	55

3.4 Gel electrophoresis of the Tat-CD147 fragment which was		
	amplified by using TatNsiIFw and Tat-CD147Rv primers	56
3.5	Restriction fragment analysis of pTat8-CD147 with Nsi I, Xho I,	
	Spe I and Xba I	58
3.6	Reamplified product of Tat-CD147 fragment from pTat8-CD147	
	using TatNsiIFw and Tat-CD147Rv primers	59
3.7	Map of pTat8-CD147 phagemid	
3.8	Comparison of the expression of phage-displayed CD147Ex via	
	Tat and Sec pathway by Sandwich ELISA	64
3.9	Detection of CD147Ex presenting on phage particles through	
	gpVIII by Sandwich ELISA	66
3.10	Western immunoblotting of phage-displayed CD147ExgpVIII	67

## **ABBREVIATIONS AND SYMBOLS**

Ab, Abs	antibody, antibodies
bp	base pair
cfu	colony forming unit
°C	degree Celsius
E. coli	Escherichia coli
g	gravity
gpIII	minor coat protein 3
gpVIII	major coat protein 8
h	hour
μg	microgram
μl	microliter
mAb	monoclonal antibody
mg	milligram
min	minute
ml	milliliter
mM	milli Molar
ng	nanogram
OD	optical density
PBS	phophate buffer saline
PEG	polyethylene glycol

RT	room temperature
rpm	round per minute
S	second
U	unit
%	percent
Φ	phage