

TABLE OF CONTENTS

	PAGE
ACKNOWLEDGEMENTS	iii
ENGLISH ABSTRACT	iv
THAI ABSTRACT	vi
LIST OF TABLES	xii
LIST OF FIGURES	xiii
ABBREVIATIONS AND SYMBOLS	xiv
CHAPTER I INTRODUCTION	
1.1 Statement and significance of the problem	1
1.2 Literature review	2
1.2.1 Adenovirus	2
1.2.1.1 Virus structure	2
1.2.1.2 Binding and entry	5
1.2.1.3 Transcription and replication	9
1.2.2 Adenoviral vector	11
1.2.3 AdEasy system	12
1.2.4 Application of adenovirus vectors	15
1.2.5 The human CD147	18
1.2.6 Antibody	19
1.2.7 Single-chain Fv	23

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright © by Chiang Mai University
All rights reserved

1.2.8 Protein translocation	24
1.3 Objectives	27

CHAPTER II MATERIALS AND MEDTHODS

2.1 Chemicals and equipments	28
2.2 Adenoviral plasmids and recombinant adenovirus expressing scFv-M61B9ER gene	28
2.3 Construction of pAdTrack-CMV vector expressing scFv-M61B9cyt gene	28
2.3.1 Primer design	28
2.3.2 Amplification of scFv-M61B9cyt gene	29
2.3.3 Purification of PCR product by QIAquick PCR Purification Kit	29
2.3.4 Ligation of the scFv-M61B9cyt gene with pAdTrack-CMV vector	30
2.3.5 Transformation of the recombinant pAdTrack-CMV vector in <i>E. coli</i> Novablue	30
2.3.6 Purification of plasmid vector by using alkaline lysis method	31
2.3.7 Analytical <i>Sal</i> I and <i>Not</i> I digestion of pAdTrack-CMV vector expressing scFv-M61B9	32
2.3.8 Proof of pAdT-scFv-M61B9cyt by using polymerase chain reaction (PCR)	32
2.4 Construction of pAdTrack-CMV vector expressing scFv-M61B9sec gene	32

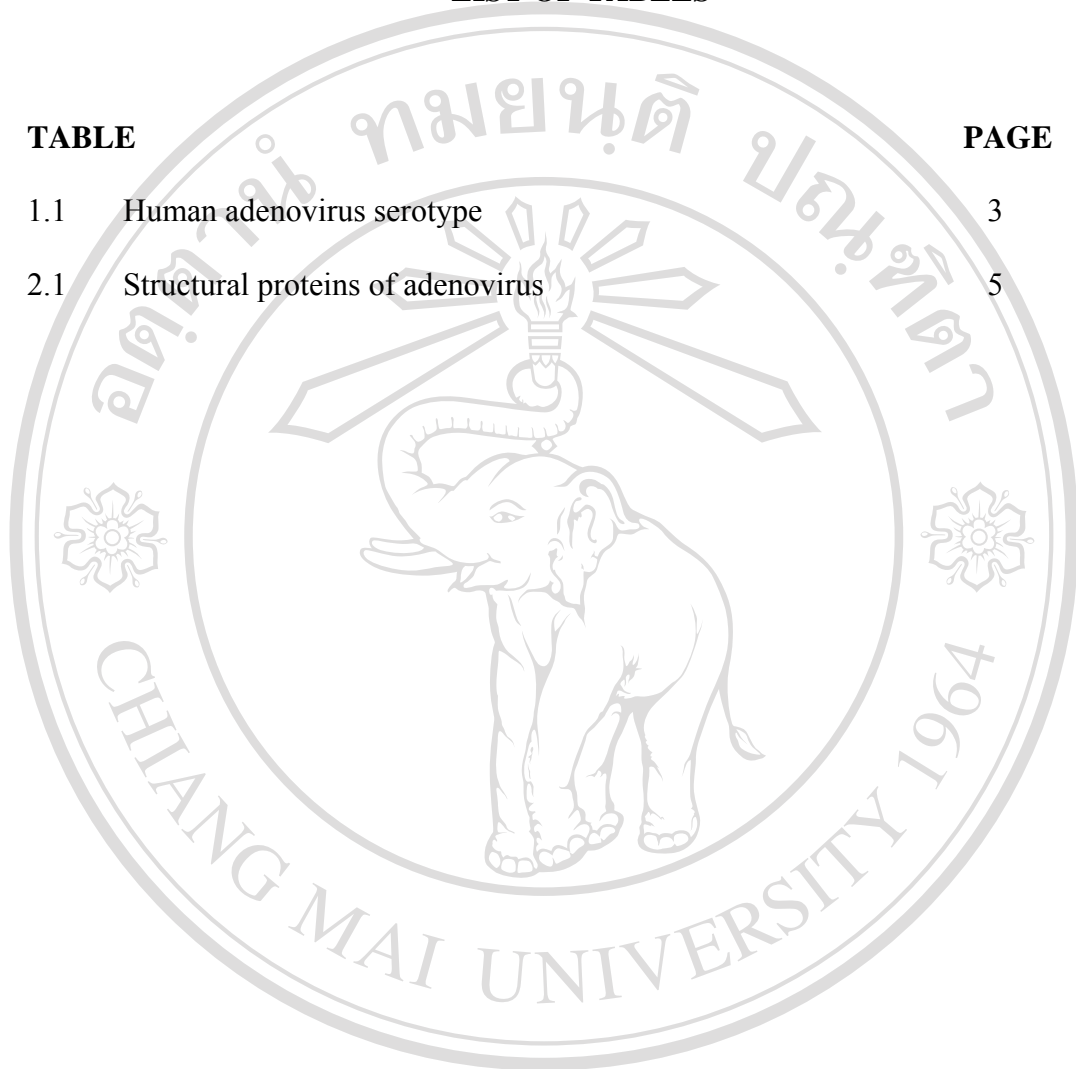
2.4.1	Primer design	32
2.4.2	Site-directed mutagenesis	33
2.5	Construction and preparation of pAdEasy vector containing scFv-M61B9 _{cyt} and scFv-M61B9 _{sec} gene	33
2.5.1	Preparation of BJ5183-pAdEasy competent cells	33
2.5.2	Transformation of pAdTrack-CMV vector containing scFv-M61B9 gene in BJ5183-pAdEasy competent cells	34
2.5.3	<i>Pac</i> I restriction analysis of pAdEasy vector containing scFv-M61B9 gene	35
2.5.4	Proof of pAdE-scFv-M61B9 _{cyt} and pAdE-scFv-M61B9 _{sec} by using polymerase chain reaction (PCR)	35
2.5.5	Transformation of pAdEasy vector containing scFv-M61B9 gene in <i>E. coli</i> DH10B	35
2.5.6	Purification of plasmid vector by using QIAGEN Plasmid MiDi Kit	36
2.6	Production of recombinant adenoviruses expressing scFv-M61B9	37
2.6.1	Cell culture	37
2.6.2	<i>Pac</i> I linearization of the pAdEasy vector containing scFv-M61B9 gene	37
2.6.3	Transfection of 293A cells with <i>Pac</i> I linearized pAdEasy vector containing scFv-M61B9 gene	37
2.7	Production of scFv-M61B9 in HeLa cell compartments	38
2.8	ScFv-M61B9 extraction by FractionPREP™ Cell Fractionation System	39

2.9	Detection of scFv-M61B9 by immunological techniques	39
2.9.1	Indirect ELISA	39
2.9.2	SDS-PAGE and Western immunoblotting	40
CHAPTER III RESULTS		
3.1	Construction of adenoviral plasmids containing scFv-M61B9 gene	42
3.2	Generation of recombinant adenovirus expressing scFv-M61B9	51
3.3	Production of scFv in each compartment of mammalian cells	53
3.4	Functional scFv produced in mammalian cells	58
CHAPTER IV DISCUSSION AND CONCLUSION		60
REFERENCES		66
APPENDICES		
APPENDIX A		74
APPENDIX B		77
CURRICULUM VITAE		84

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
 Copyright© by Chiang Mai University
 All rights reserved

LIST OF TABLES

TABLE		PAGE
1.1	Human adenovirus serotype	3
2.1	Structural proteins of adenovirus	5



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

LIST OF FIGURES

FIGURE	PAGE
1.1 Structure of adenovirus	4
1.2 Cell-entry pathway of the adenovirus	8
1.3 Transcription of the adenovirus genome	10
1.4 Schematic outline of the AdEasy system	14
1.5 Structural regions of an antibody molecule	22
3.1 PCR product of scFv-M61B9cyt gene	43
3.2 Characterization of pAdTrack-CMV containing scFv-M61B9cyt	44
3.3 PCR product of scFv-M61B9cyt amplified from pAdT-scFv-M61B9cyt	45
3.4 The point mutation of pAdT-scFv-M61B9ER	47
3.5 Restriction fragment analysis of pAdE-scFv-M61B9	48
3.6 ScFv-M61B9 gene amplification of pAdE-scFv-M61B9	49
3.7 Schematic illustration of recombinant adenoviral plasmid vector	50
3.8 Adenovirus-producing foci after transfection of 293A cells	52
3.9 Transduction of the adenovirus vector	55
3.10 The detection of scFv-M61B9 produced by HeLa cells	56
3.11 The localization of scFv-M61B9 in HeLa cells	57
3.12 The binding activity and specificity of scFv-M61B9	59

ABBREVIATIONS AND SYMBOLS

%	percent
°C	degree Celsius
Ab	antibody
bp	base pair
C _H	constant domain of the heavy chain
C _L	constant domain of the light chain
<i>E. coli</i>	<i>Escherichia coli</i>
ELISA	enzyme-linked immunosorbent assay
Fab	antigen binding fragment
Fv	variable fragment
g	gravity
gm	gram
hr	hour
HCl	hydrochloric acid
HRP	horseradish peroxidase
Ig	immunoglobulin
kb	kilo base pair
kDa	kilodaltons
M	molar
mAb	monoclonal antibody
mg	milligram

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

Copyright © by Chiang Mai University

All rights reserved

min	minute
ml	milliliter
ng	nanogram
OD	optical density
PBS	phosphate buffer saline
PCR	polymerase chain reaction
rpm	round per minute
RT	room temperature
scFv	single-chain variable fragment
TMB	3,3',5,5'-tetramethylbenzidine
U	unit
V _H	variable domain of the heavy chain
V _L	variable domain of the light chain
μg	microgram
μl	microliter

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
 Copyright© by Chiang Mai University
 All rights reserved