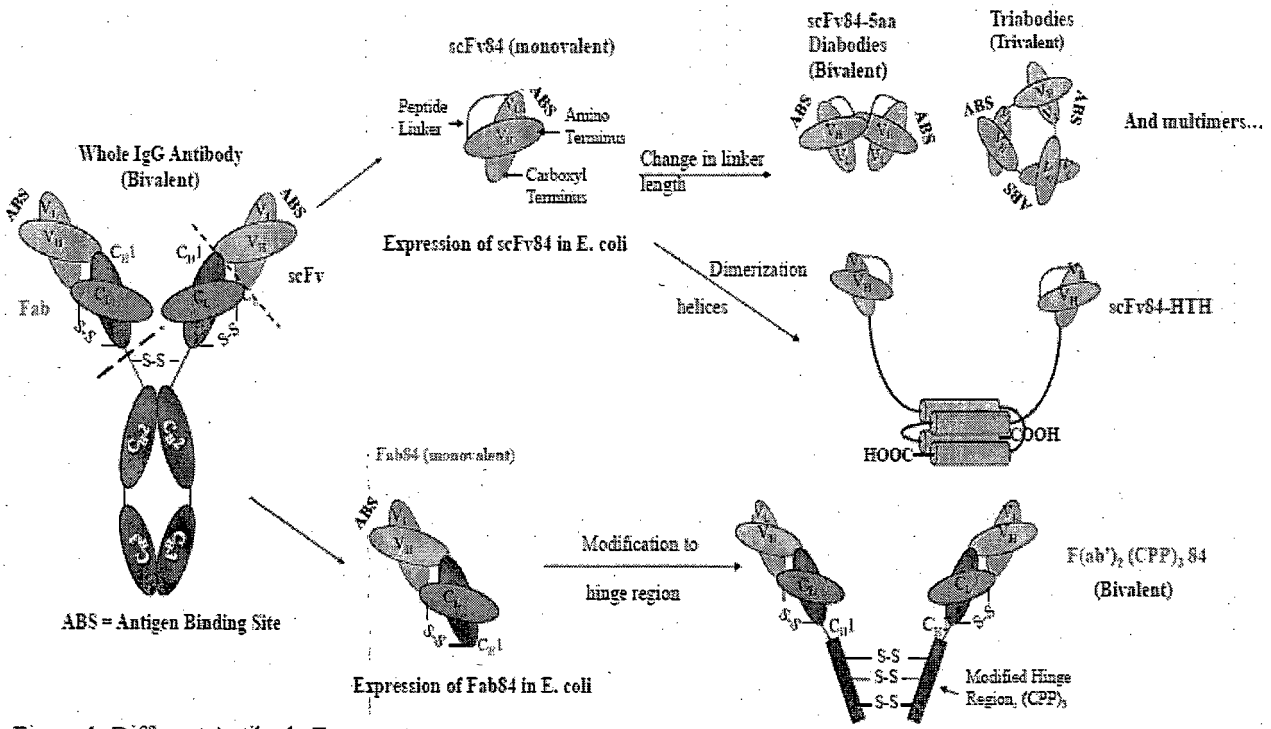


Figure 1. Different Antibody Fragments

Figure 1

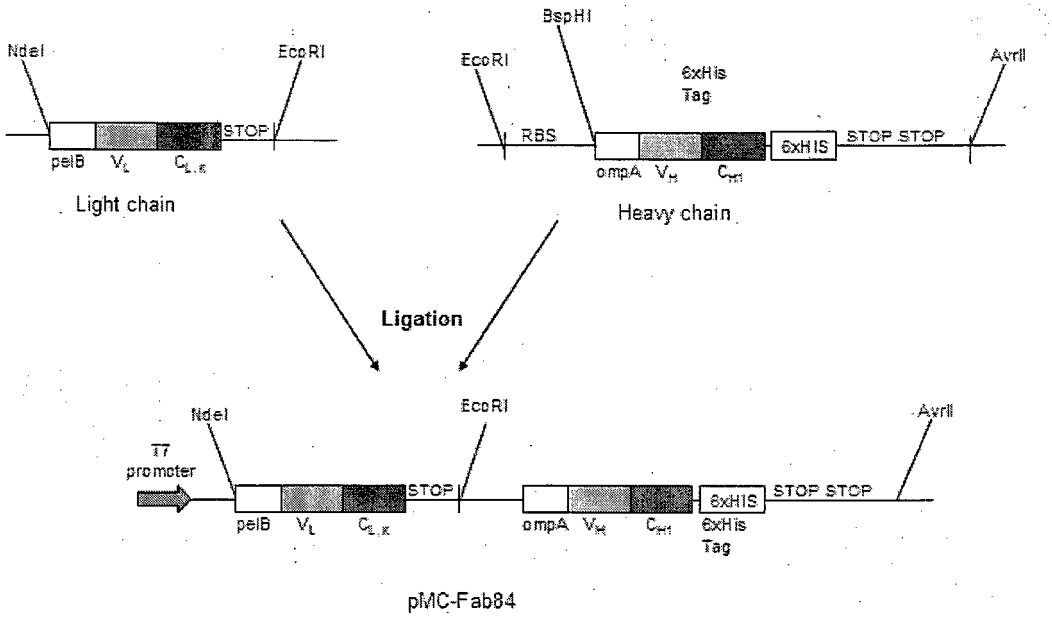

NdeI
EcoRI
STOP
pelB
Light chain
BspHI
EcoRI
6xHis Tag
AvrII
RBS
6xHIS
STOP STOP
ompA
Heavy chain
Ligation
NdeI
EcoRI
AvrII
T7 promoter
STOP
pelB
ompA
6xHIS
STOP STOP
6xHis Tag
pMC-Fab84

Figure 2a

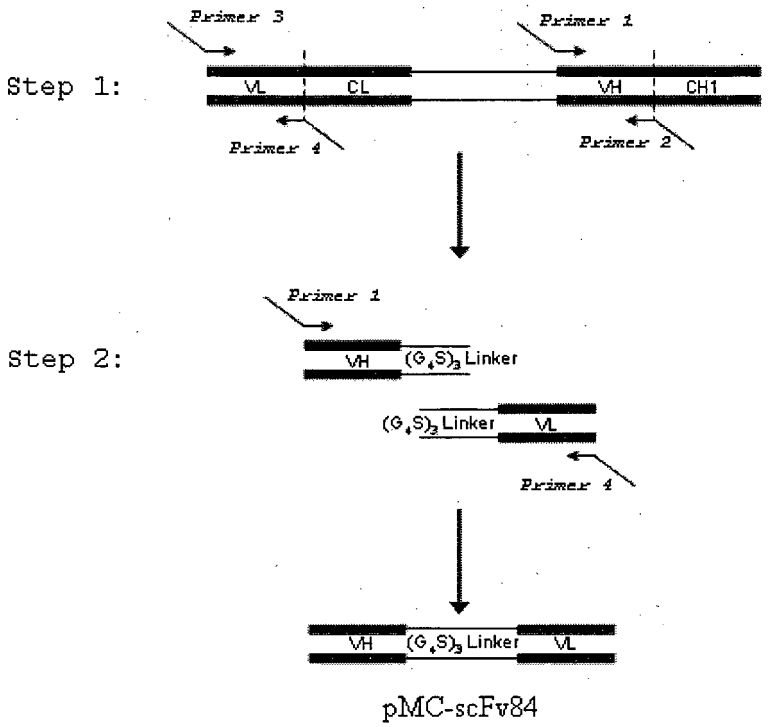


Figure 2b

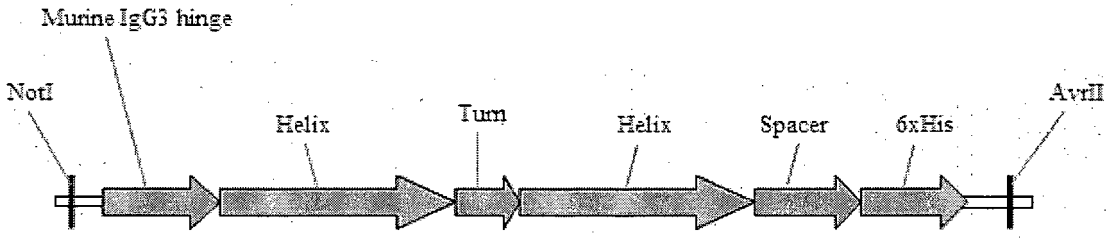


Figure 3

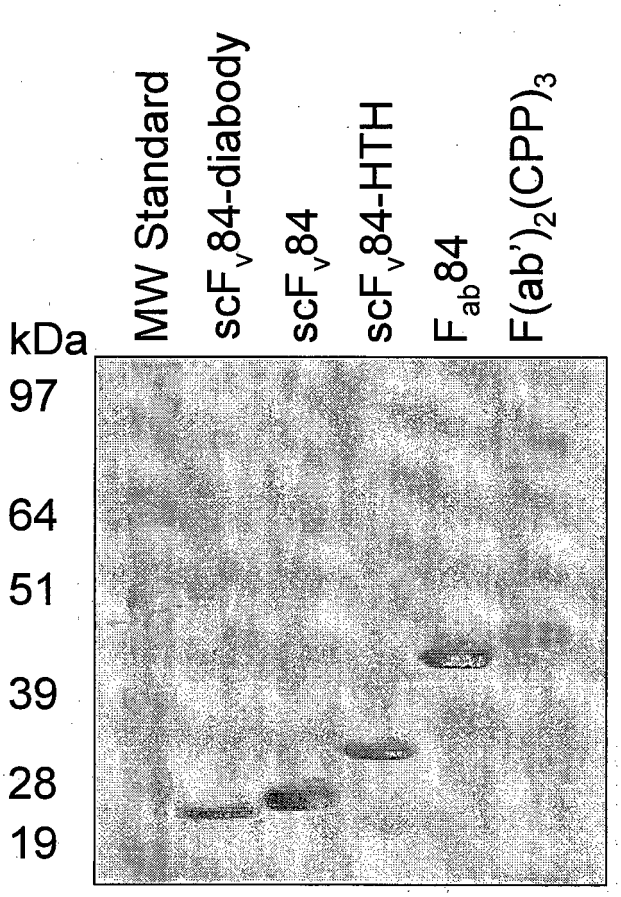

kDa
97
64
51
39
28
19
MW Standard
scF$_v$84-diabody
scF$_v$84
scF$_v$84-HTH
F$_{ab}$84
F(ab')$_2$(CPP)$_3$

Figure 4a

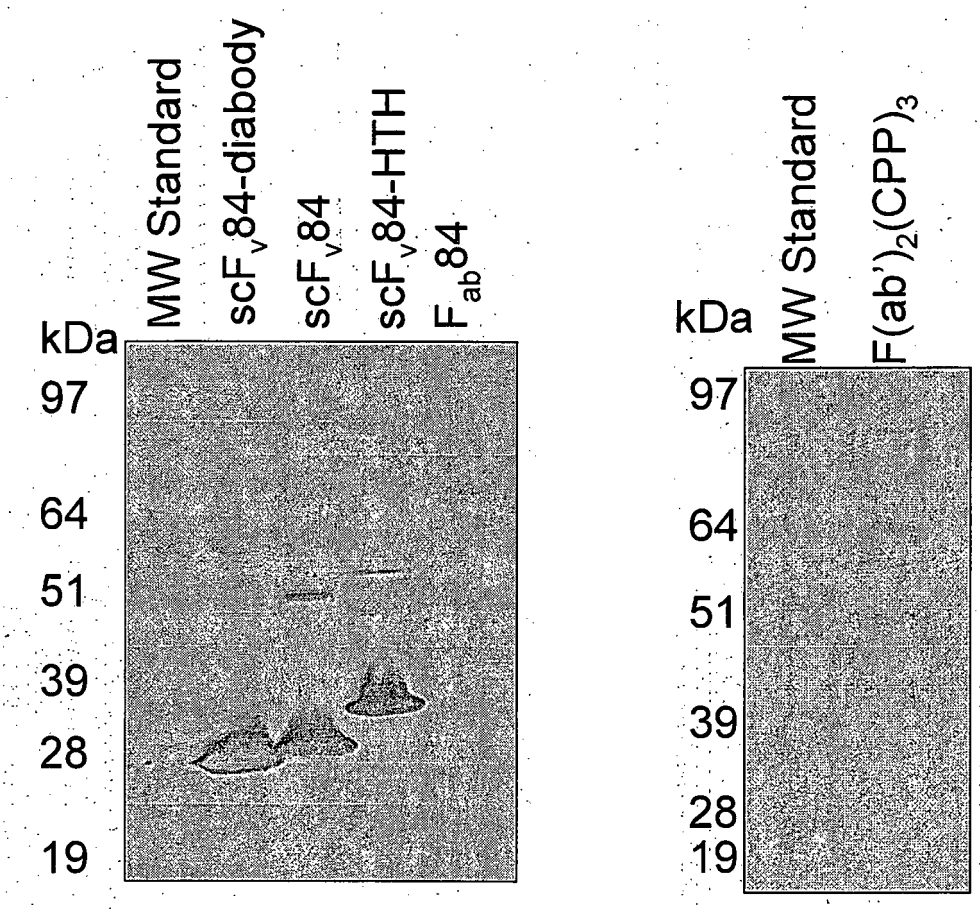

kDa
MW Standard
scF$_v$84-diabody
scF$_v$84
scF$_v$84-HTH
F$_{ab}$84
97
64
51
39
28
19
kDa
MW Standard
F(ab')$_2$(CPP)$_3$
97
64
51
39
28
19

Figure 4b

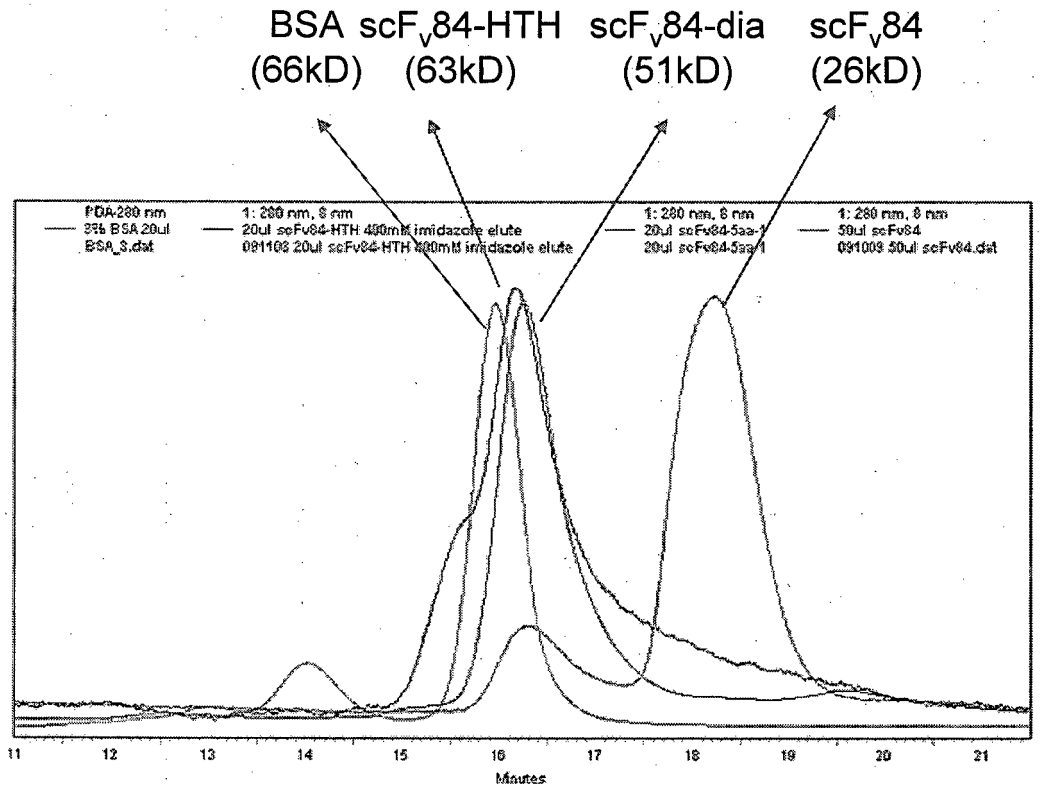

BSA (66kD)
scFv84-HTH (63kD)
scFv84-dia (51kD)
scFv84 (26kD)
PDA 280 nm
3% BSA 20ul
BSA_3.dat
1: 280 nm, 8 nm
20ul scFv84-HTH 400mM imidazole elute
091103 20ul scFv84-HTH 400mM imidazole elute
1: 280 nm, 8 nm
20ul scFv84-5aa-1
20ul scFv84-5aa-1
1: 280 nm, 8 nm
50ul scFv84
091009 50ul scFv84.dat
11
12
13
14
15
16
17
18
19
20
21
Minutes

Figure 5

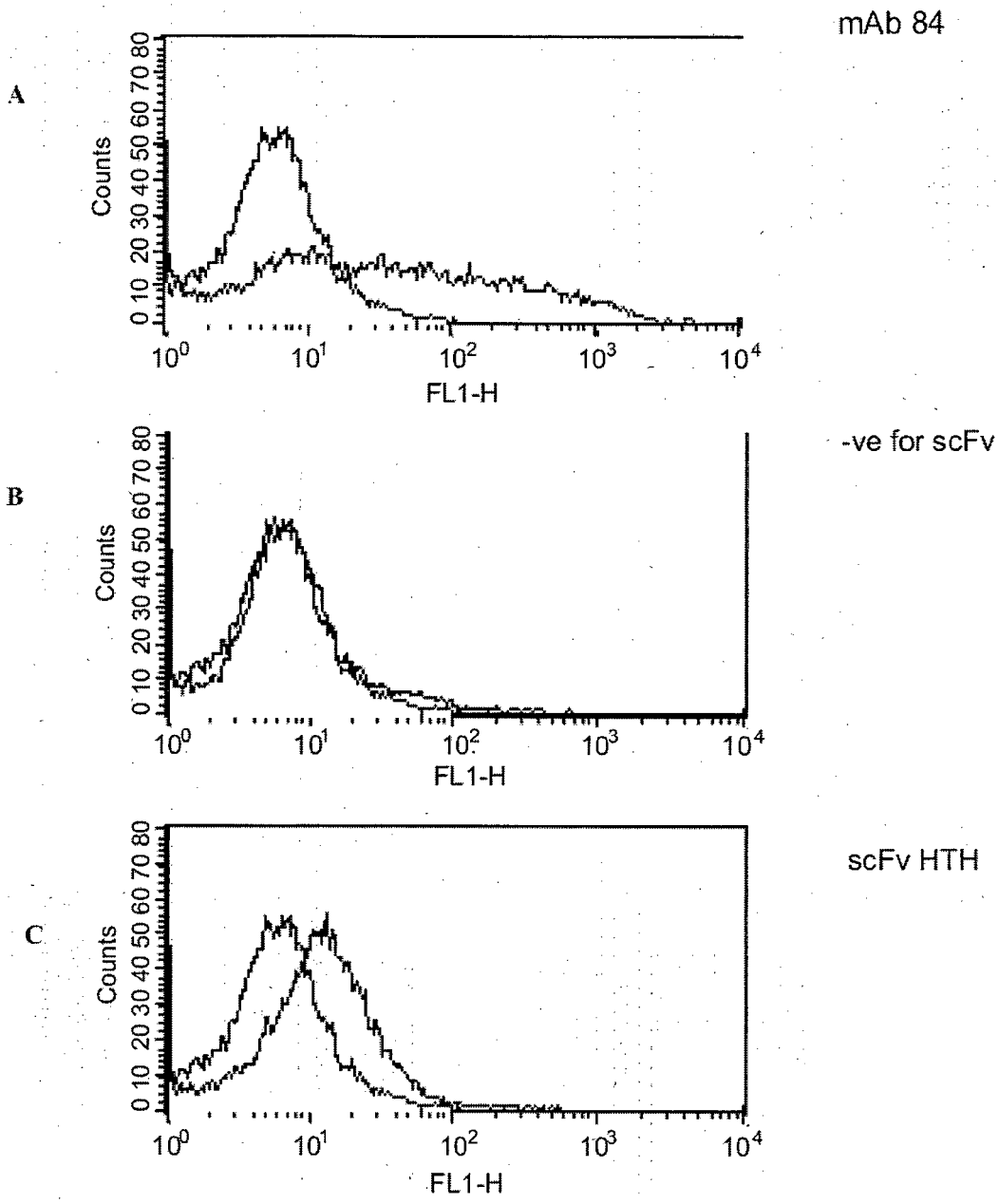

A
mAb 84
Counts
0 10 20 30 40 50 60 70 80
10^0 10^1 10^2 10^3 10^4
FL1-H
B
-ve for scFv
Counts
0 10 20 30 40 50 60 70 80
10^0 10^1 10^2 10^3 10^4
FL1-H
C
scFv HTH
Counts
0 10 20 30 40 50 60 70 80
10^0 10^1 10^2 10^3 10^4
FL1-H

Figure 6

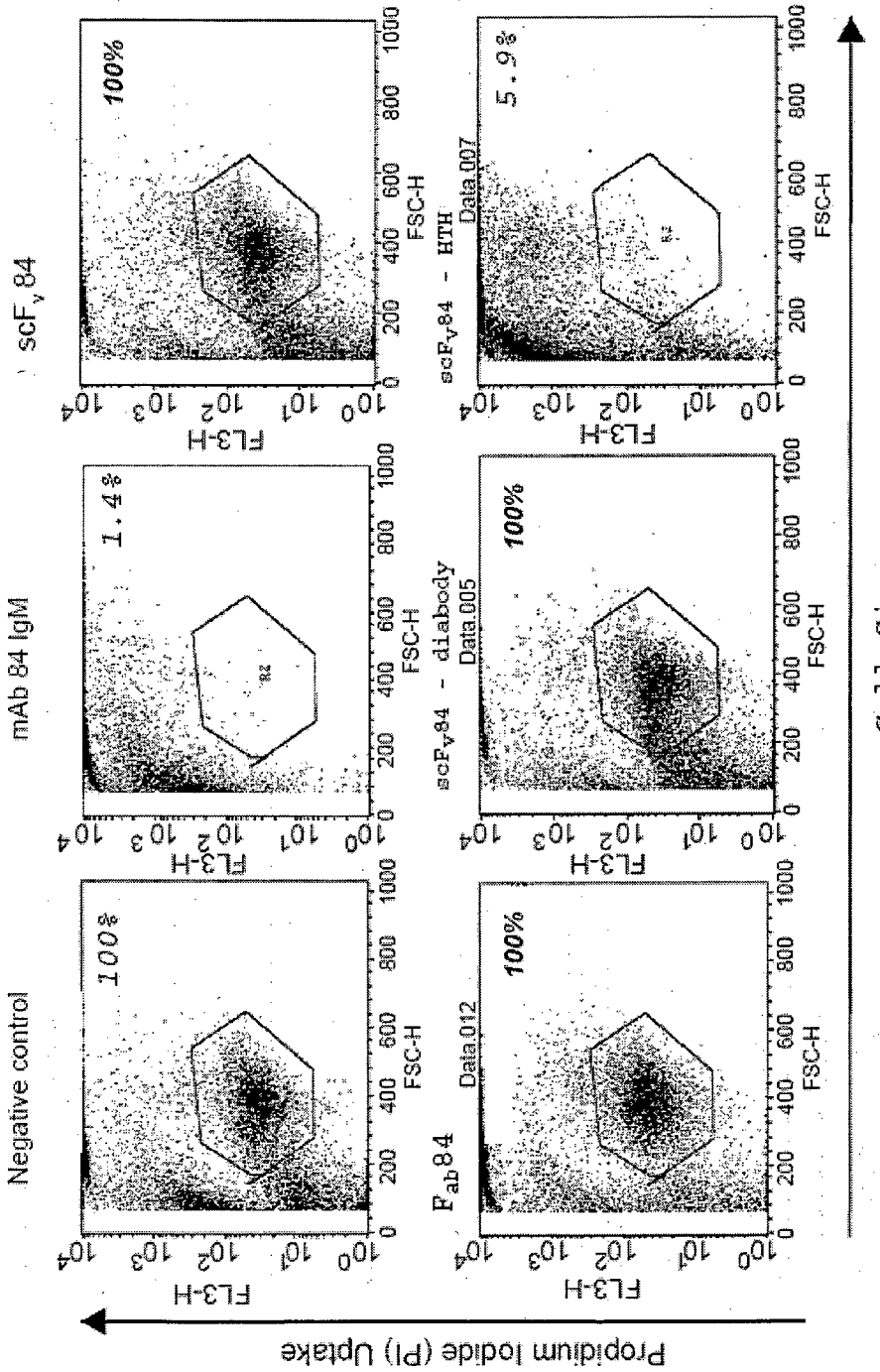

Negative control
mAb 84 IgM
scF$_v$84
F$_{ab}$84
scF$_v$84 - diabody
scF$_v$84 - HTH
Data 012
Data 005
Data 007
100%
1.4%
5.9%
FL3-H
FSC-H
Propidium Iodide (PI) Uptake
Cell Size

Figure 7a

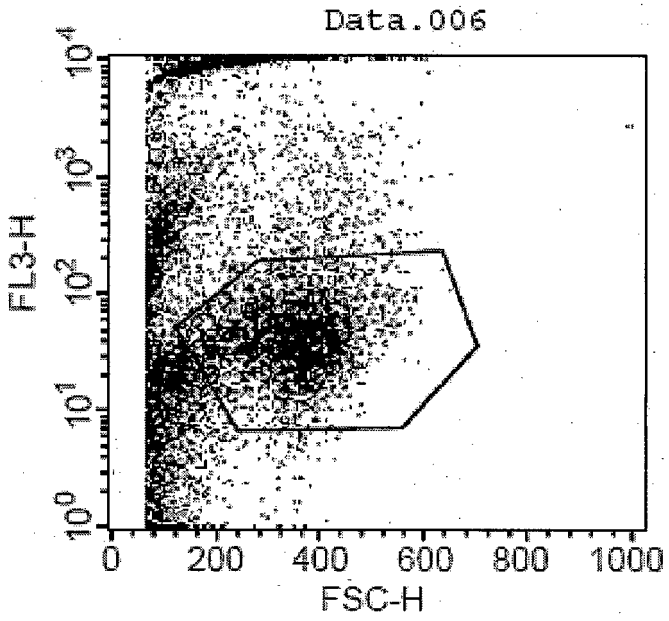


Figure 7a cont…

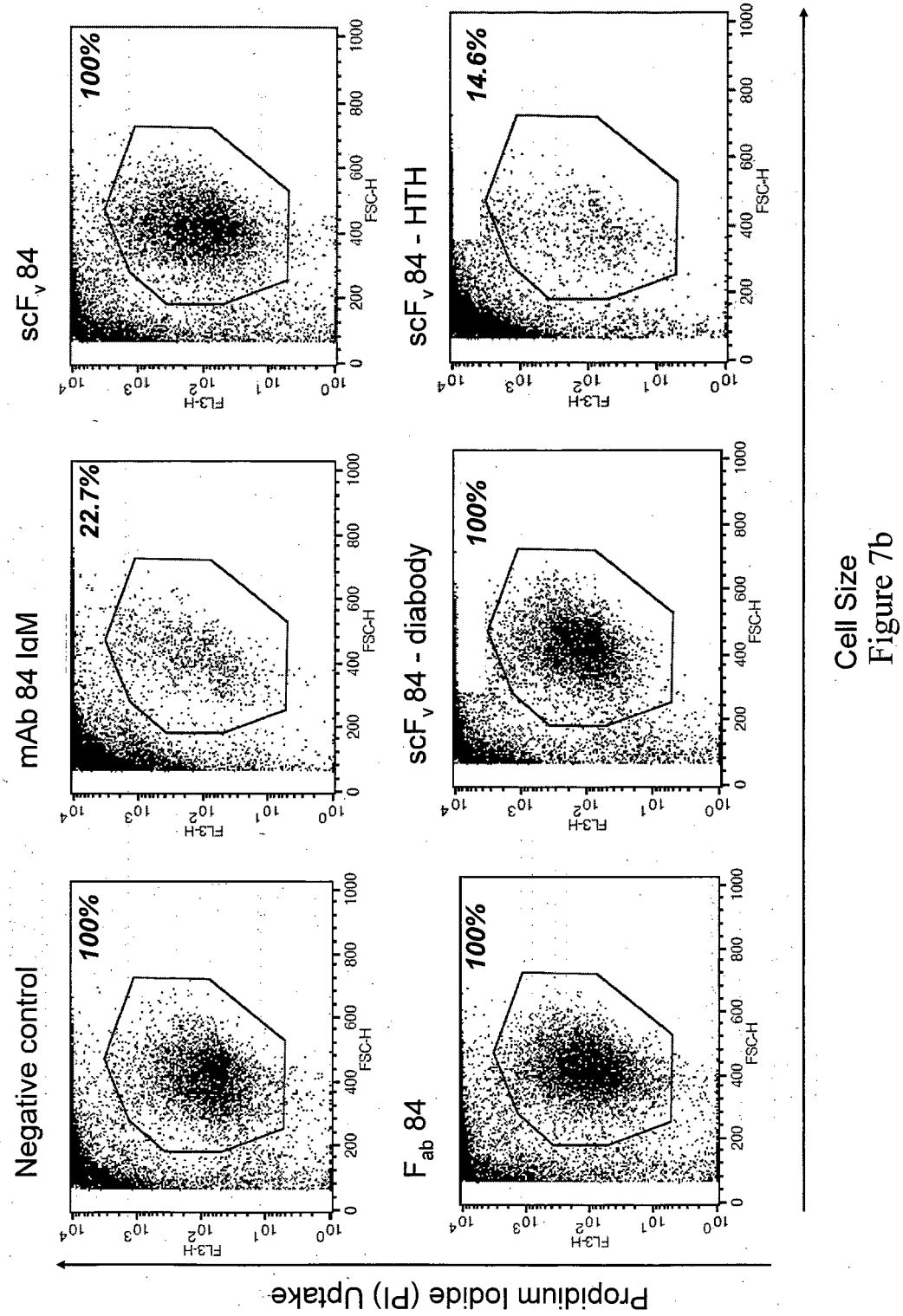

Negative control
100%
mAb 84 IgM
22.7%
scFv 84
100%
Fab 84
100%
scFv 84 - diabody
100%
scFv 84 - HTH
14.6%
FL3-H
FSC-H
Propidium Iodide (PI) Uptake
Cell Size

Figure 7b

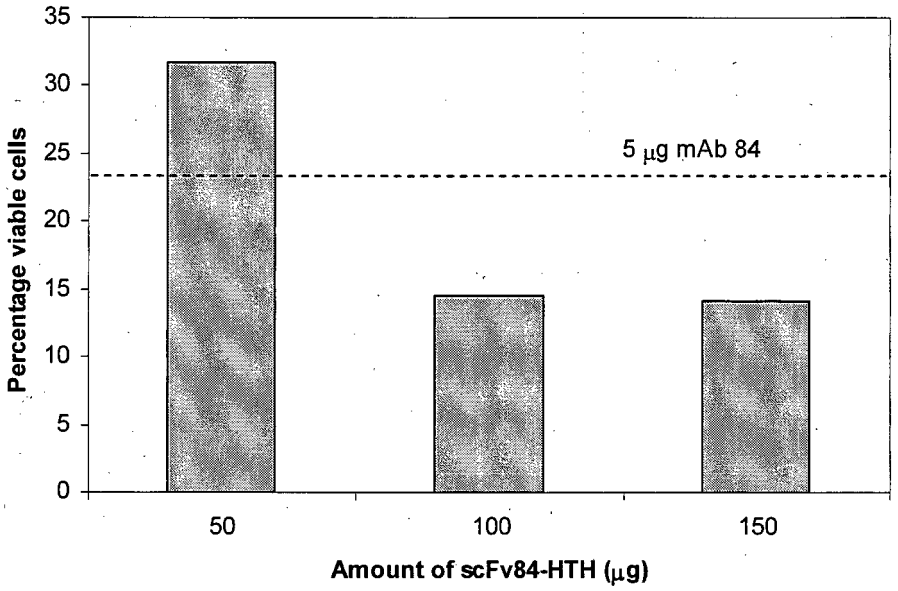

35
30
25
20
15
10
5
0
Percentage viable cells
5 μg mAb 84
50
100
150
Amount of scFv84-HTH (μg)

Figure 7c

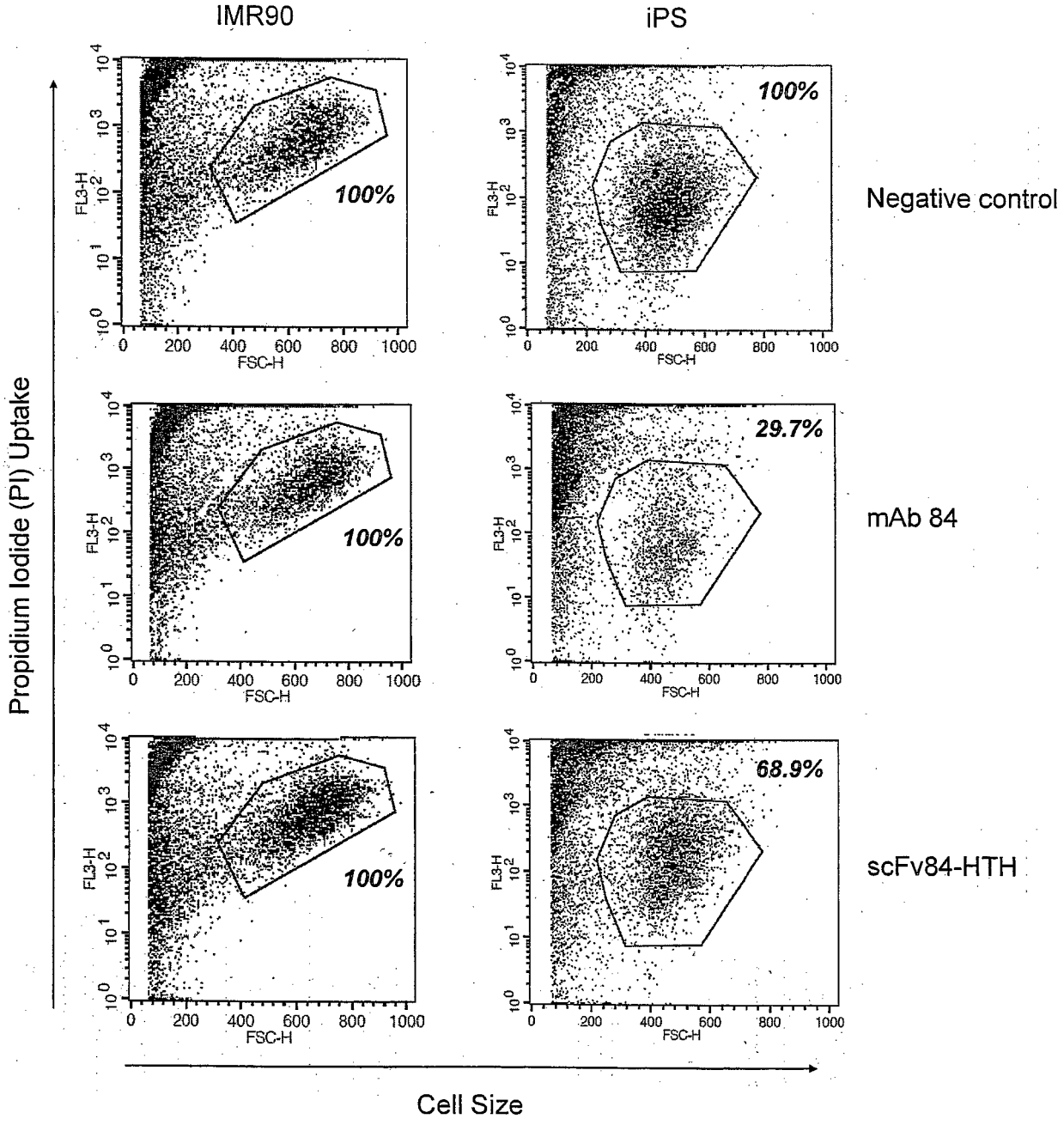

IMR90
iPS
Propidium Iodide (PI) Uptake
Cell Size
FL3-H
FSC-H
100%
100%
Negative control
100%
29.7%
mAb 84
100%
68.9%
scFv84-HTH

Figure 8

Primer Name	Primer Sequence
c547t-F	5' tctaaatacattcaaatatgtatccgcttatgaattaattcttagaaaaactcatcg 3'
c547t-R	5' cgatgagtttttctaagaattaattcataagcggatacatatttgaatgtatttaga 3'
c1422t-F	5' ctgtttatgtaagcagacagttttattgtttatgaccaaaatccctt 3'
c1422t-R	5' aagggattttggtcataaacaataaaactgtctgcttacataaacag 3'
c4845t-F	5' gccgaaacaagcgcttatgagcccgaagtgg 3'
c4845t-R	5' ccacttcgggctcataagcgcttgtttcggc 3'
scFv84-NcoI_VH_F 1	5' gcccagccggccatggcccaggttcagctgcagcagag 3'
scFv84-VH_linker_R 2	5' cgatccgccaccgccagagccacctccgcctgaaccgcctccacc gctgctaacggtcacggtggtgcc 3'
scFv84-linker_VL_F 3	5' ggtggaggcggttcaggcggaggtggctctggcggtggcggatcg gatattgaactgacccagagccc 3'
scFv84-VL_NotI_R 4	5' gtgatgtgcggccgcacgtttgatttccagtttgg 3'
F(ab')2-84-VH_AgeI_F	5' ccggaaccggtgaccgtgag 3'
F(ab')2-84-VH_30nt_AvrII_R	5'tagcagcctaggttattaatgatgatgatgatgcgccgccgccgggcacggcgggcaggtatgggttttatc acagcttttcggttccacttttttatc 3'
F(ab)2-(CPP)3_F	5' gccgtgcccgccgtgcgcggcggcgc 3'
F(ab)2-(CPP)3_R	5' gcgccgccgcgcacggcgggcacggc 3'
scFv-5_F	5' cgtgaccgttagcagcggtggaggcggttcagatattgaactgacccagagcccggc 3'
scFv-5_R	5' gccgggctctgggtcagttcaatatctgaaccgcctccaccgctgctaacggtcacg 3'
scFv-10_F	5' ggtggaggcggttcaggcggaggtggctctgatattgaactgacccagagcccggc 3'
scFv-10_R	5' gccgggctctgggtcagttcaatatcagagccacctccgcctgaaccgcctccacc 3'
scFv-0_VH_F	5' gccggccatggcccaggttcagctgcagcagagc 3'
scFv-0_VHVL_R	5' ggtcagttcaatatcgctgctaacggtcacg 3'
scFv-0_VL R (same primer as *scFv84-VL_NotI_R 4*)	5' gtgatgtgcggccgcacgtttgatttccagtttgg 3'

Figure 9

scFv84

Amino acid

qvqlqqsggglvqpggsmklscvasgftfsnywmnwvrqspekglewvaeirlksnnyathyaesvkgrftisrddskss
vylqmnnlraedtgiyyctgerawgqgttvtvssggggsggggsggggsdieltqspaimsaspgekvtmtcsasssvn
ymywyqqkpgssprlliydtsnlasgvpvrfsgsgsgtsysltisrmeaedaatyycqqwssypytfgggtkleikr
(SEQ ID NO: 1)

Nucleotide

Caggttcagctgcagcagagcggtggcggcctggtgcagccgggcggtagcatgaaactgagctgcgtggcgagcggt
tttacctttagcaactattggatgaattgggtgcgccagagcccggaaaaaggcctggaatgggtggcggaaattcgtctga
aaagcaataactatgcgacccattatgccgaaagcgtgaaaggtcgctttaccattagccgcgatgatagcaaaagcagc
gtgtatctgcagatgaacaatctgcgcgcggaagataccggcatttattattgcaccggcgaacgcgcgtggggccaggg
caccaccgtgaccgttagcagcggtggaggcggttcaggcggaggtggctctggcggtggcggatcggatattgaactg
acccagagcccggccattatgagcgcgagcccgggcgaaaaagtgaccatgacctgcagcgcgagcagcagcgtga
actatatgtattggtatcagcagaaaccgggcagcagcccgcgcctgctgatttatgataccagcaacctggccagcggtg
tgccggtgcgctttagcggtagcggcagcggcaccagctatagcctgaccattagccgtatggaagcggaagatgcggc
gacctattattgccagcagtggagcagctatccgtatacctttggcggtggcaccaaactggaaatcaaacgt
(SEQ ID NO: 13)

Figure 10

scFv84 Amino acid sequences

VH
qvqlqqsggglvqpggsmklscvasgftfsnywmnwvrqspekglewvaeirlksnnyathyaesvkgrftisrddskss
vylqmnnlraedtgiyyctgerawgqgttvtvss
(SEQ ID NO: 9)

Glysine-serine linker
ggggsggggsggggs
(SEQ ID NO: 12)

VL
dieltqspaimsaspgekvtmtcsasssvnymywyqqkpgssprlliydtsnlasgvpvrfsgsgsgtsysltisrmeaed
aatyycqqwssypytfgggtkleikr[aaa]
(SEQ ID NO: 8)

[optional sequence encoded by optional NotI site for cloning]

Murine IgG3 hinge
pkpstppgss
(SEQ ID NO: 10)

HTH with His-tag
geleellkhlkellkgprkgeleellkhlkellkggsggaphhhhhh
(SEQ ID NO: 11)

Figure 11

scFv84 Nucleotide sequences

VH
caggttcagctgcagcagagcggtggcggcctggtgcagccgggcggtagcatgaaactgagctgcgtggcgagcggtt
ttacctttagcaactattggatgaattgggtgcgccagagcccggaaaaaggcctggaatgggtggcggaaattcgtctga
aaagcaataactatgcgacccattatgccgaaagcgtgaaaggtcgctttaccattagccgcgatgatagcaaaagcagc
gtgtatctgcagatgaacaatctgcgcgcggaagataccggcatttattattgcaccggcgaacgcgcgtggggccaggg
caccaccgtgaccgttagcagc
(SEQ ID NO: 15)

Glysine-serine linker
ggtggaggcggttcaggcggaggtggctctggcggtggcggatcg
(SEQ ID NO: 18)

VL
gatattgaactgacccagagcccggccattatgagcgcgagcccgggcgaaaaagtgaccatgacctgcagcgcgag
cagcagcgtgaactatatgtattggtatcagcagaaaccgggcagcagcccgcgcctgctgatttatgataccagcaacct
ggccagcggtgtgccggtgcgctttagcggtagcggcagcggcaccagctatagcctgaccattagccgtatggaagcg
gaagatgcggcgacctattattgccagcagtggagcagctatccgtatacctttggcggtggcaccaaactggaaatcaaa
cgt[gcggccgca]
(SEQ ID NO: 14)

[optional NotI site for cloning]

Murine IgG3 hinge
ccgaaaccgagcaccccgccgggcagcagc
(SEQ ID NO: 16)

HTH with His-tag and stop codon
ggcgaactggaagaactgctgaaacatctgaaagaactgctgaaaggcccgcgtaaaggcgaattagaggaactgctg
aaacacttaaaagaattactgaaaggcggcagcggtggagcaccacatcatcatcatcattaataa
(SEQ ID NO: 17)

Figure 12

Fab84 Amino acid sequences

Heavy chain
VH
qvqlqqsggglvqpggsmklscvasgftfsnywmnwvrqspekglewvaeirlksnnyathyaesvkgrftisrddskss
vylqmnnlraedtgiyyctgerawgqgttvtvss
(SEQ ID NO: 9)

CH1
astkgpsvfplapsskstsggtaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtqtyicnv
nhkpsntkvdkkvepksc
(SEQ ID NO: 24)

Short hinge with CPP
dkthtcppcppc
(SEQ ID NO: 25)

His-tag
aaahhhhhh
(SEQ ID NO: 26)

Light chain
VL
dieltqspaimsaspgekvtmtcsasssvnymywyqqkpgssprlliydtsnlasgvpvrfsgsgsgtsysltisrmeaed
aatyycqqwssypytfgggtkleikr
(SEQ ID NO: 8)

CL
tvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslsstltlskadyekhkv
yacevthqglsspvtksfnrgec
(SEQ ID NO: 27)

Figure 13

Fab84 Nucleotide sequences

Heavy chain
VH
caggttcagctgcagcagagcggtggcggcctggtgcagccgggcggtagcatgaaactgagctgcgtggcgagcggtt
ttacctttagcaactattggatgaattgggtgcgccagagcccggaaaaaggcctggaatgggtggcggaaattcgtctga
aaagcaataactatgcgacccattatgccgaaagcgtgaaaggtcgctttaccattagccgcgatgatagcaaaagcagc
gtgtatctgcagatgaacaatctgcgcgcggaagataccggcatttattattgcaccggcgaacgcgcgtggggccaggg
caccaccgtgaccgttagcagc
(SEQ ID NO: 15)

CH1
gccagcaccaaaggtccgagcgtgtttccgctggccccgagcagcaaaagcaccagcggcggtaccgcggcgctggg
ctgtctggtgaaagattattttccggaaccggtgaccgtgagctggaatagcggtgccctgaccagcggtgttcatacctttcc
ggccgtgctgcagagcagcggcctgtatagcctgagcagcgtggtgaccgtgccgagcagcagcctgggcacccagac
ctatatctgcaatgttaatcataaaccgagcaacaccaaagttgataaaaaagtggaaccgaaaagctgt
(SEQ ID NO: 28)

His-tag
gcggcggcgcatcatcatcatcatcat
(SEQ ID NO: 29)

Light chain
VL
gatattgaactgacccagagcccggccattatgagcgcgagcccgggcgaaaaagtgaccatgacctgcagcgcgag
cagcagcgtgaactatatgtattggtatcagcagaaaccgggcagcagcccgcgcctgctgatttatgataccagcaacct
ggccagcggtgtgccggtgcgctttagcggtagcggcagcggcaccagctatagcctgaccattagccgtatggaagcg
gaagatgcggcgacctattattgccagcagtggagcagctatccgtatacctttggcggtggcaccaaactggaaatcaaa
cgt
(SEQ ID NO: 14)

CL
accgttgcggcgccgagcgtgtttattttcccgccgagcgatgaacagctgaaaagcggcaccgcgagcgtggtgtgcct
gctgaataatttttatccgcgcgaagccaaagtgcagtggaaagtggataatgcgctgcagagcggcaatagccaggaa
agcgtgaccgaacaggatagcaaagatagcacctatagcctgagcagcaccctgaccctgagcaaagccgattatgaa
aaacataaagtgtatgcgtgtgaagtgacccatcagggcctgagcagcccggttaccaaaagctttaaccgtggtgaatg
c
(SEQ ID NO: 30)

Figure 14

Sequence for heavy chain of Fab84 with leucine zipper extension

QVQLQQSGGGLVQPGGSMKLSCVASGFTFSNYWMNWVRQSPEKGLEWVAEIRLKSN
NYATHYAESVKGRFTISRDDSKSSVYLQMNNLRAEDTGIYYCTGERAWGQGTTVTVSS
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCAAAPKPSTPPGSSR
MKQLEDKVEELLSKNYHLENEVARLKKLVGERGSGGAPHHHHHH

(SEQ ID NO: 31)

Sequence for light chain of Fab84 (identical to monovalent Fab84)

DIELTQSPAIMSASPGEKVTMTCSASSSVNYMYWYQQKPGSSPRLLIYDTSNLASGVP
VRFSGSGSGTSYSLTISRMEAEDAATYYCQQWSSYPYTFGGGTKLEIKRTVAAPSVFIF
PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL
SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

(SEQ ID NO: 32)

Figure 15

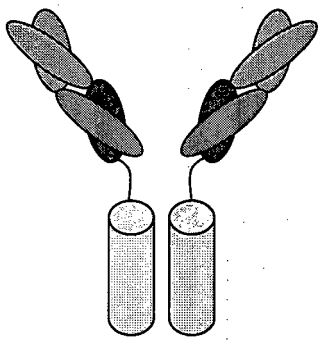


Figure 16

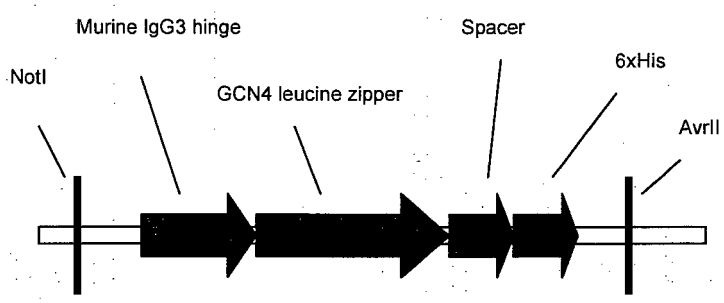


Figure 17

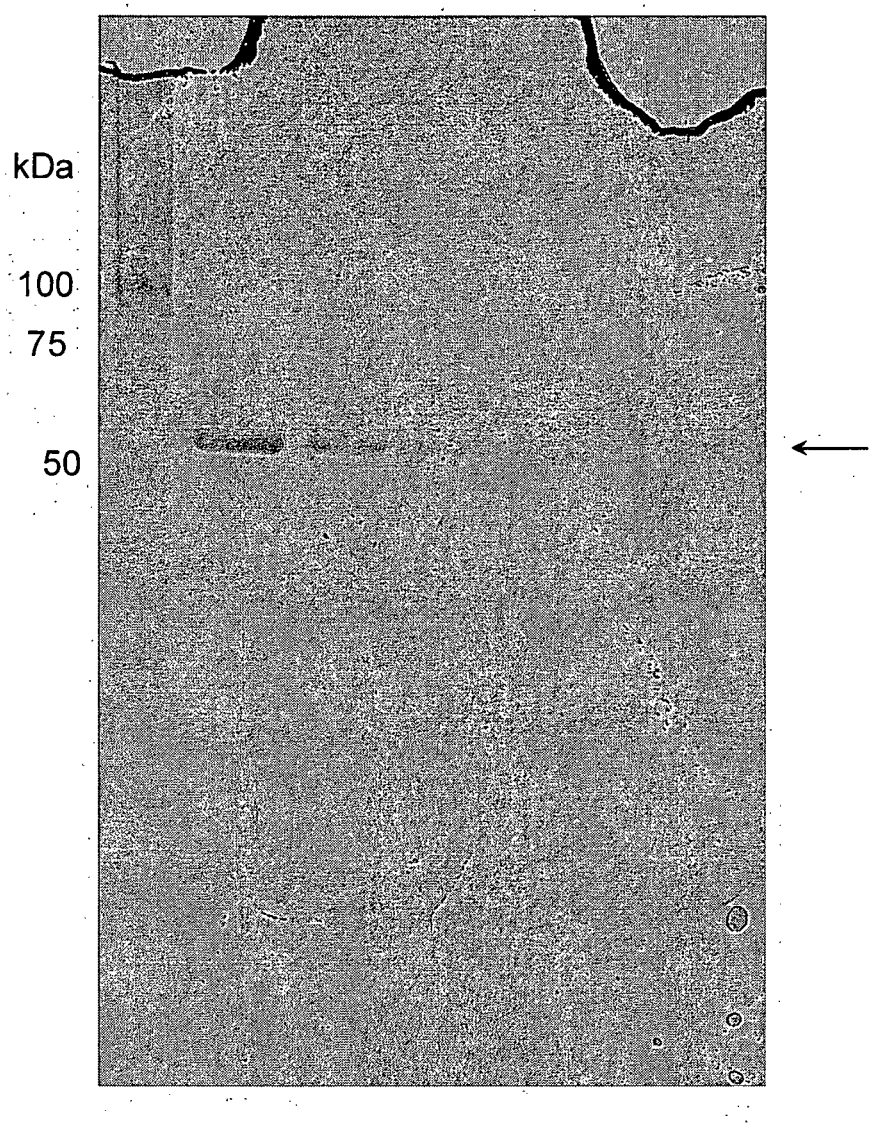
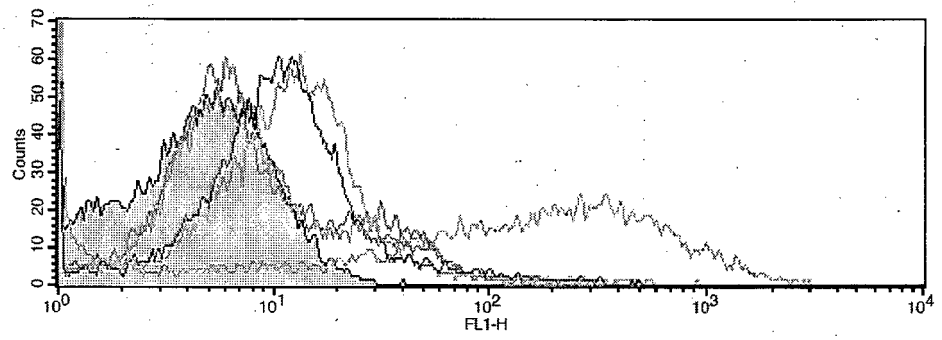


Figure 18



Key	Name	Parameter	Gate
mAb -ve	Data.001	FL1-H	No Gate
5ug mAb84	Data.002	FL1-H	No Gate
scFv -ve	Data.003	FL1-H	No Gate
Blank buffer PBSI	Data.006	FL1-H	No Gate
100ug Fab84-LZ	Data.013	FL1-H	No Gate
200ug Fab84-LZ	Data.014	FL1-H	No Gate

Figure 19

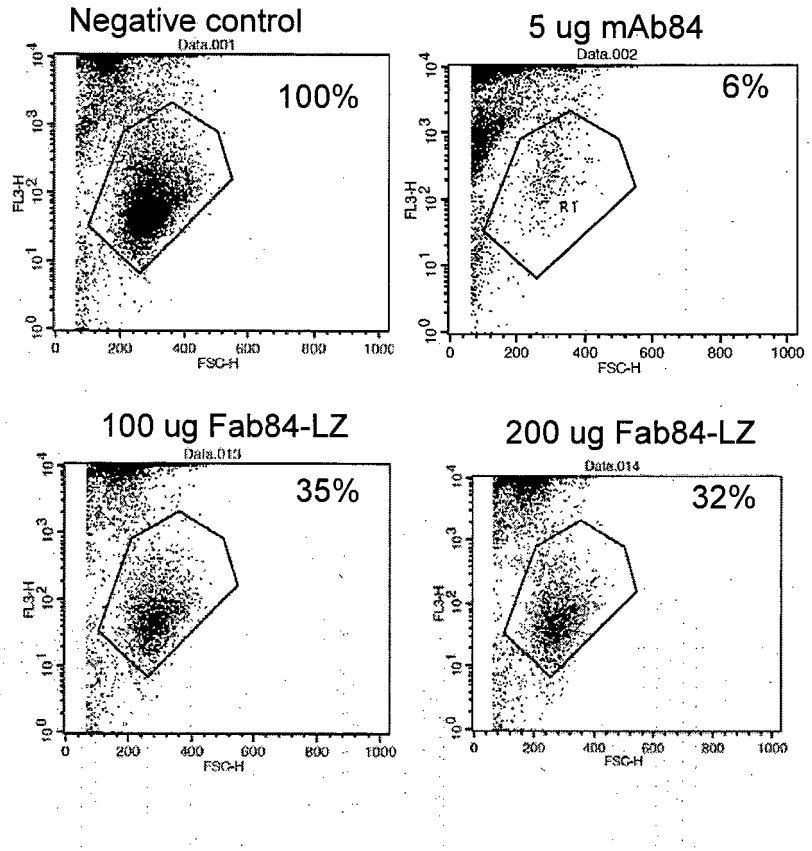

Negative control
Data.001
100%
5 ug mAb84
Data.002
6%
R1
100 ug Fab84-LZ
Data.013
35%
200 ug Fab84-LZ
Data.014
32%
FL3-H
FSC-H

Figure 20