

## TABLE OF CONTENTS

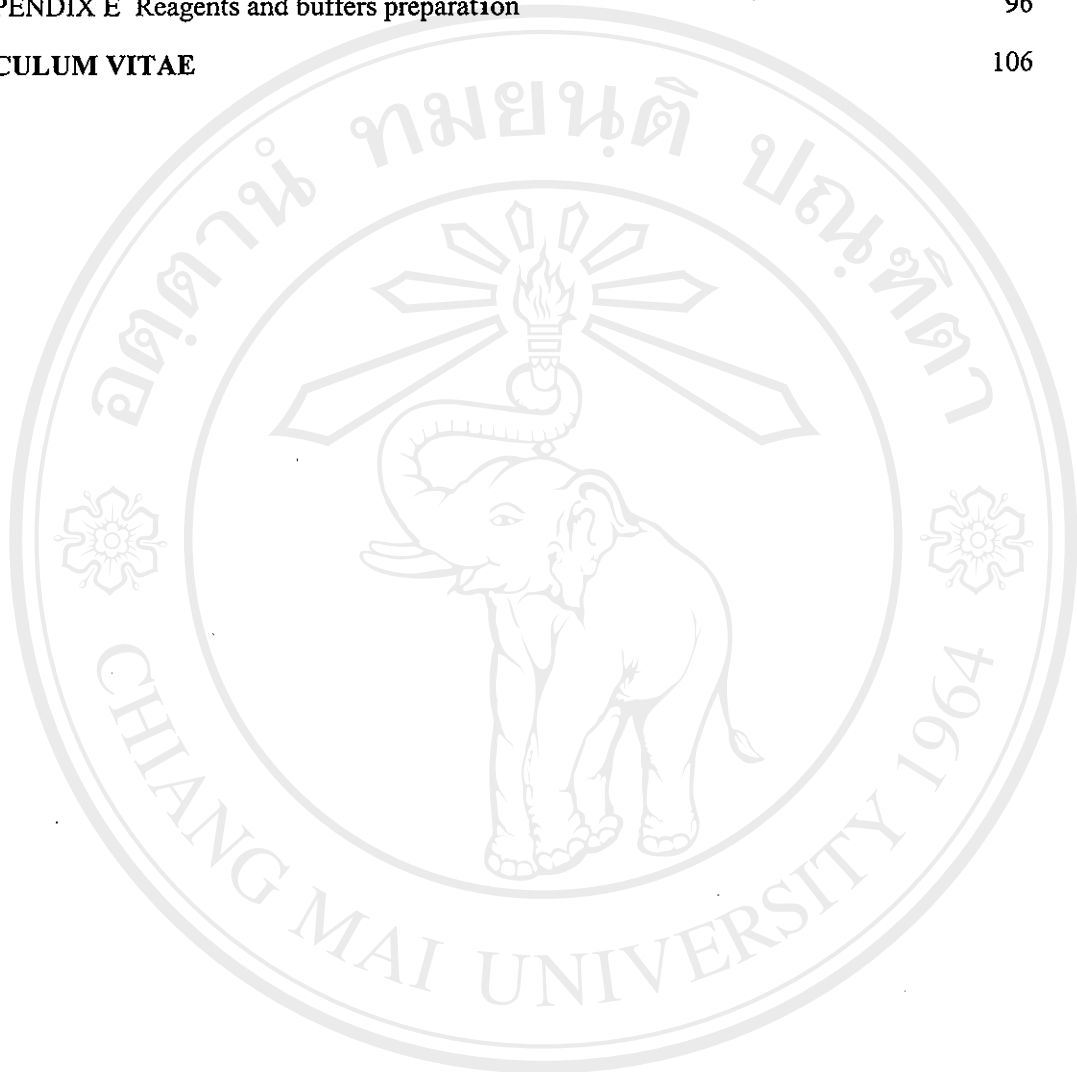
	PAGE
<b>ACKNOWLEDGEMENTS</b>	iii
<b>ABSTRACT</b>	iv
<b>TABLE OF CONTENTS</b>	x
<b>LIST OF TABLES</b>	xv
<b>LIST OF ILLUSTRATIONS</b>	xvi
<b>ABBREVIATIONS</b>	xviii
<b>CHAPTER I: INTRODUCTION</b>	1
1.1 Statement of problems	1
1.2 Literature reviews	2
1.2.1 Introduction to immunology	2
1.2.1.1 Innate immunity	3
1.2.1.1.1 The physical and chemical barriers	3
1.2.1.1.2 Cells involved in body defense	5
1.2.1.1.3 Blood protein complements	7
1.2.1.2 Adaptive immunity	8
1.2.1.2.1 Humoral immunity (HMI)	8
1.2.1.2.2 Cell-mediated immunity (CMI)	12
1.2.2 Cell cooperation	15
1.2.2.1 Extracellular signaling molecules in cell cooperation	15
1.2.2.2 T cell activation and signal transduction	17
1.2.2.2.1 MHC-TCR complex mediated-T cell activation	17
1.2.2.2.2 CD3-mediated T cell activation	19
1.2.3 Apoptosis	20
1.2.3.1 The receptor-dependent pathway	21
1.2.3.2 The receptor-independent pathway	22

1.2.4	Leukocyte surface molecules	24
1.2.5	CD147 molecule	25
1.3	Objectives	28
<b>CHAPTER II: MATERIALS AND METHODS</b>		29
2.1	Chemicals, antibodies, cell lines and instruments	29
2.2	Production of ascitic fluids containing anti-CD147 mAbs	29
2.3	Purification of anti-CD147 mAbs from ascitic fluids	29
2.4	Measurement of activity and specificity of the purified anti-CD147 mAbs	30
2.4.1	Measurement of activity of the purified mAbs using U937 cell line	30
2.4.2	Measurement of the specificity of the purified mAbs by using CD147 expressing COS cells	31
2.4.2.1	Preparation of COS cells	31
2.4.2.2	COS cell transfection	31
2.4.2.3	Staining of transfected COS cells by indirect immunofluorescent method	32
2.5	Conjugation of purified anti-CD147 mAb with fluorescein isothiocyanate (FITC)	32
2.6	Titration of FITC-conjugated anti-CD147 mAbs for epitope mapping	33
2.7	Production of plasmid DNA encoding domain 1 or domain 2 of CD147 molecule	33
2.7.1	Transformation of plasmid DNA into <i>E. coli</i>	33
2.7.2	Isolation of plasmid DNA	34
2.7.3	Characterization of isolated plasmid DNA	35
2.7.4	Large-scale production of plasmid DNA	35
2.8	Epitope mapping	36
2.8.1	Epitope mapping of anti-CD147 mAbs by cross-blocking analysis using U937 cell line	36

2.8.2	Epitope mapping of anti-CD147 mAbs using domain 1 or domain 2 of CD147 expressing COS cells	36
2.8.2.1	COS cell transfection	36
2.8.2.2	Intracellular staining of transfected COS cells by indirect immunofluorescent method	37
2.9	Characterization of anti-CD147 mAbs by western blotting	37
2.9.1	Preparation of cell lysate	37
2.9.2	Protein separation by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and blotting	37
2.9.3	Immunodetection	38
2.10	Characterization of anti-CD147 mAbs by immunoprecipitation	38
2.10.1	Biotinylation and preparation of cell lysate	38
2.10.2	Preclearing lysates	39
2.10.3	Immunoprecipitation	39
2.10.4	Chemiluminescence detection	39
2.11	Functional study of CD147 molecule	40
2.11.1	Induction of apoptosis by anti-CD147 mAbs	40
2.11.2	Study the effect of anti-CD147 mAbs on cell proliferation	40
2.11.2.1	Titration of anti-CD3 mAb for induction of T cell proliferation	40
2.11.2.1.1	Immobilization of anti-CD3 mAb	40
2.11.2.1.2	Preparation of peripheral blood mononuclear cells	41
2.11.2.1.3	T cell proliferation assay	41
2.11.2.2	Functional study of CD147 molecule involving the regulation of anti-CD3 mAb induced cell proliferation	41
2.11.2.2.1	Immobilization of anti-CD3 mAb	41
2.11.2.2.2	Functional study of CD147 molecule involving the regulation of anti-CD3 mAb induced cell proliferation	42
2.11.2.2.3	Titration of PHA for induction of T cell proliferation	42

2.11.2.2.4	Functional study of CD147 molecule involving the regulation of PHA induced cell proliferation	42
<b>CHAPTER III: RESULTS</b>		44
3.1	Production and purification of mAbs to CD147 molecule	44
3.2	Determination of activity and specificity of the purified anti-CD147 mAbs	44
3.3	Determination of epitopes recognized by anti-CD147 mAbs	45
3.3.1	Cross-blocking analysis using U937 cells	45
3.3.2	Localization of epitope recognized by anti-CD147 mAbs	51
3.3.2.1	Production of plasmid DNA encoding domain 1 or domain 2 of CD147 molecule	51
3.3.2.2	Localization of epitope recognized by anti-CD147 mAbs	54
3.4	Characterization of anti-CD147 mAb by western blotting and immunoprecipitation	58
3.4.1	Western blotting	58
3.4.2	Immunoprecipitation	61
3.5	Functional study of CD147 molecule involving the induction of apoptosis	65
3.6	Functional study of CD147 molecule that involves anti-CD3 mAb induced cell proliferation	65
3.6.1	Titration of anti-CD3 mAb for induction of T cell proliferation	65
3.6.2	Functional study of CD147 molecule involving the regulation of anti-CD3 mAb induced cell proliferation	70
3.7	Functional study of CD147 molecule involving PHA induced cell proliferation	72
<b>CHAPTER IV: DISCUSSION AND CONCLUSION</b>		75
<b>REFERENCES</b>		82
<b>APPENDICES</b>		89
APPENDIX A List of the chemicals and materials used in this study		90
APPENDIX B List of antibodies used in this study		93

APPENDIX C List of cell lines used in this study	94
APPENDIX D List of instruments used in this study	95
APPENDIX E Reagents and buffers preparation	96
<b>CIRRICULUM VITAE</b>	<b>106</b>



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่  
Copyright© by Chiang Mai University  
All rights reserved

**LIST OF TABLES**

<b>TABLE</b>		<b>PAGE</b>
1.1	The effector functions of antibody isotypes	10
3.1	F/P ratio of FITC-conjugated anti-CD147 mAbs	48
3.2	Cross-blocking analysis of anti-CD147 mAbs	50
3.3	Summary of epitopes recognized by anti-CD147 mAbs	64

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่  
Copyright© by Chiang Mai University  
All rights reserved

## LIST OF ILLUSTRATIONS

FIGURE	PAGE
1.1 T cell signaling leads to activation	18
1.2 Insufficient TCR aggregation results in the generation of a partial signal	20
1.3 Schematic representation of apoptotic stimuli, signaling pathways, and effector mechanisms	23
3.1 The immunofluorescence profiles of activity of purified anti-CD147 mAbs	46
3.2 Reactivity of purified anti-CD147 mAbs with CD147 expressing COS cells	47
3.3 Titration for optimal concentration of FITC-conjugated anti-CD147 mAbs	49
3.4 Characterization of the plasmid DNA encoding domain 1 or domain 2 of CD147 molecule isolated from various bacterial colonies	52
3.5 Characterization of the plasmid DNA encoding domain 1 or domain 2 of CD147 molecule isolated from the selected bacterial colonies	53
3.6 Reactivity of mAbs M6-2F9 (a), M6-2B1 (b), M6-1F3 (c), M6-1D4 (d), M6-1B9 (e), M6-1E9(f), and MEM-M6/6 (g) with intact CD147 expressing COS cells	55
3.7 Reactivity of mAbs M6-2F9 (a), M6-2B1 (b), M6-1D4 (c), M6-1B9 (d) and M6-1E9 (e) with CD147 domain 1 expressing COS cells	56
3.8 Reactivity of mAb MEM-M6/6 with CD147 domain 2 expressing COS cells (a) and mAb MT8 with CD8 expressing COS cells (b)	57
3.9 Analysis of anti-CD147 mAbs by Western blotting under non-reducing condition	59
3.10 Analysis of anti-CD147 mAbs by Western blotting under reducing condition	60
3.11 Immunoprecipitation of cell surface molecule recognized by anti-CD147 mAbs under reducing condition	62

3.12 Immunoprecipitation of cell surface molecule recognized by anti-CD147 mAbs under non-reducing condition	63
3.13 The immunofluorescence profiles of anti-CD147 mAbs for apoptotic induction of U937 cell line	66
3.14 The immunofluorescence profiles of anti-CD147 mAbs for apoptotic induction of Sup-T1 cell line	67
3.15 The immunofluorescence profiles of anti-CD147 mAbs for apoptotic induction of KG1a cell line	68
3.16 Titration of immobilized mAb OKT3 concentration for T cells proliferation assay	69
3.17 Involvement of CD147 molecule in the regulation of CD3-mediated T cells proliferation	71
3.18 Titration of PHA for T cells proliferation assay	73
3.19 Involvement of CD147 molecule in the regulation of PHA-mediated cells proliferation	74



## ABBREVIATION

%	Percent
$\beta$	Beta
$\alpha$	Alpha
$\gamma$	Gamma
$\mu$	Micro
$\mu\text{Ci}$	Microcurie
$\mu\text{g}$	Microgram
$\mu\text{l}$	Microliter
$\mu\text{M}$	Micromolar
2ME	2-mercaptoethanol
ADCC	Antibody-dependent cell cytotoxicity
APCs	Antigen presenting cells
APS	Ammonium persulfate
BCR	B cell receptors
BSA	Bovine serum albumin
CD	Cluster of differentiation
cDNA	Complementary deoxyribonucleic acid
cm	Centimeter
CMI	Cell-mediated immunity
CRP	C-reactive protein
CSF	Colony-stimulating factors
CTLs	Cytotoxic T-lymphocytes
DEAE-Dextran	Diethylaminoethyl-Dextran
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid

EDTA	Ethylenediaminetetraacetic acid
EMMPRIN	Extracellular matrix metalloproteinase inducer
EtBr	Ethidium bromide
FACS	Fluorescence-activated cell sorter
FCS	Fetal calf serum
FITC	Fluorescein isothiocyanate
g	Gram or gravity
HCl	Hydrochloric acid
HMI	Humoral-mediated immunity
IFN	Interferon
Ig	Immunoglobulin
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
Igs	Immunoglobulins
IL	Interleukin
IMDM	Iscove's modified Dulbecco's medium
kDa	Kilodalton
LPS	Lipopolysaccharides
LT	Lymphotoxin
M	Molarity
mAb	Monoclonal antibody
mAbs	Monoclonal antibodies
MALT	Mucosa-associated lymphoid tissue
MBL	Mannose-binding lectin
MEM	Minimum essential medium
mg	Milligram
MHC	Major histocompatibility complex

MHC-I	Major histocompatibility complex class I
MHC-II	Major histocompatibility complex class II
min	Minute
ml	Milliliter
mM	Millimolar
mm <sup>3</sup>	Cubic millimeter
MW	Molecular weight
N	Normality
NaN <sub>3</sub>	Sodium azide
ng	Nanogram
NK cell	Natural Killer cell
nm	Nanometer
OD	Optical density
°C	Degree Celsius
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline
PHA	Phytohaemagglutinin
PMSF	Phenylmethylsulfonyl fluoride
rpm	Revolutions per minute
SALT	Skin-associated lymphoid tissue
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
TCRs	T cell receptors
T <sub>H</sub> 1	T-helper type 1
T <sub>H</sub> 2	T-helper type 2
TNF	Tumor necrosis factor