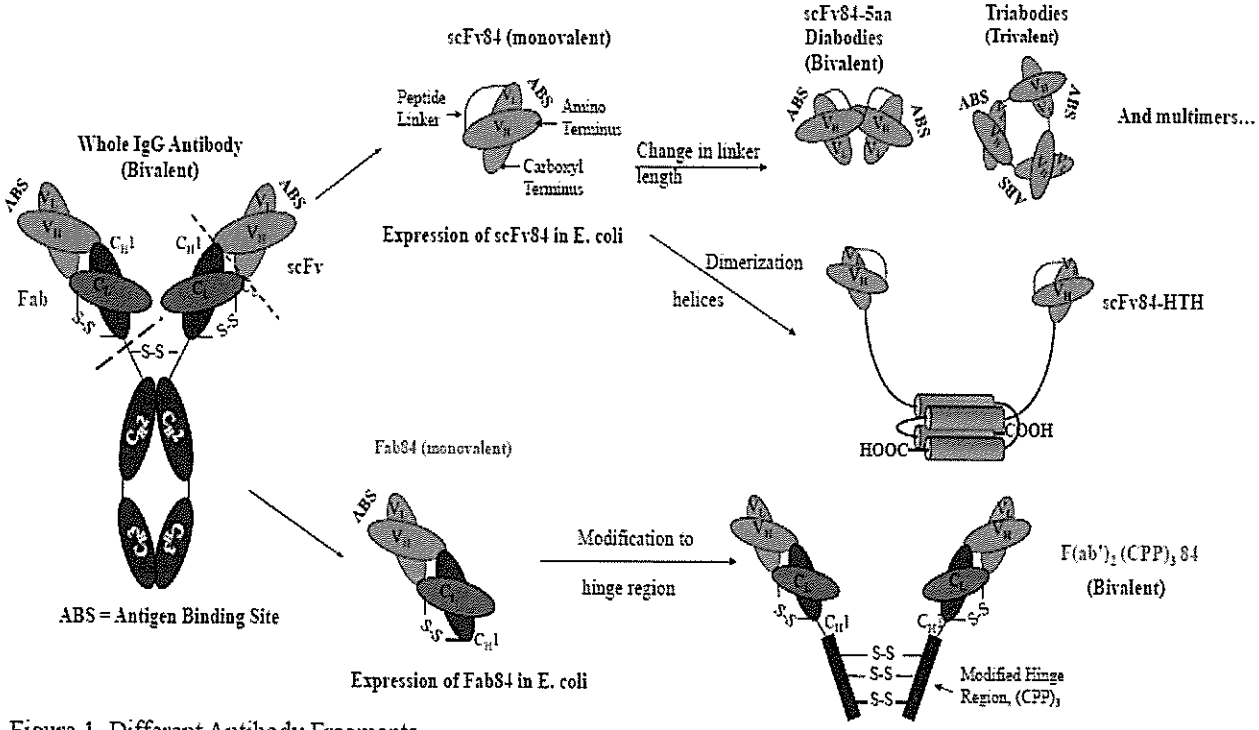


Figure 1. Different Antibody Fragments

Figure 1

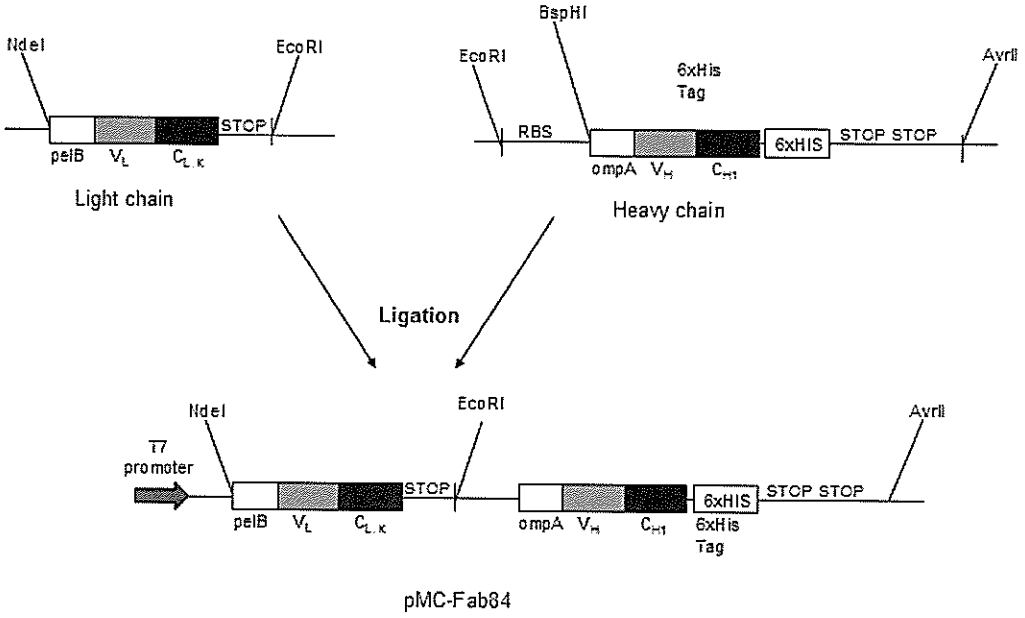
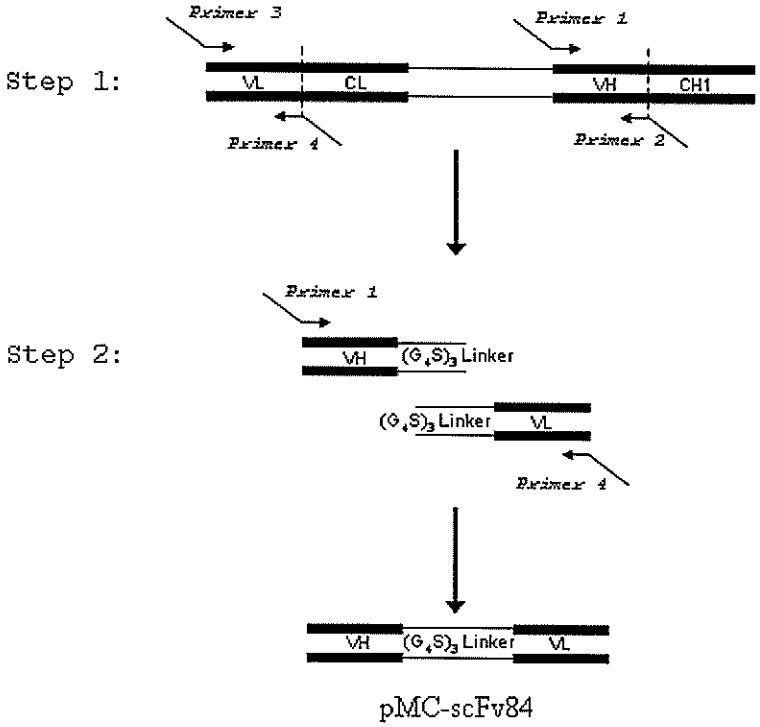

NdeI
EcoRI
STOP
pelB
V_L
$C_{L,K}$
Light chain
BspHI
EcoRI
6xHis
Tag
AvrII
RBS
6xHIS
STOP STOP
ompA
V_H
C_{H1}
Heavy chain
Ligation
NdeI
EcoRI
AvrII
T7
promoter
STOP
pelB
V_L
$C_{L,K}$
ompA
V_H
C_{H1}
6xHIS
STOP STOP
6xHis
Tag
pMC-Fab84

Figure 2a



pMC-scFv84

Figure 2b

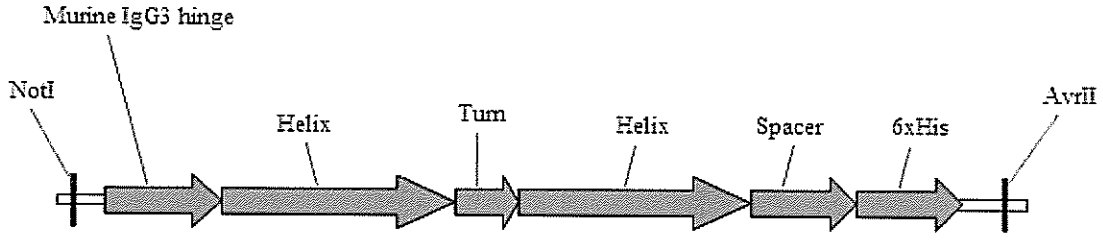


Figure 3

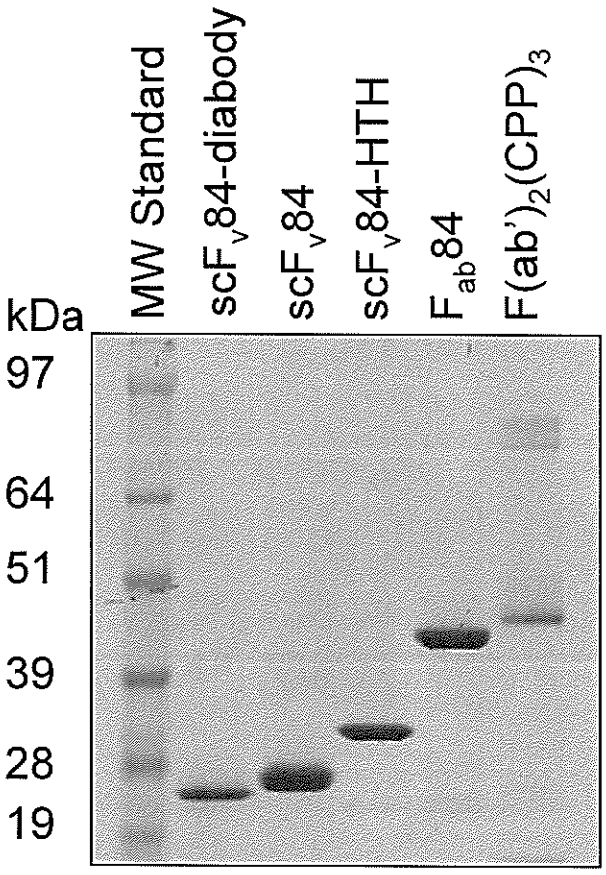

kDa
97
64
51
39
28
19
MW Standard
scF$_v$84-diabody
scF$_v$84
scF$_v$84-HTH
F$_{ab}$84
F(ab')$_2$(CPP)$_3$

Figure 4a

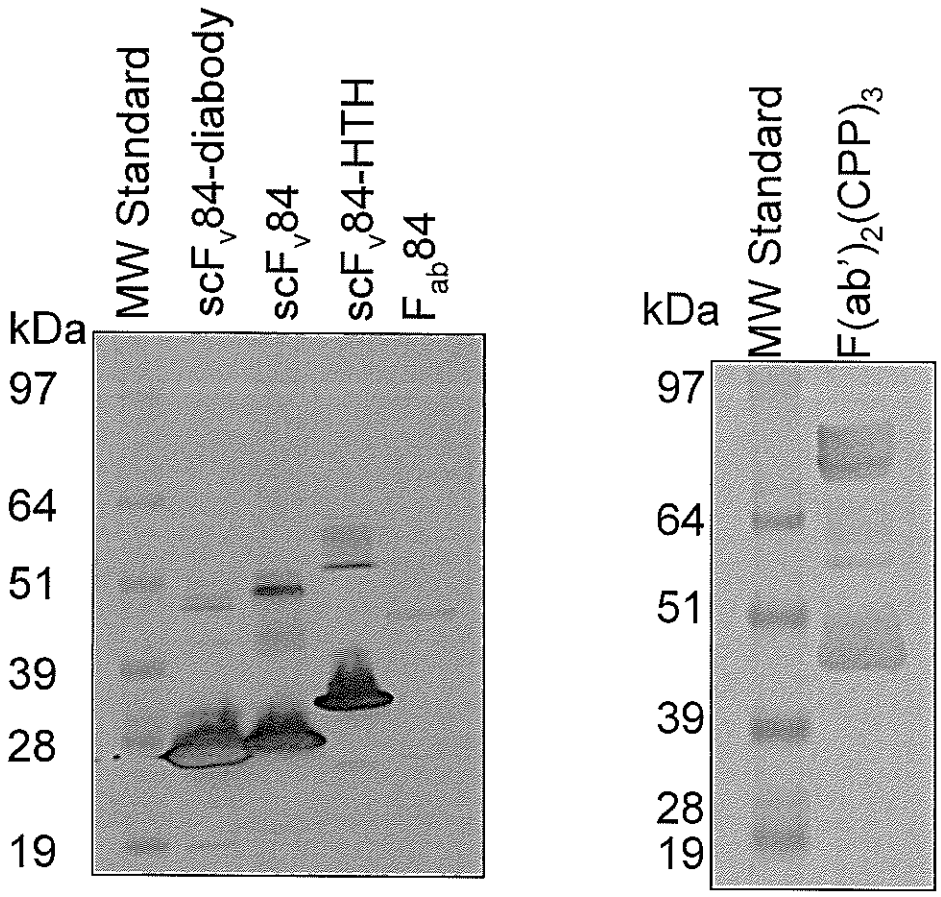

kDa
MW Standard
scFv84-diabody
scFv84
scFv84-HTH
Fab84
97
64
51
39
28
19
kDa
MW Standard
F(ab')2(CPP)3
97
64
51
39
28
19

Figure 4b

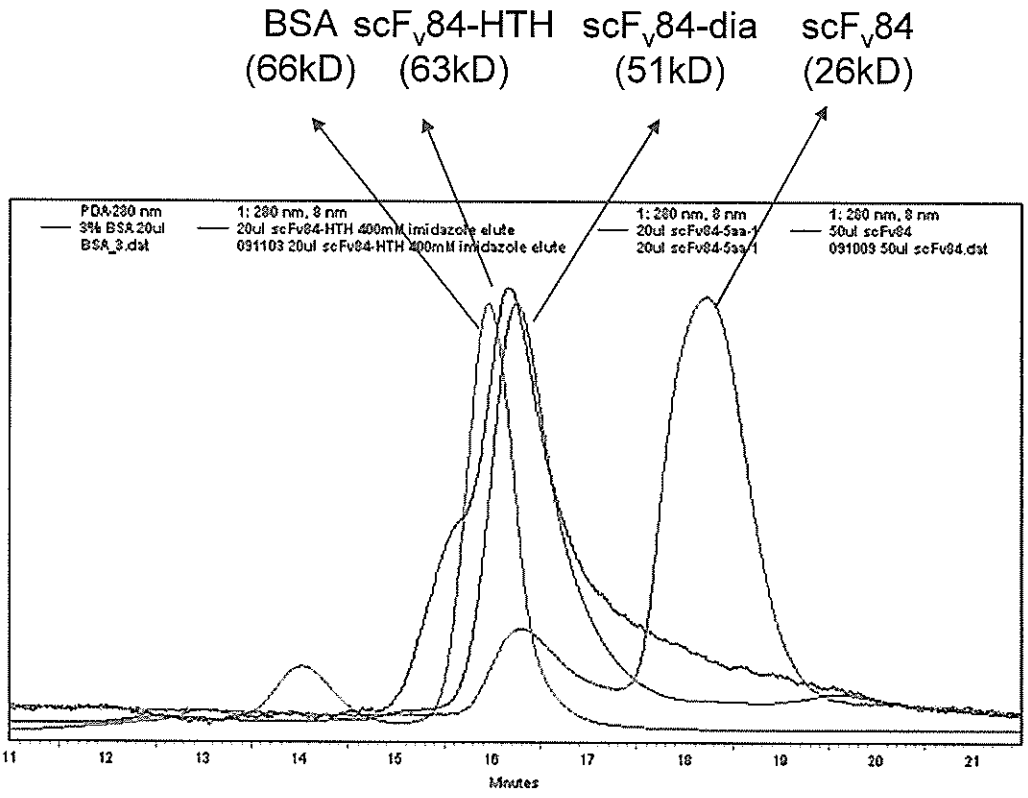

BSA (66kD)
scFv84-HTH (63kD)
scFv84-dia (51kD)
scFv84 (26kD)
PDA-280 nm
3% BSA 20ul
BSA_3.dat
1: 280 nm, 8 nm
20ul scFv84-HTH 400mM imidazole elute
091103 20ul scFv84-HTH 400mM imidazole elute
1: 280 nm, 8 nm
20ul scFv84-5aa-1
20ul scFv84-5aa-1
1: 280 nm, 8 nm
50ul scFv84
091009 50ul scFv84.dat
11
12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

Figure 5

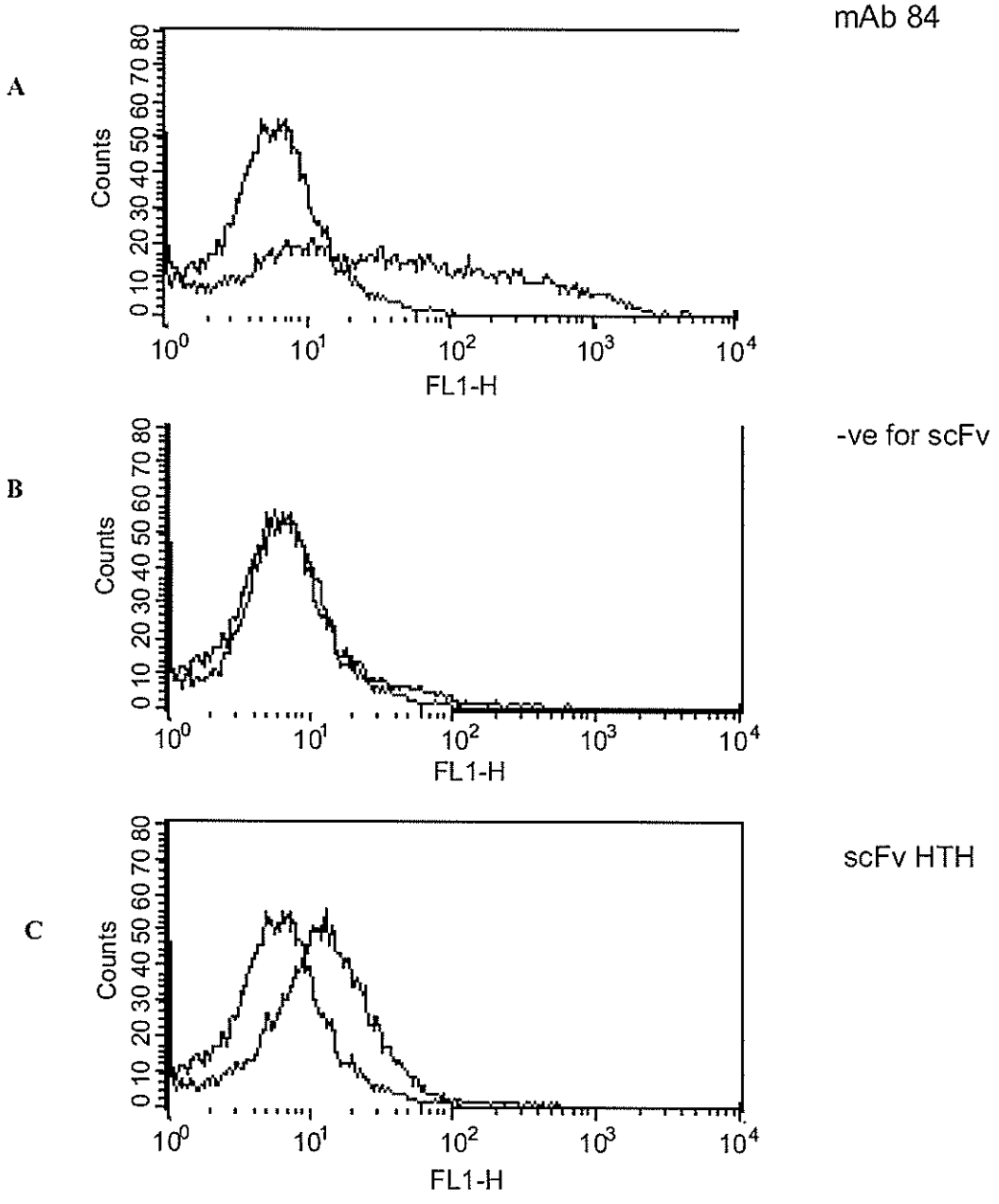

A
mAb 84
Counts
0 10 20 30 40 50 60 70 80
10^0 10^1 10^2 10^3 10^4
FL1-H
B
-ve for scFv
Counts
0 10 20 30 40 50 60 70 80
10^0 10^1 10^2 10^3 10^4
FL1-H
C
scFv HTH
Counts
0 10 20 30 40 50 60 70 80
10^0 10^1 10^2 10^3 10^4
FL1-H

Figure 6

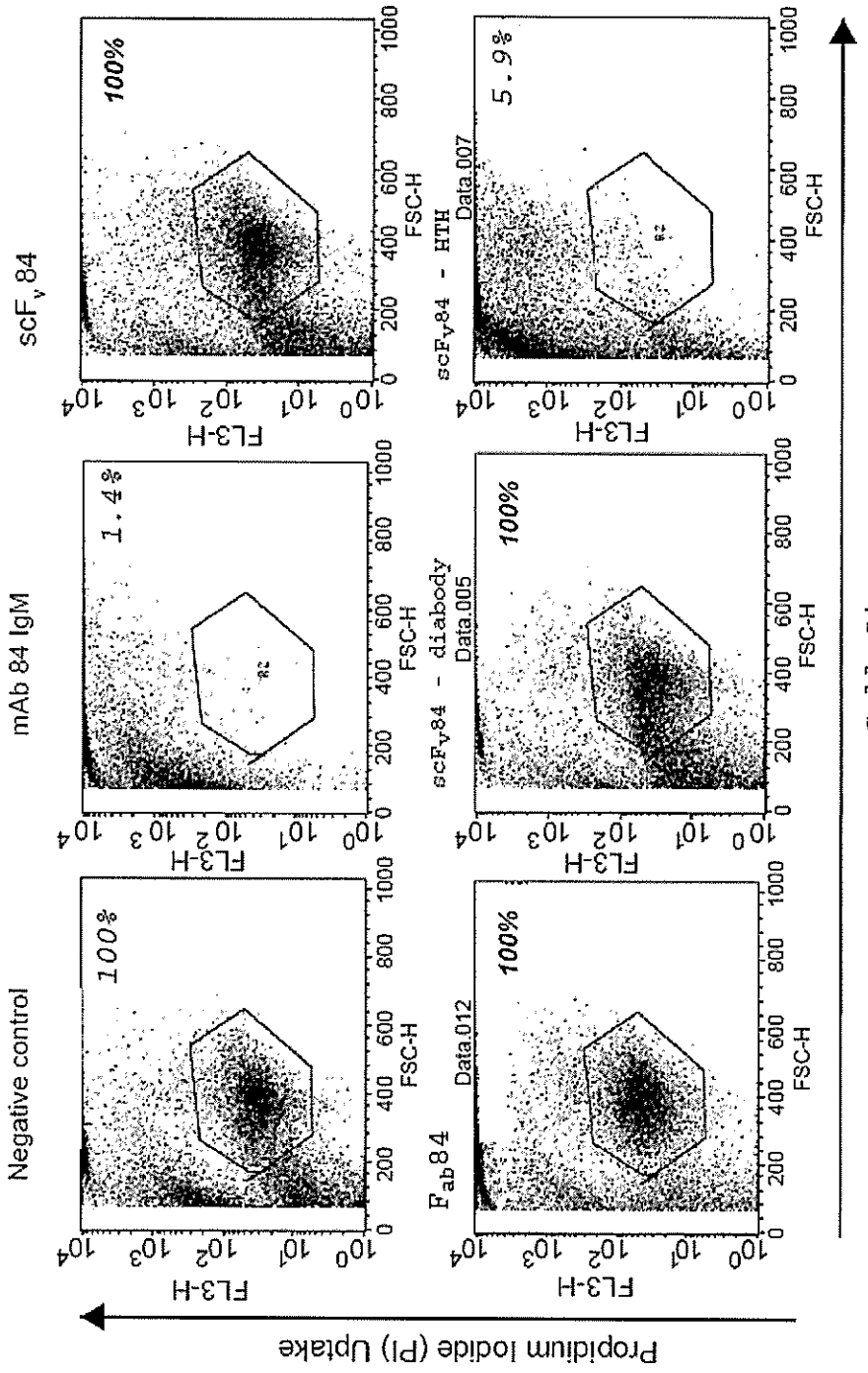

Negative control
mAb 84 IgM
scF$_v$84
Fab84
scFv84 - diabody
scFv84 - HTH
Data.012
Data.005
Data.007
100%
1.4%
5.9%
R2
FL3-H
FSC-H
Propidium Iodide (PI) Uptake
Cell Size

Figure 7a

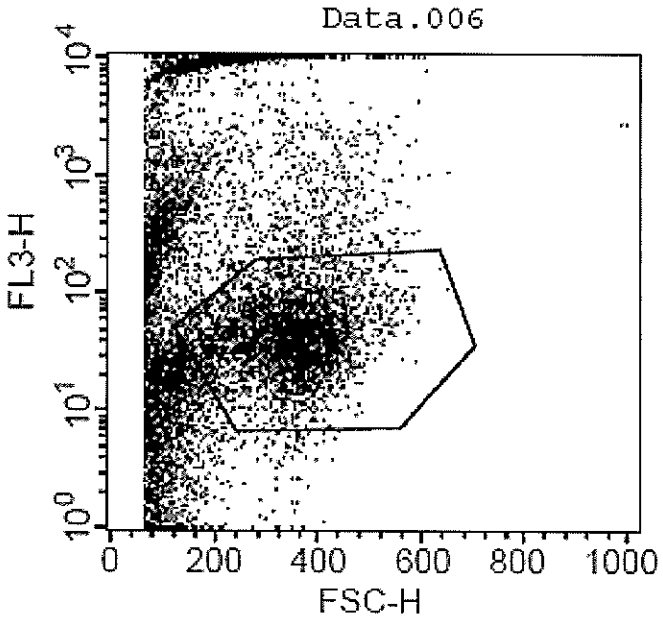


Figure 7a cont...

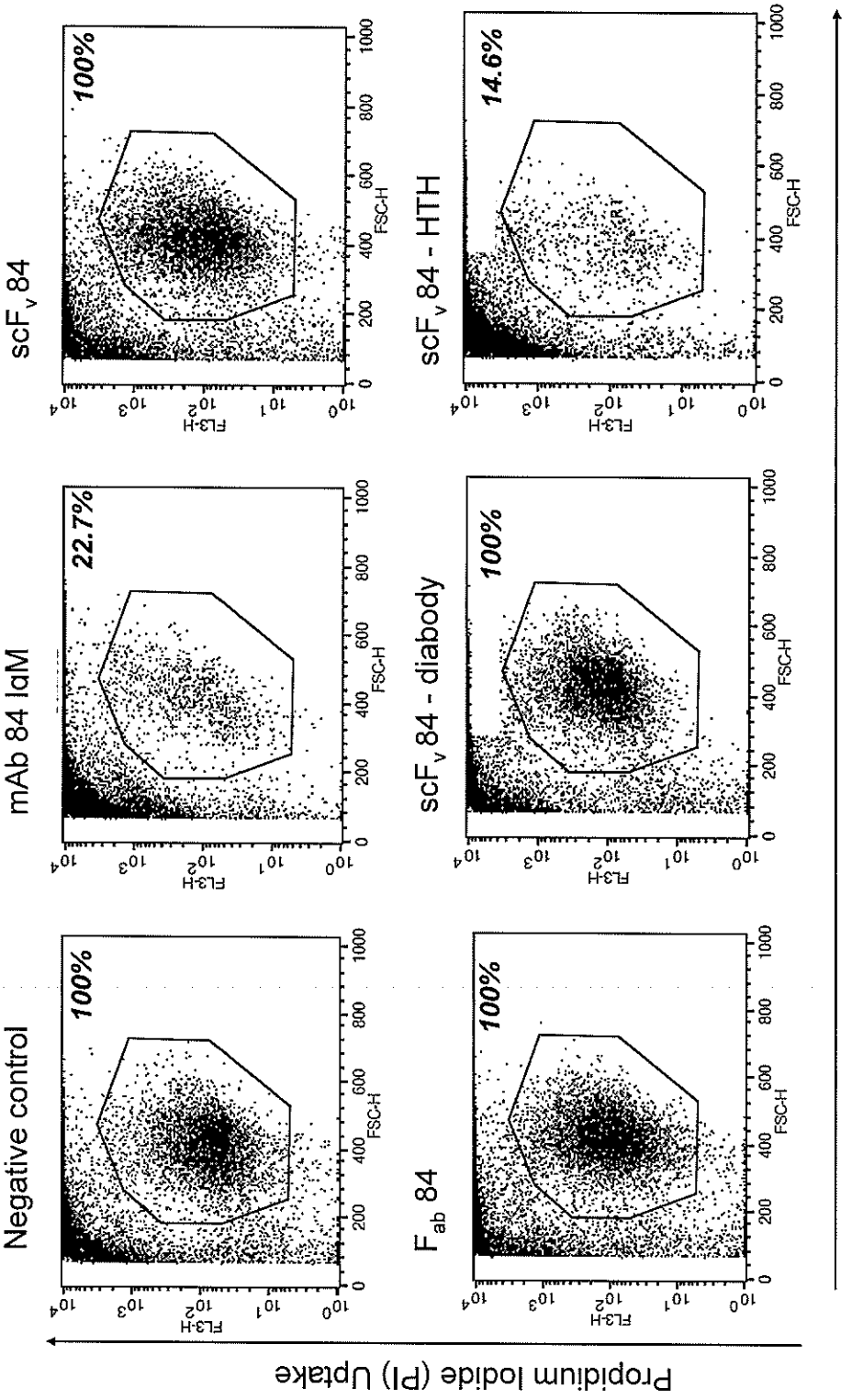

Negative control
mAb 84 IgM
scF$_v$84
F$_{ab}$ 84
scF$_v$84 - diabody
scF$_v$84 - HTH
100%
22.7%
14.6%
R1
FL3-H
FSC-H
Propidium Iodide (PI) Uptake
Cell Size

Figure 7b

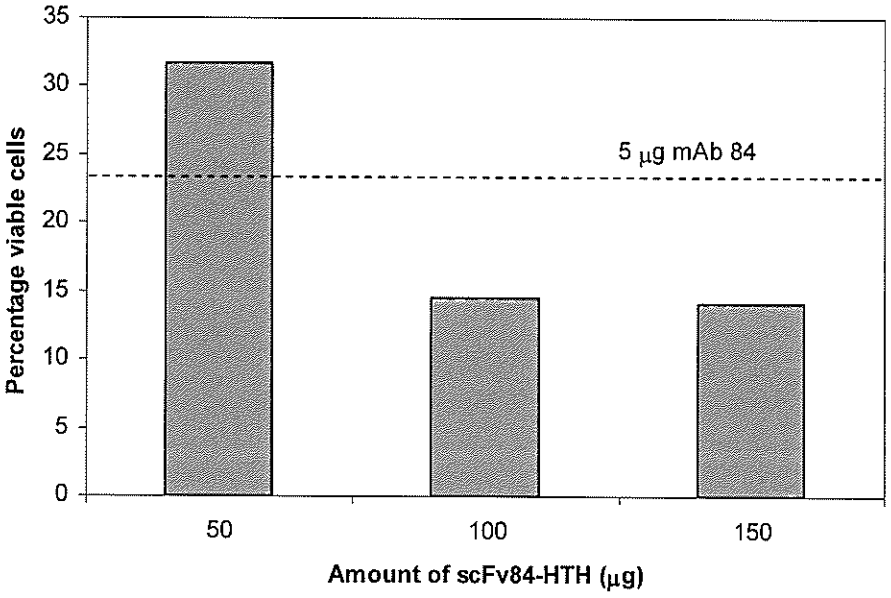

35
30
25
20
15
10
5
0
Percentage viable cells
5 μg mAb 84
50
100
150
Amount of scFv84-HTH (μg)

Figure 7c

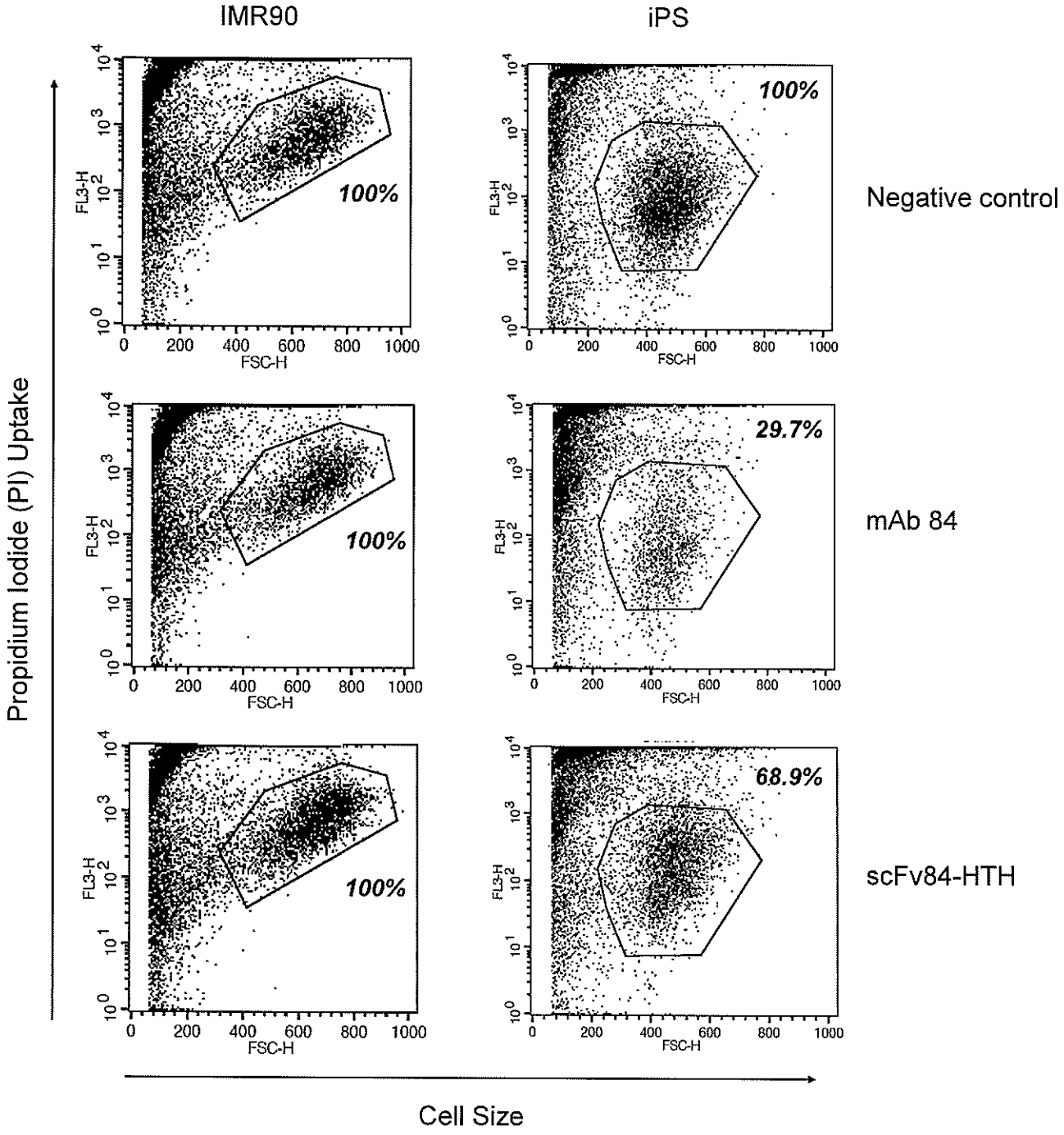

IMR90
iPS
Negative control
mAb 84
scFv84-HTH
100%
100%
29.7%
100%
68.9%
100%
FL3-H
FSC-H
Propidium Iodide (PI) Uptake
Cell Size

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
ggggsggggsgggs
(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

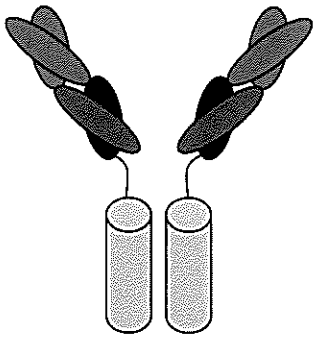


Figure 16

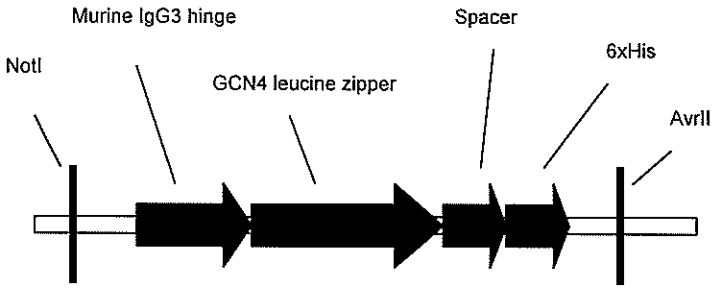


Figure 17

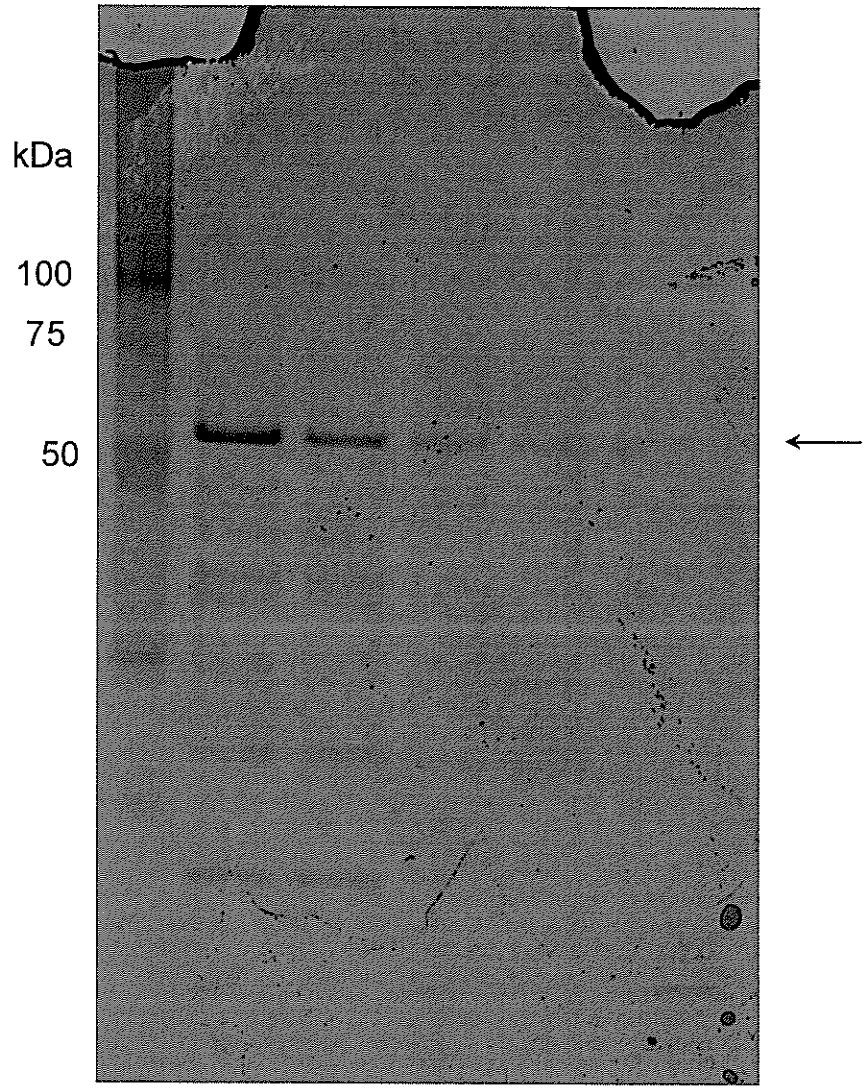
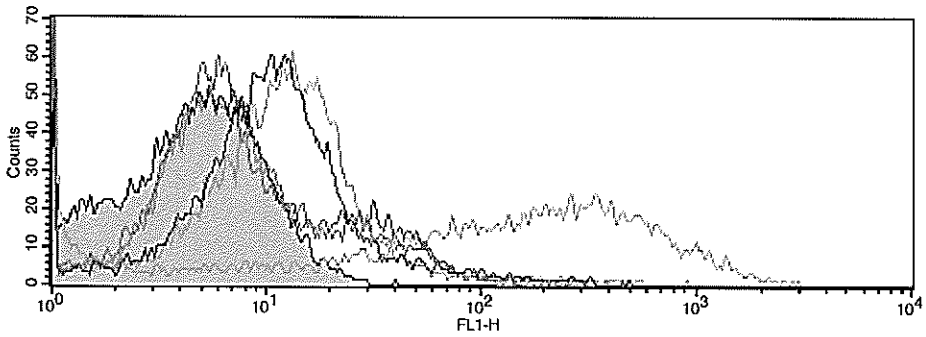


Figure 18



	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

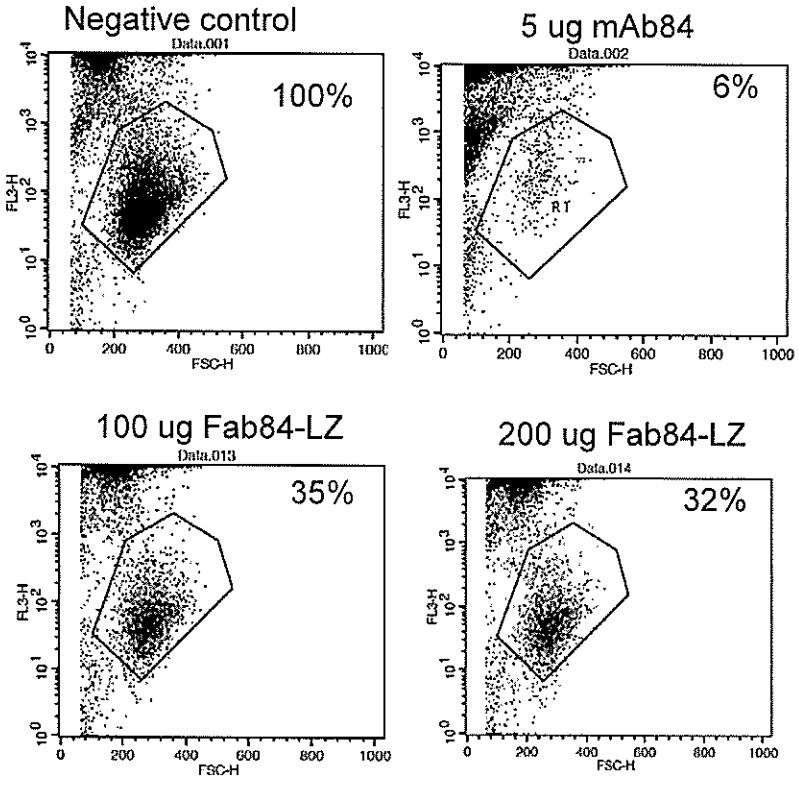

Negative control
Data.001
100%
5 ug mAb84
Data.002
6%
R1
100 ug Fab84-LZ
Data.013
35%
200 ug Fab84-LZ
Data.014
32%
FL3-H
FSC-H

Figure 20