Figures

Fig. 1

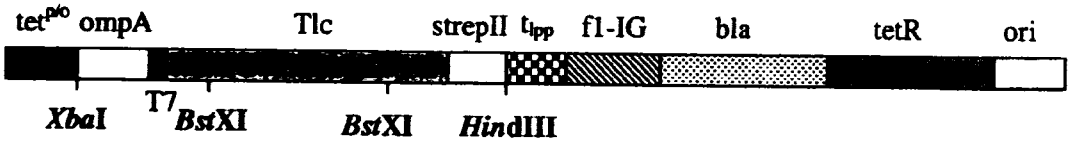


Fig. 2

MKKTAIAIAV ALAGFATVAQ *ADASMTGGQQ MG*ASDEEIQD VSGTWYLKAM
TVDSRCPRAY YGSVTPMTLT TLEGGNLEAK VTMQRIGRSQ EVKAVLEKTD
EPGKYTASGG RHVAYIIRSH VKDHYIFYSE GLCPGQPVPG VWLVGRDPKN
NLEALEDFEK AAGARGLSTE SILIPRQSET SSPG**SAWSHP QFEK**

Fig. 3

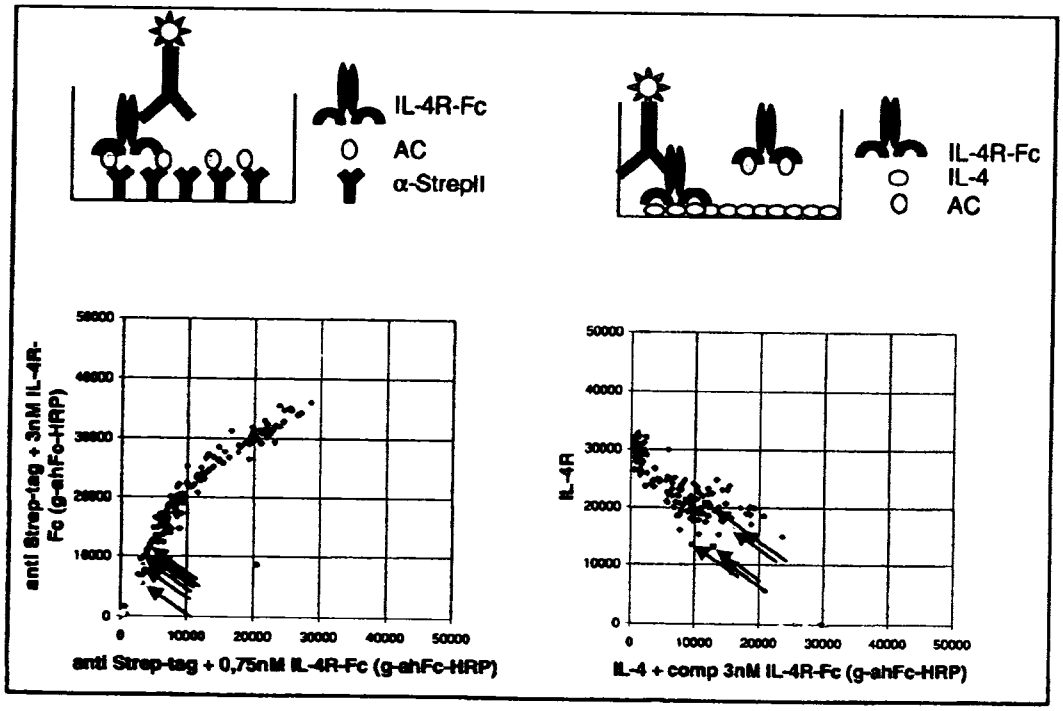


Fig. 4

S191.5 K12:
MKKTAIAIAVALAGFATVAQAASDEEIQDVSGTWYLKAMTVDSRCPRAYYKSVTPMTLTTLEGGNLEAKFTAQ
RNGRWQELKLVLEKTDEPGKYAASGGRHVAYIIRSHVKDHYIFYSEGLCPGQPVPGVWLVGRDPKNNLEALED
FEKAAGARGLSTESILIPRQSETSSPGSAWSHPQFEK

S148.3 J14AM2C2:
MKKTAIAIAVALAGFATVAQAASDEEIQDVSGTWYLKAMTVDSRCPRAYYESVTPMTLTTLEGGNLEAKFTLQ
RRGRWQEGKLVLEKTDEPGKYTASGGRHVAYIIRSHVKDHYIFYSEGLCPGQPVPGVWLVGRDPKNNLEALED
FEKAAGARGLSTESILIPRQSETSSPGSAWSHPQFEK

S191.4 B24:
MKKTAIAIAVALAGFATVAQAASDEEIQDVSGTWYLKAMTVDSRCPRAYYSSVTPMTLTTLEGGNLEAKFTAQ
RSGRWQEYKLVLEKTDEPGKYTASGGRHVAYIIRSHVKDHYIFHSEGLCPGQPVPGVWLVGRDPKNNLEALED
FEKAAGARGLSTESILIPRQSETSSPGSAWSHPQFEK

S191.4 K19:
MKKTAIAIAVALAGFATVAQAASDEEIQDVSGTWYLKAMTVDSRCPRAHYSSVTPMTLTTLEGGNLEAKLTLQ
RAGRWQEGKIVLEKTDEPGKYTASGGRHVAYIIRSHVKDHYIFYSEGLCPGQPVPGVWLVGRDPKNNLEALED
FEKAAGARGLSTESILIPRQSETSSPGSAWSHPQFEK

S191.5 H16:
MKKTAIAIAVALAGFATVAQAASDEEIQDVSGTWYLKAMTVDSRCPRAYYDSVTPMTLTTLEGGNLEAKGTLQ
RKGRPQEMKLVLEKTDEPGKYTASGGRHVAYIIRSHVKDHYIFYSEGLCPGQPVPGVWLVGRDPKNNLEALED
FEKAAGARGLSTESILIPRQSETSSPGSAWSHPQFEK

S197.8 D22:
MKKTAIAIAVALAGFATVAQAASDEEIQDVSGTWYLKAMTVDSRCPRAYYGSVTPMTLTTLEGGNLEAKLTLQ
RSGRWQESKVVLEKTDEPGKYTASGGRHVAYIIRSHVKDHYIFYSEGLCPGQPVPGVWLVGRDPKNNLEALED
FEKAAGARGLSTESILIPRQSETSSPGSAWSHPQFEK

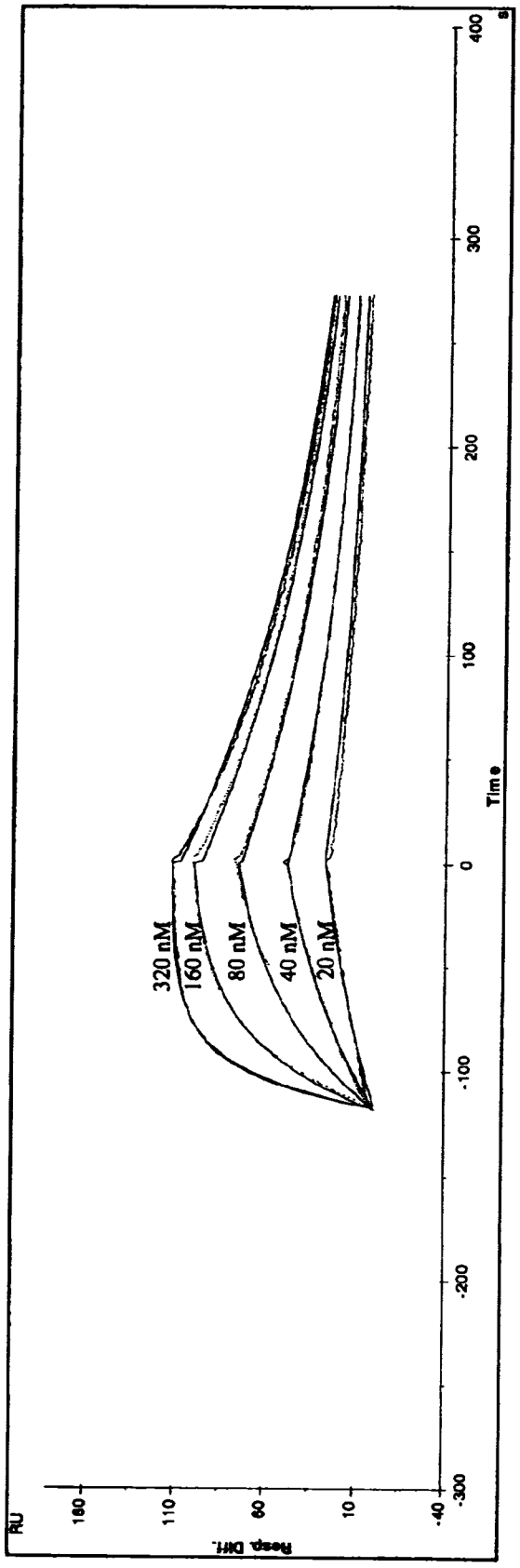

RU
Resp. Diff.
180
110
80
10
-40
-300
-200
-100
0
100
200
300
400
Time
320 nM
160 nM
80 nM
40 nM
20 nM

Fig. 5

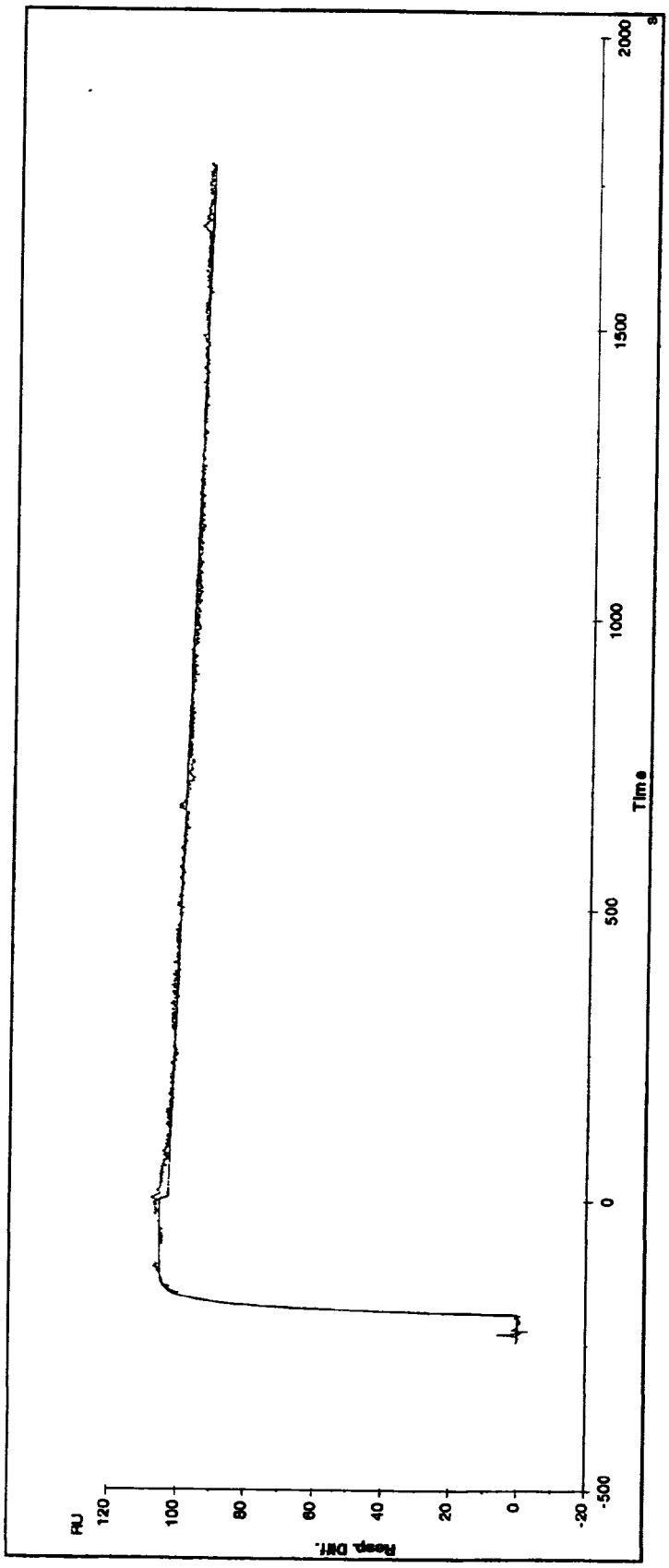

2000
1500
1000
500
0
-500
Time
s
120
100
80
60
40
20
0
-20
RU
Resp. Diff.

Fig. 6

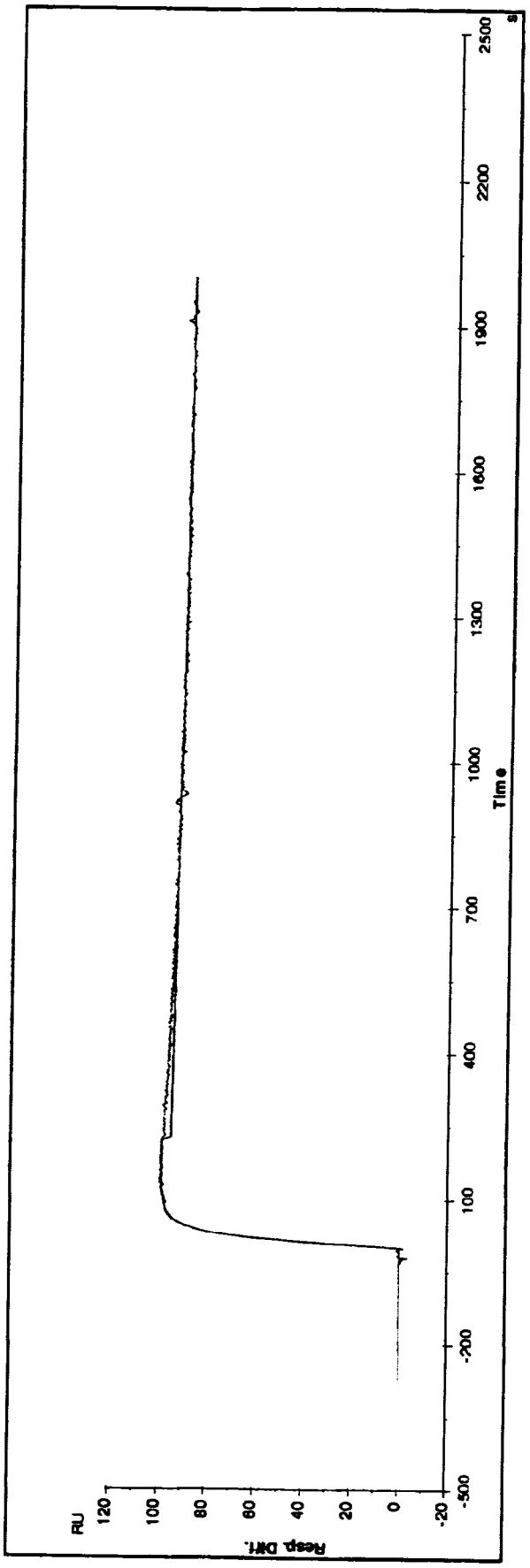

RU
120
100
80
60
40
20
0
-20
Resp. Dif.
-500
-200
100
400
700
1000
1300
1600
1900
2200
2500
Time
s

Fig. 7

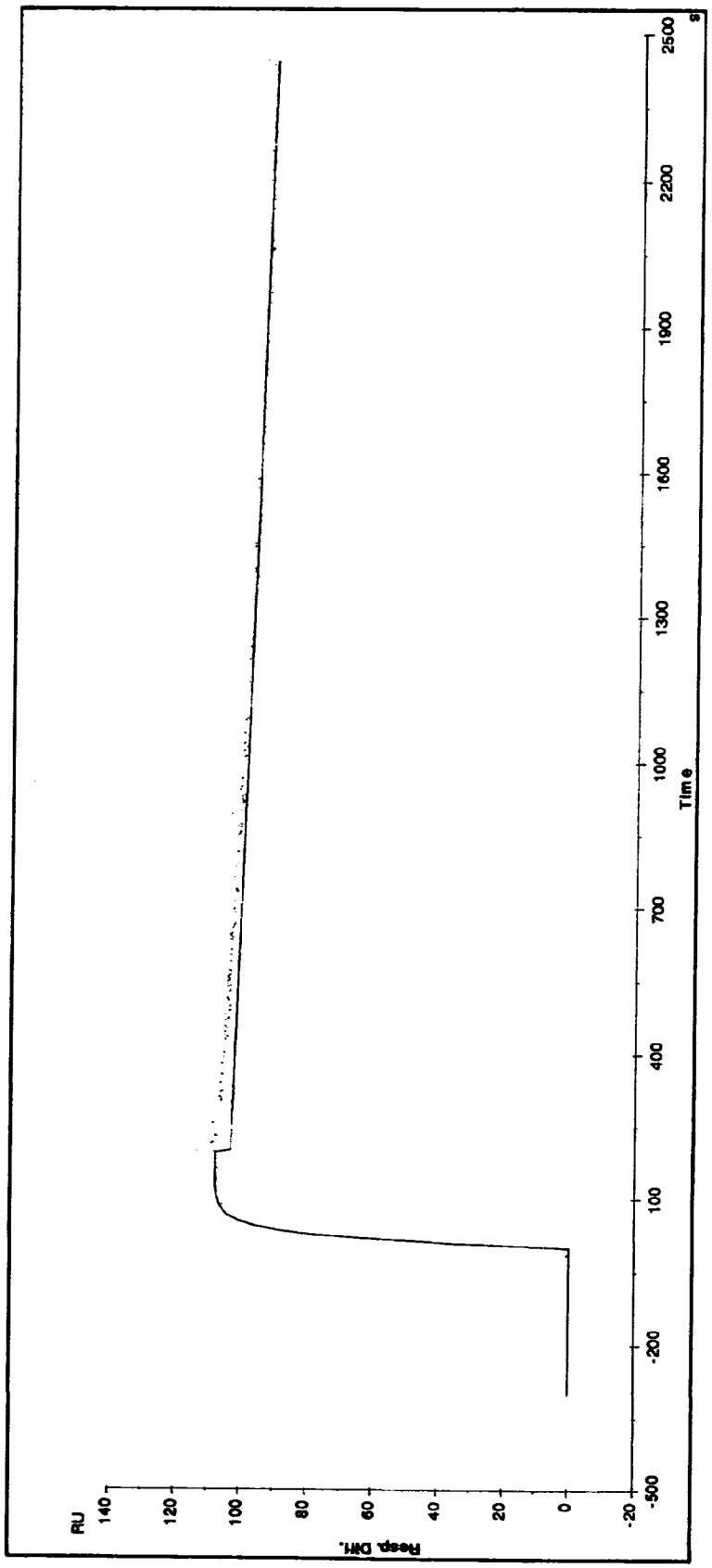

RU
140
120
100
80
60
40
20
0
-20
Resp. Diff.
-500
-200
100
400
700
1000
1300
1600
1900
2200
2500
Time
s

Fig. 8

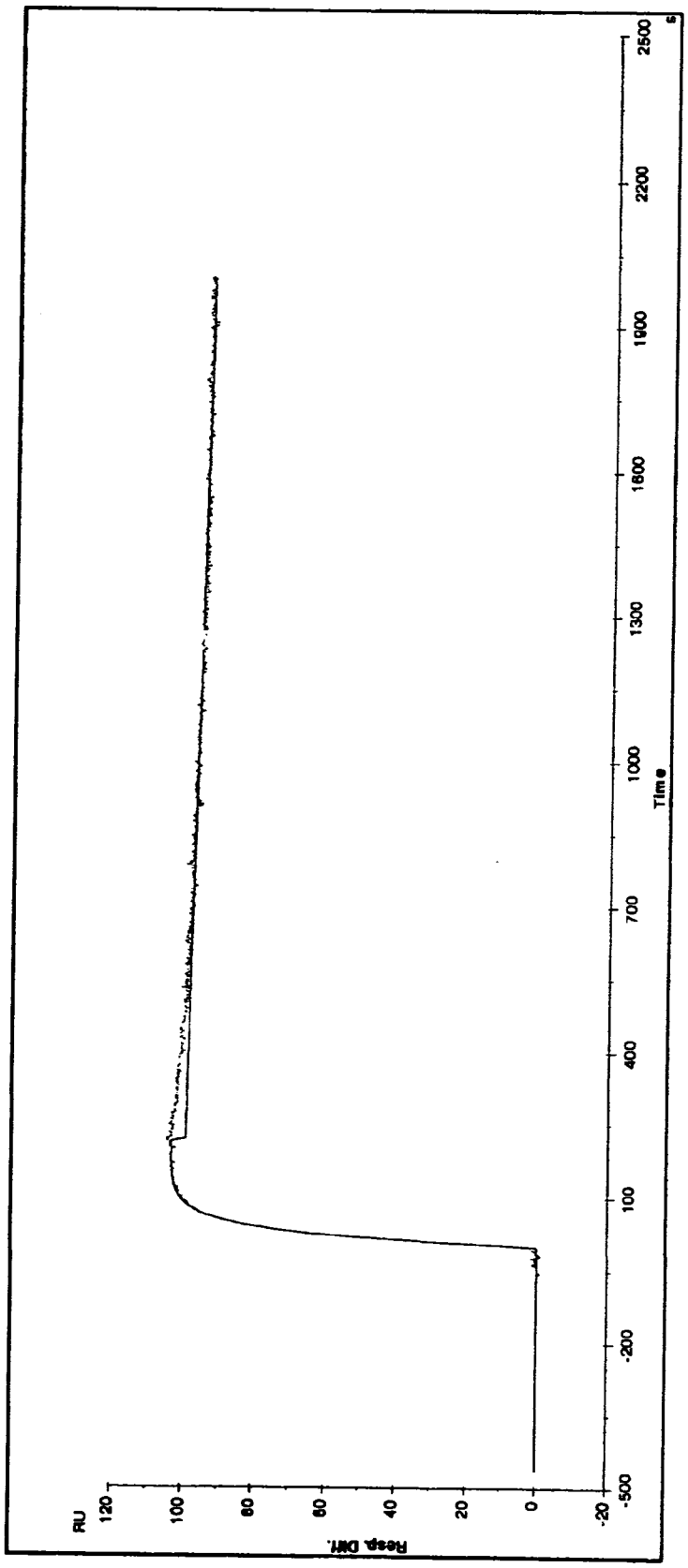

RU
120
100
80
60
40
20
0
-20
Resp. Dif.
-500
-200
100
400
700
1000
1300
1600
1900
2200
2500
Time
s

Fig. 9

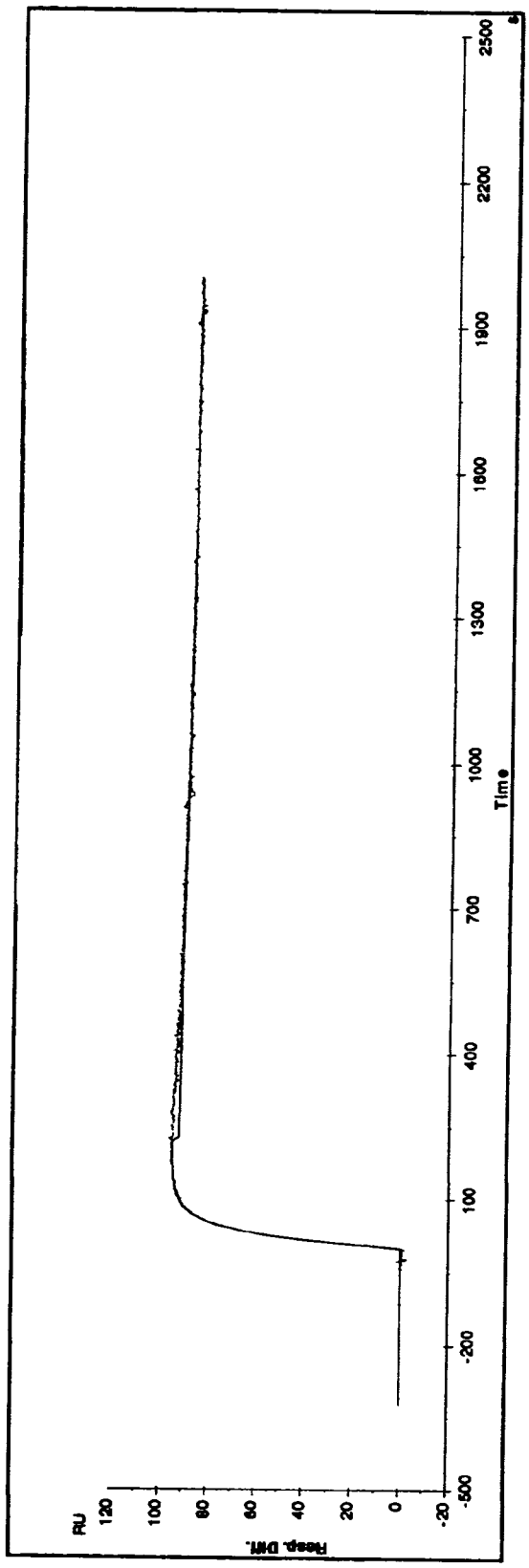

RU
120
100
80
60
40
20
0
-20
Resp. Diff.
-500
-200
100
400
700
1000
1300
1600
1900
2200
2500
Time

Fig. 10

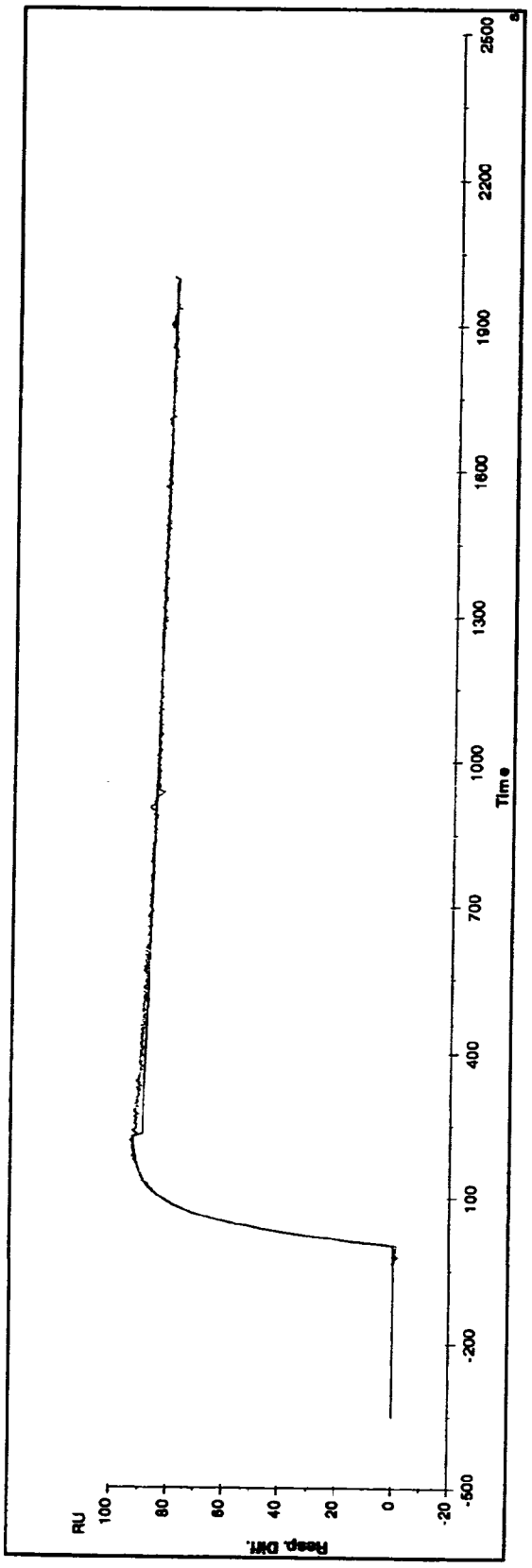

RU
100
80
60
40
20
0
-20
Resp. Dif.
-500
-200
100
400
700
1000
1300
1600
1900
2200
2500
Time

Fig. 11

Fig. 12

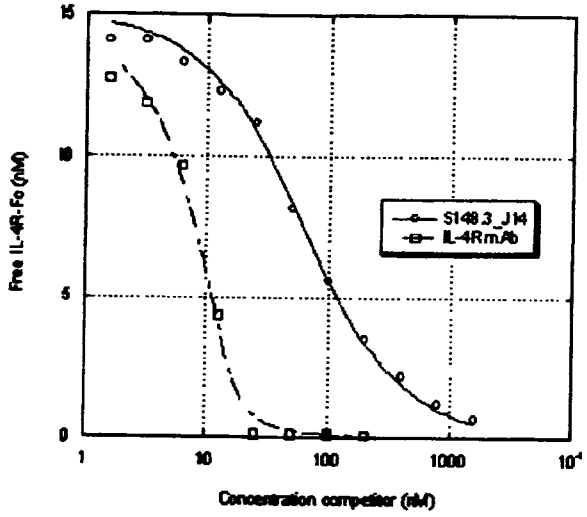
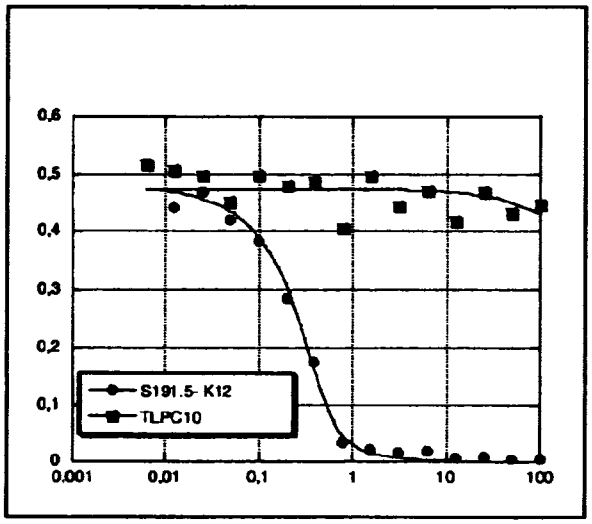
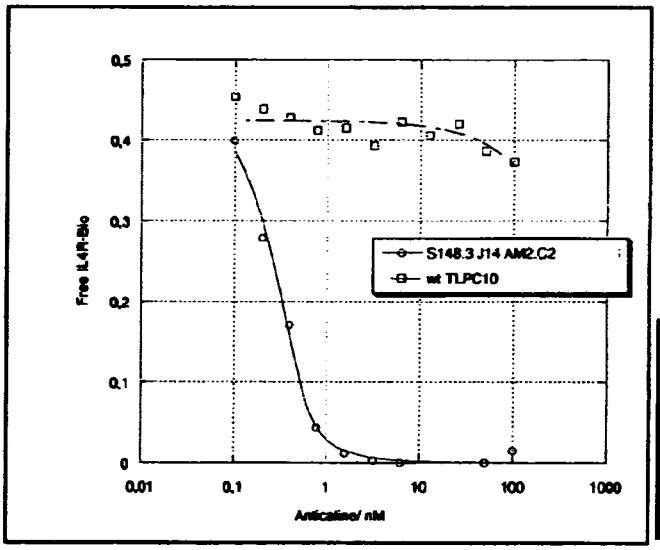

15
10
5
0
1
10
100
1000
S148.3_J14
IL-4RmAb
Free IL-4R-Fc (nM)
Concentration competitor (nM)

Fig. 13



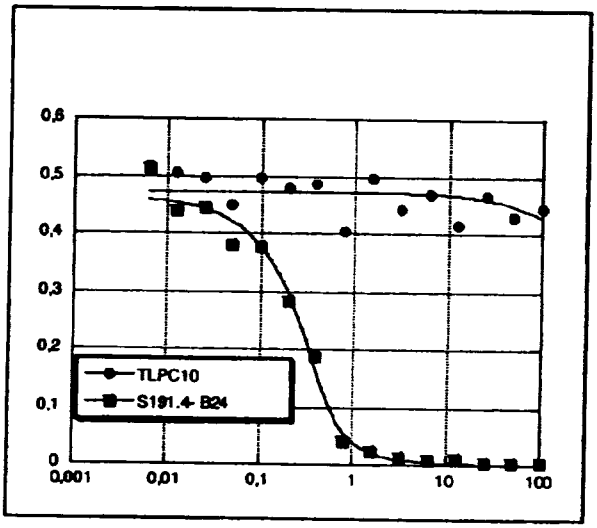
y = 0.5*(-m0+m2-m1+sqrt((-m0...		
	Value	Error
m1	0,03715	0,012294
m2	0,47887	0,0082153
Chisq	0,0045061	NA
R	0,99618	NA

Fig. 14



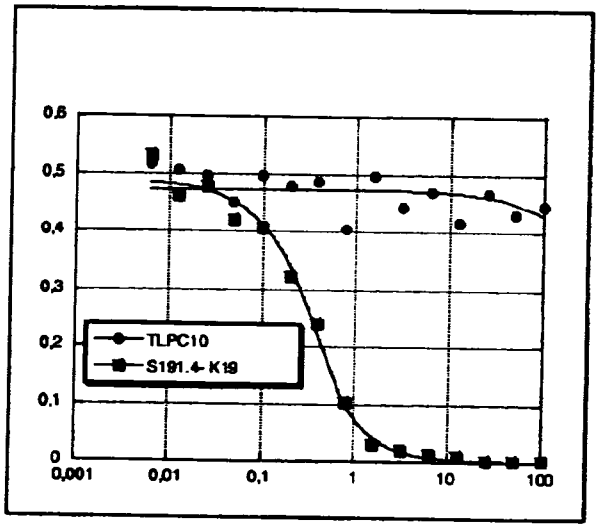
y = 0.5*(-m0+m2-m1+sqrt((-m0...		
	Value	Error
m1	0,037134	0,0082609
m2	0,47752	0,0089865
Chisq	0,00098348	NA
R	0,99744	NA

Fig. 15



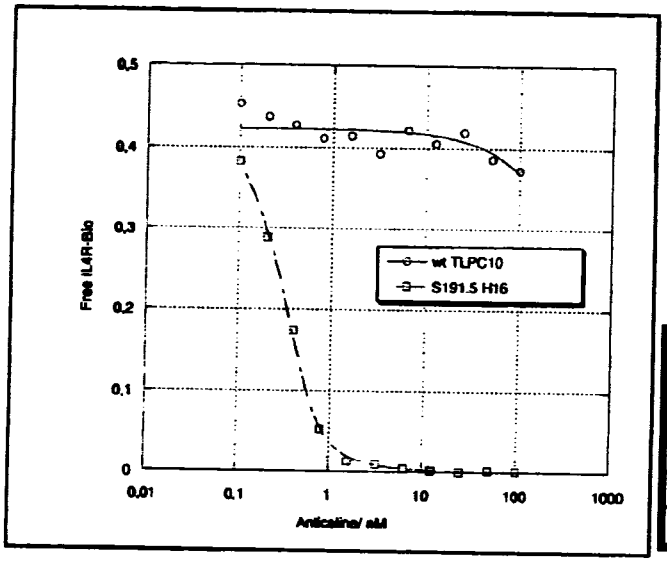
y = 0.5*(-m0+m2-m1+sqrt((-m0...		
	Value	Error
m1	0,052369	0,01679
m2	0,46505	0,0095265
Chisq	0,0058732	NA
R	0,99474	NA

Fig. 16



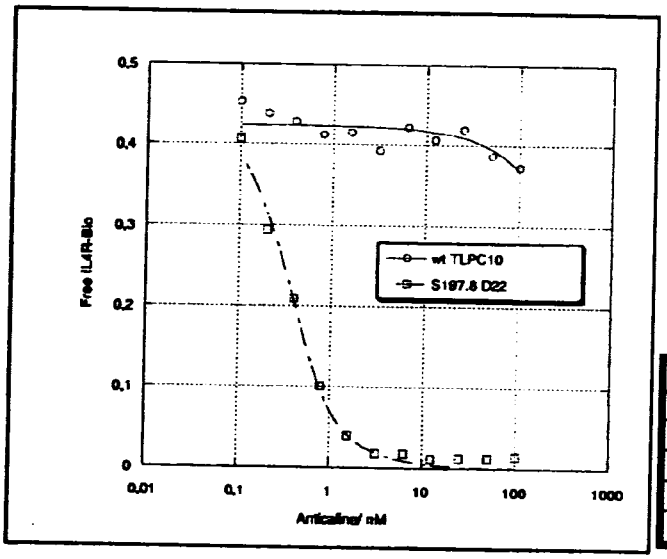
y = 0.5*(-m0+m2-m1+sqrt((-m0...		
	Value	Error
m1	0,10445	0,020528
m2	0,49066	0,0083334
Chisq	0,0043991	NA
R	0,99645	NA

Fig. 17



y = 0.5*(-m0+m2-m1+sqrt((-m0...		
	Value	Error
m1	0,048704	0,0040592
m2	0,46864	0,0039151
Chisq	0,00017517	NA
R	0,99953	NA

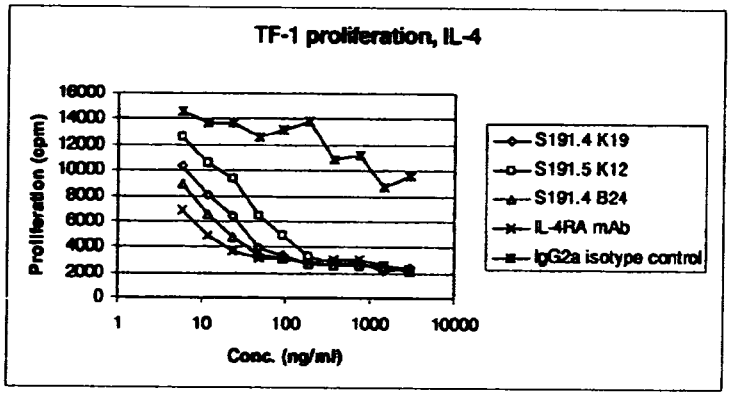
Fig. 18



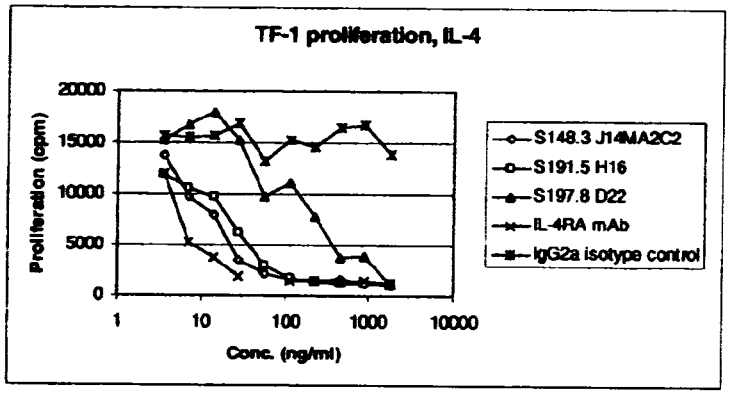
y = 0.5*(-m0+m2-m1+sqrt((-m0...		
	Value	Error
m1	0,10652	0,018248
m2	0,46605	0,011723
Chisq	0,001392	NA
R	0,99636	NA

Fig. 19

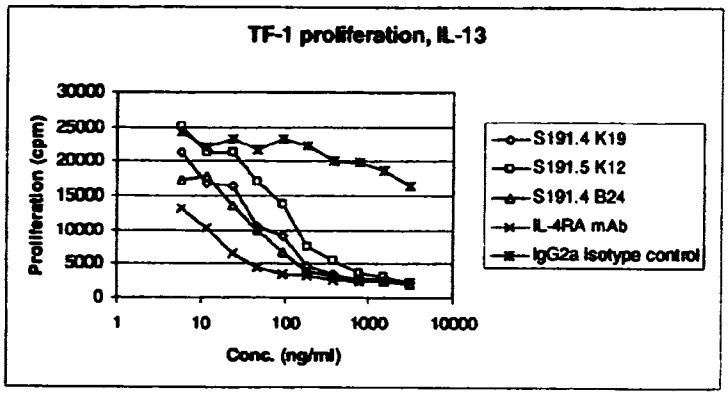
a)


TF-1 proliferation, IL-4
Proliferation (cpm)
16000
14000
12000
10000
8000
6000
4000
2000
0
1
10
100
1000
10000
Conc. (ng/ml)
S191.4 K19
S191.5 K12
S191.4 B24
IL-4RA mAb
IgG2a isotype control

b)


TF-1 proliferation, IL-4
Proliferation (cpm)
20000
15000
10000
5000
0
1
10
100
1000
10000
Conc. (ng/ml)
S148.3 J14MA2C2
S191.5 H16
S197.8 D22
IL-4RA mAb
IgG2a isotype control

c)


TF-1 proliferation, IL-13
Proliferation (cpm)
30000
25000
20000
15000
10000
5000
0
1
10
100
1000
10000
Conc. (ng/ml)
S191.4 K19
S191.5 K12
S191.4 B24
IL-4RA mAb
IgG2a isotype control

d)

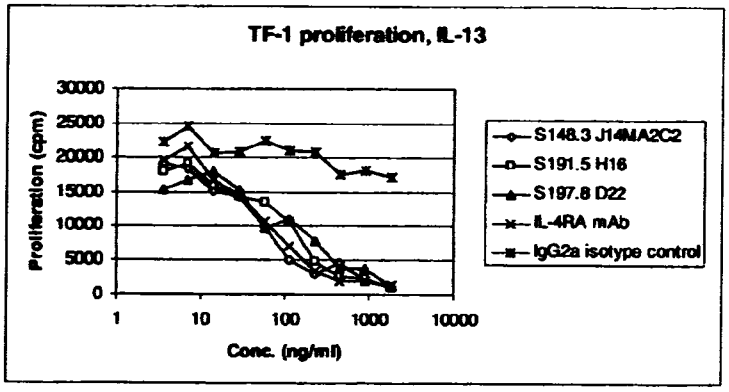

TF-1 proliferation, IL-13
Proliferation (cpm)
30000
25000
20000
15000
10000
5000
0
1
10
100
1000
10000
Conc. (ng/ml)
S148.3 J14MA2C2
S191.5 H16
S197.8 D22
IL-4RA mAb
IgG2a isotype control

Fig. 20

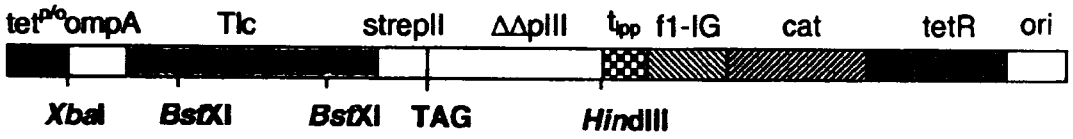

tet^p/o
ompA
Tlc
strepII
ΔΔpIII
t_lpp
f1-IG
cat
tetR
ori
XbaI
BstXI
BstXI
TAG
HindIII

Fig. 21

Fig. 21a)

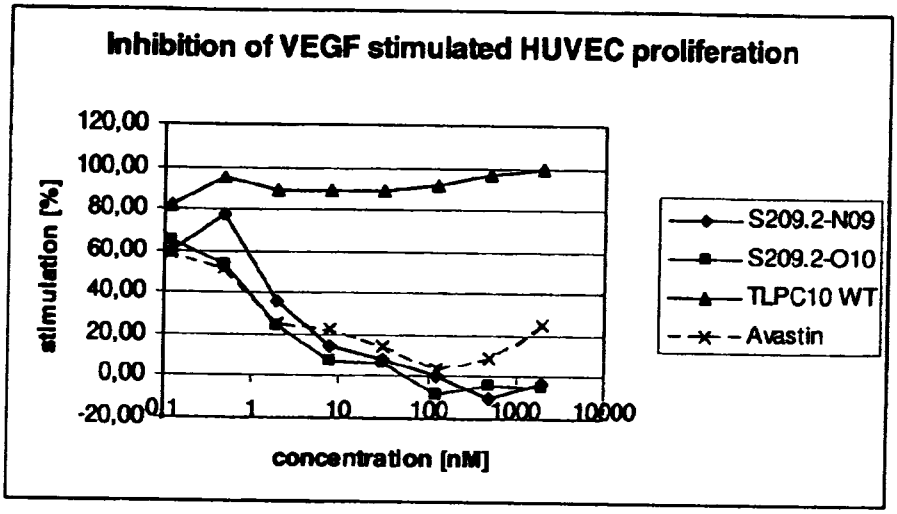


Fig. 21b)

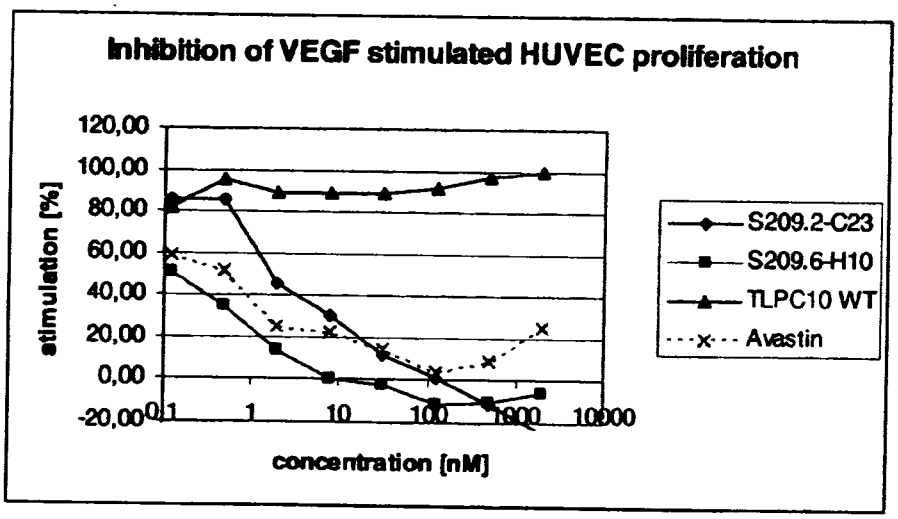


Fig. 21c)

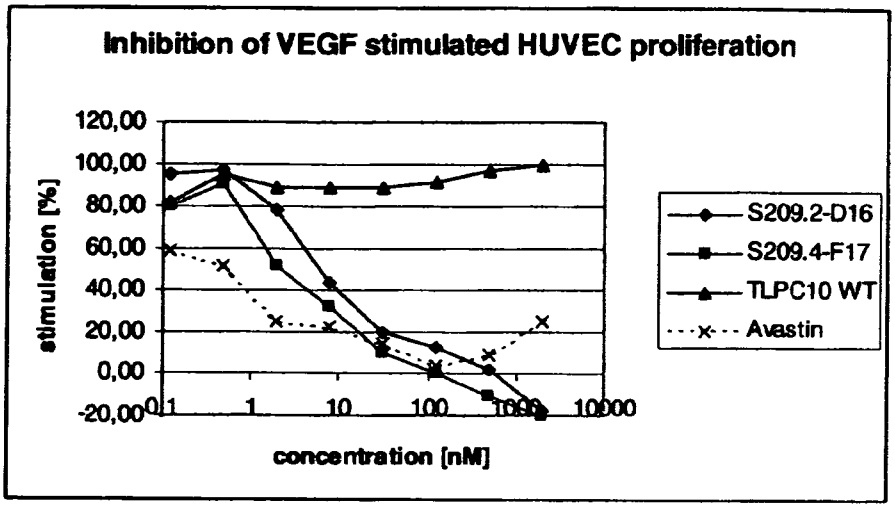


Fig. 21d)

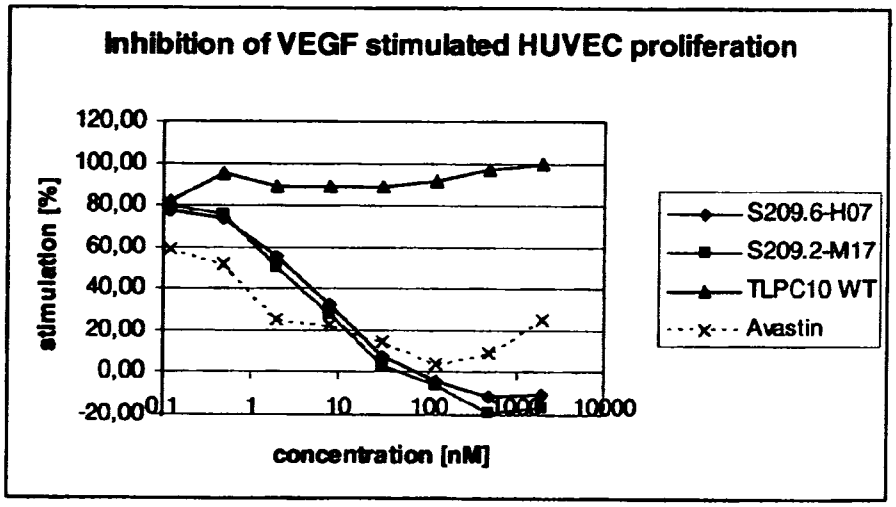


Fig. 22

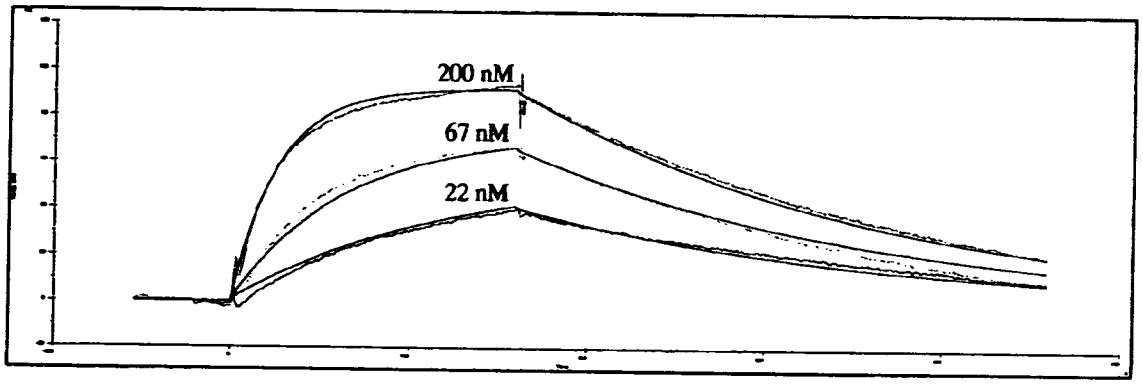

200 nM
67 nM
22 nM

Fig. 23

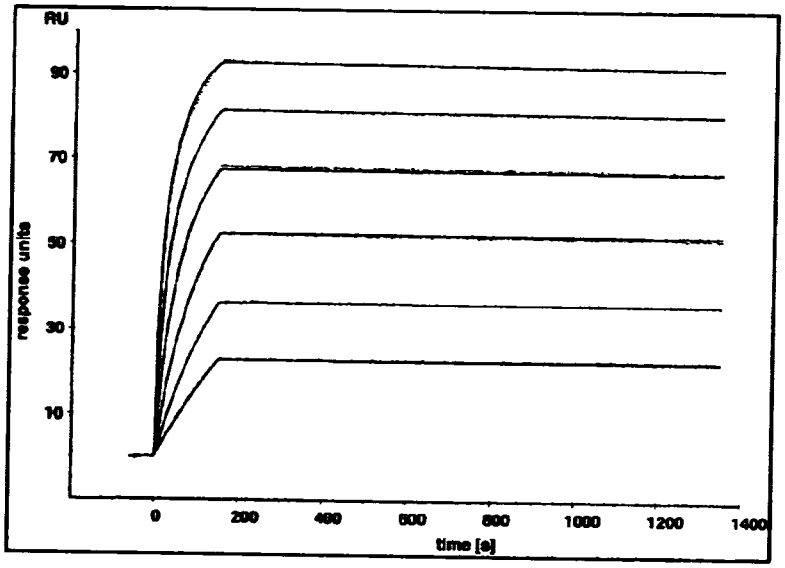

RU
response units
90
70
50
30
10
0
200
400
600
800
1000
1200
1400
time [s]

Fig. 24

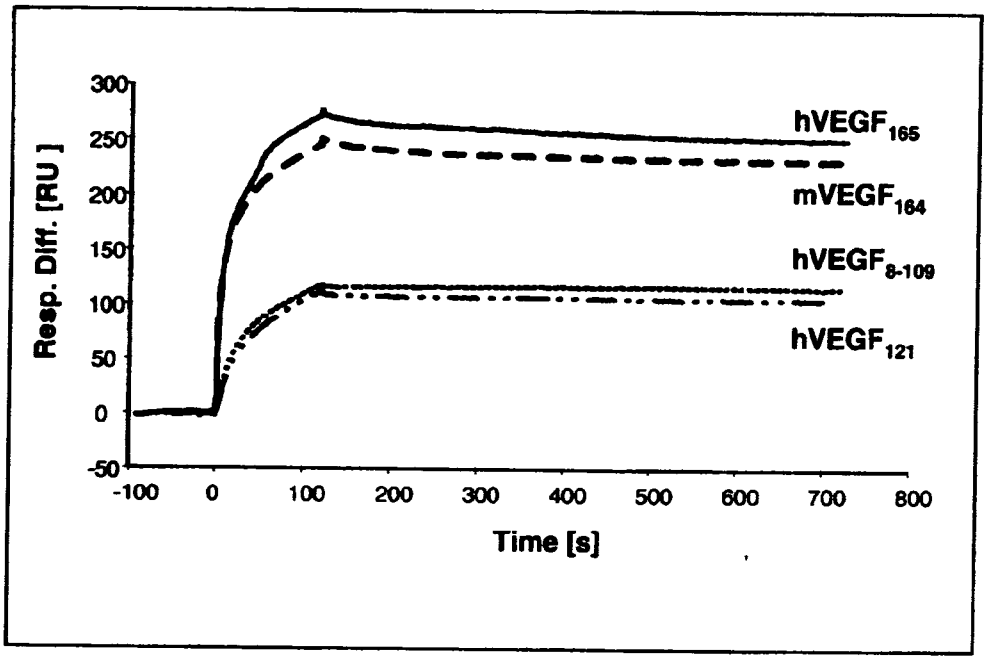
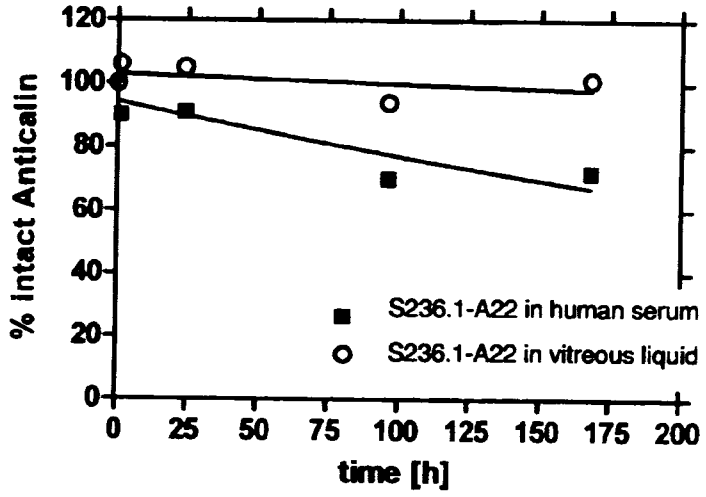

Resp. Diff. [RU]
300
250
200
150
100
50
0
-50
-100
0
100
200
300
400
500
600
700
800
Time [s]
hVEGF165
mVEGF164
hVEGF8-109
hVEGF121

Fig. 25

a)


% Intact Anticalin
120
100
80
60
40
20
0
0 25 50 75 100 125 150 175 200
time [h]
S236.1-A22 in human serum
S236.1-A22 in vitreous liquid

b)

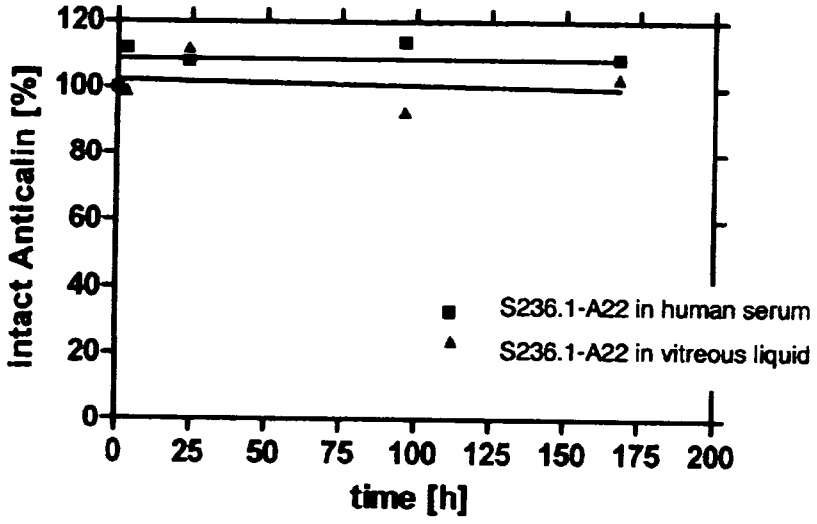

Intact Anticalin [%]
120
100
80
60
40
20
0
0 25 50 75 100 125 150 175 200
time [h]
S236.1-A22 in human serum
S236.1-A22 in vitreous liquid

Fig. 26

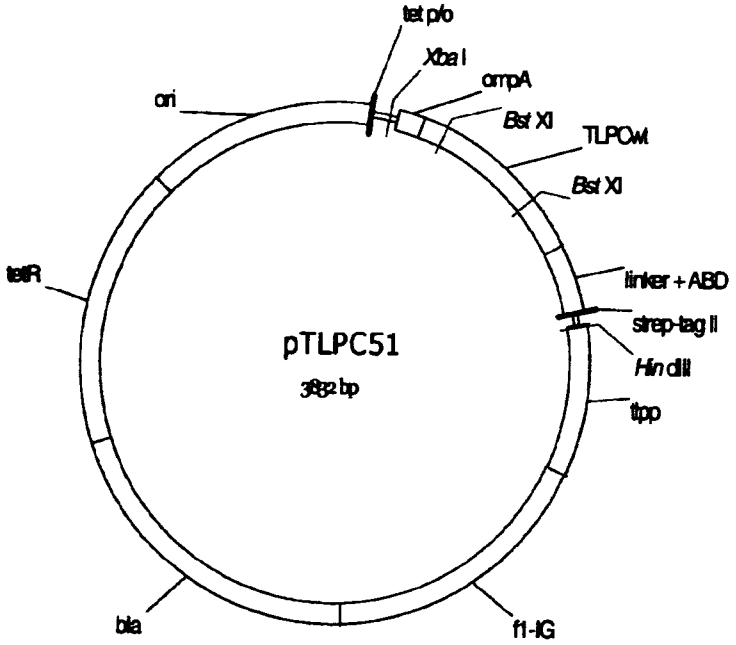
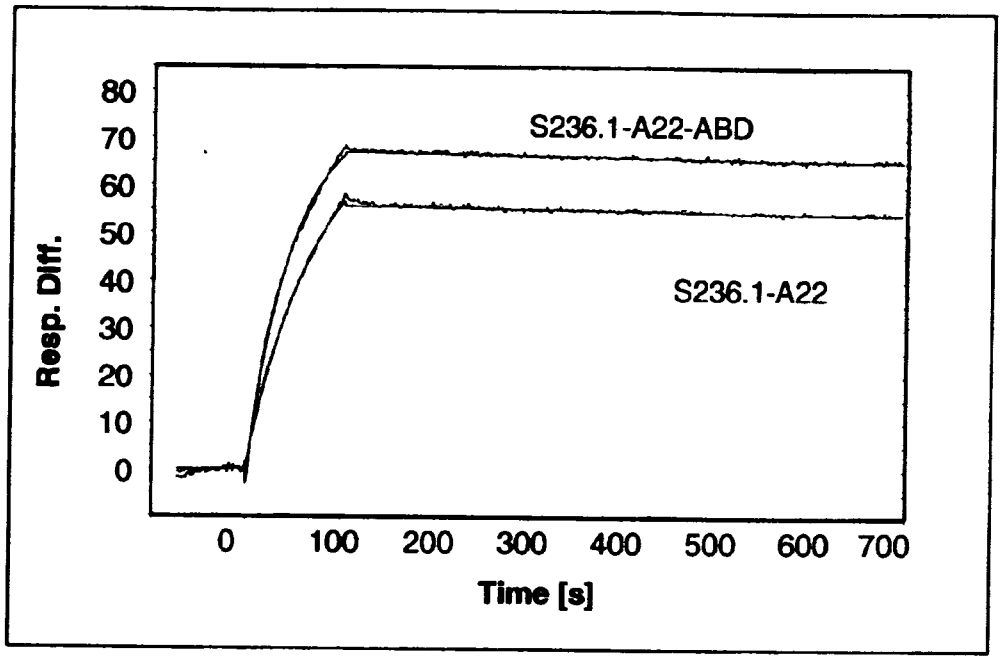

tet p/o
Xba I
ompA
Bst XI
TLPCwt
Bst XI
linker + ABD
strep-tag II
Hin dIII
ttpp
f1-IG
bla
tetR
ori
pTLPC51
3982 bp

Fig. 27



Biacore-measurements

S236.1-A22 (tear lipocalin mutein)	KD: 200pM
S236.1-A22-ABD (tear lipocalin mutein-ABD)	KD: 260pM

Fig. 28

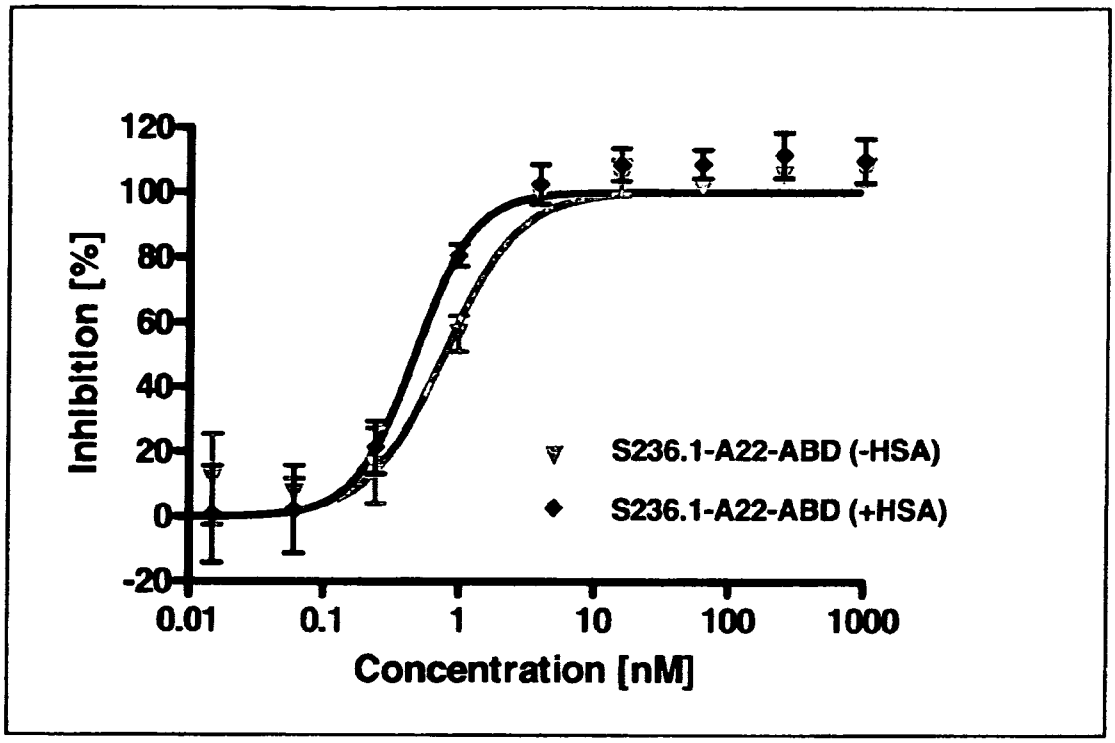
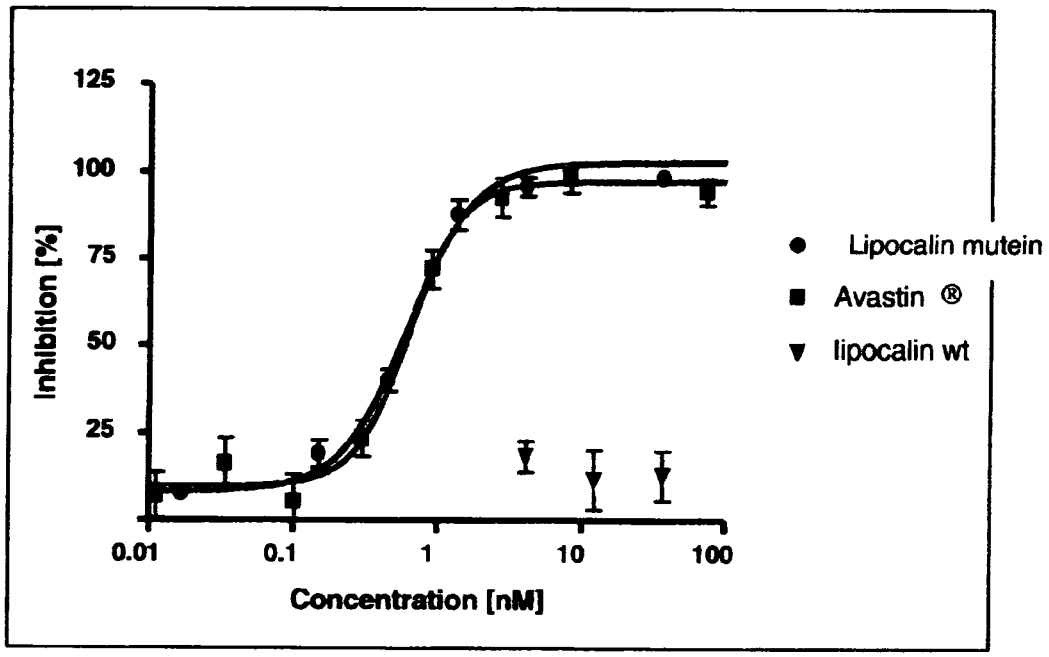
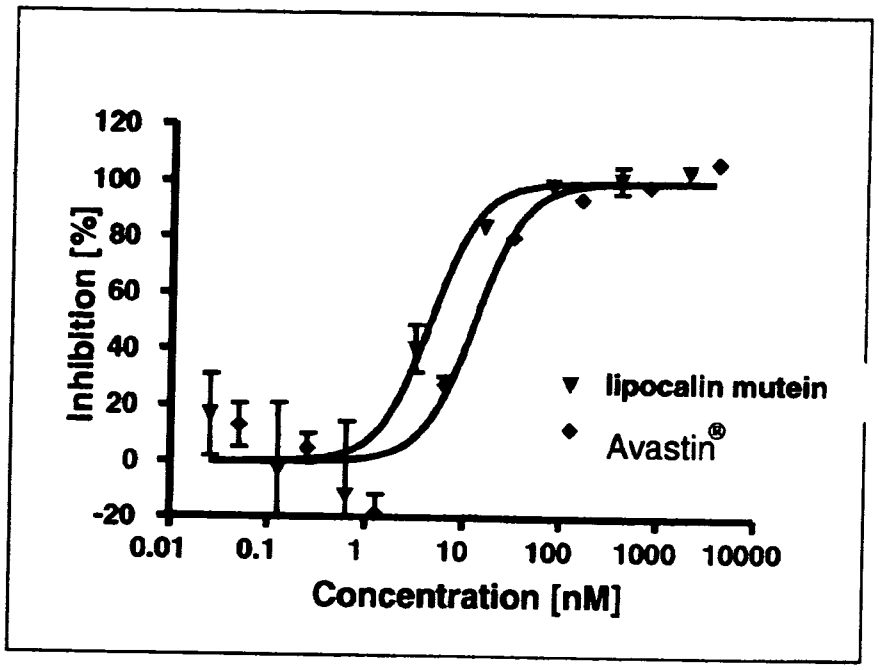

120
100
80
60
40
20
0
-20
Inhibition [%]
0.01
0.1
1
10
100
1000
Concentration [nM]
S236.1-A22-ABD (-HSA)
S236.1-A22-ABD (+HSA)

Fig. 29



Compound	**IC50** [nM]
S236.1-A22 (lipocalin mutein)	0.51
Avastin	0.56

Fig. 30



Compound	**IC50** [nM]
S236.1-A22 (lipocalin mutein)	4.5
Avastin	13

Fig. 31

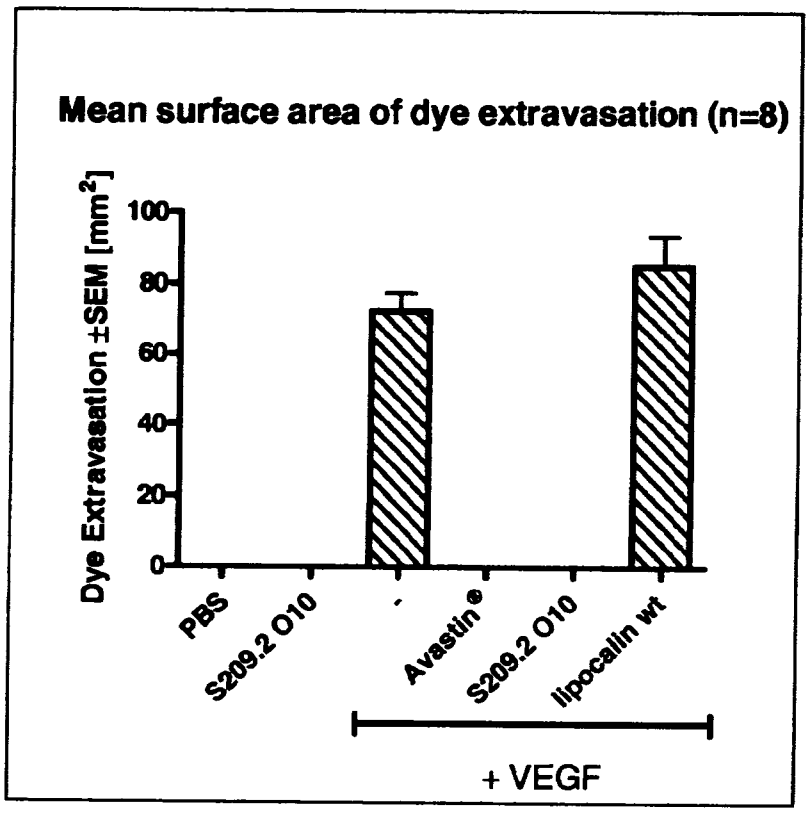

Mean surface area of dye extravasation (n=8)
Dye Extravasation ±SEM [mm²]
100
80
60
40
20
0
PBS
S209.2 O10
-
Avastin®
S209.2 O10
lipocalin wt
+ VEGF

Fig. 32

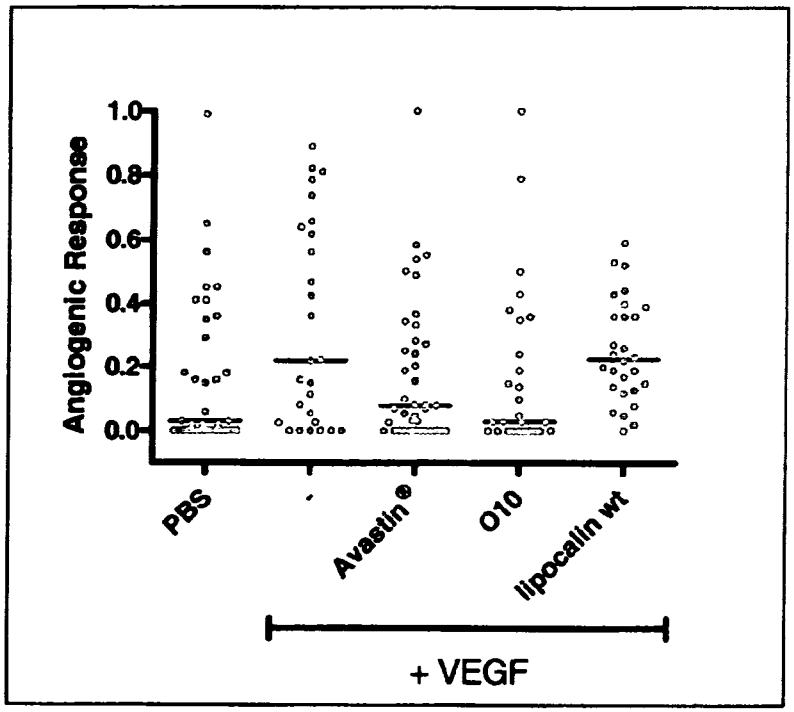

Angiogenic Response
1.0
0.8
0.6
0.4
0.2
0.0
PBS
Avastin®
O10
lipocalin wt
+ VEGF

Fig. 33

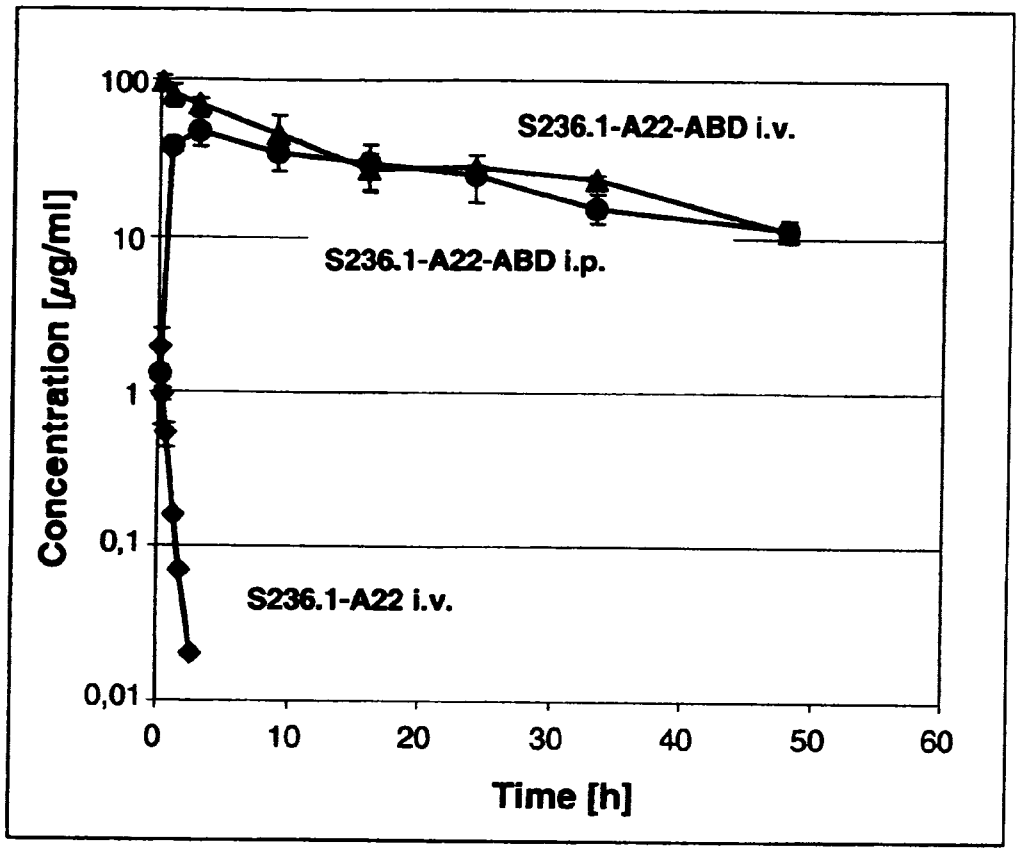

100
10
1
0,1
0,01
Concentration [µg/ml]
S236.1-A22-ABD i.v.
S236.1-A22-ABD i.p.
S236.1-A22 i.v.
0
10
20
30
40
50
60
Time [h]

Fig. 34

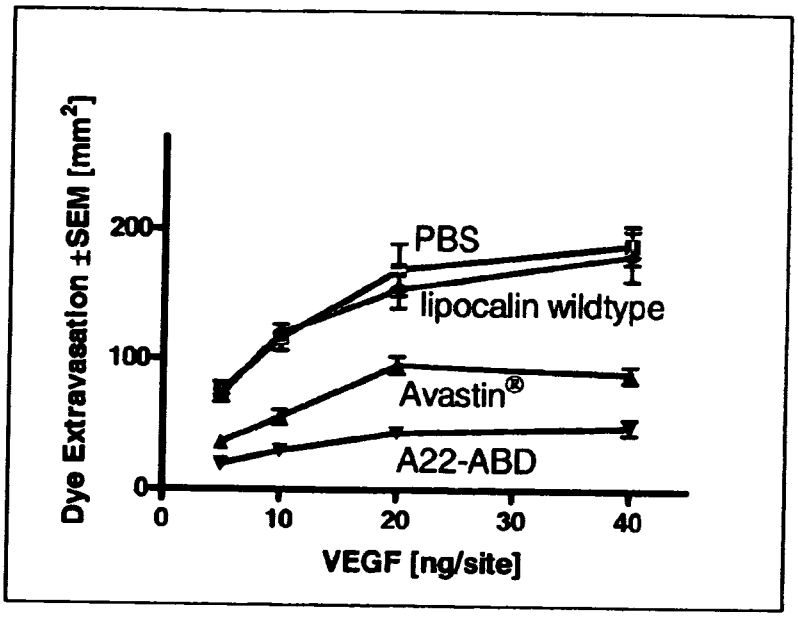

Dye Extravasation ±SEM [mm²]
200
100
0
0
10
20
30
40
VEGF [ng/site]
PBS
lipocalin wildtype
Avastin®
A22-ABD

Fig. 35

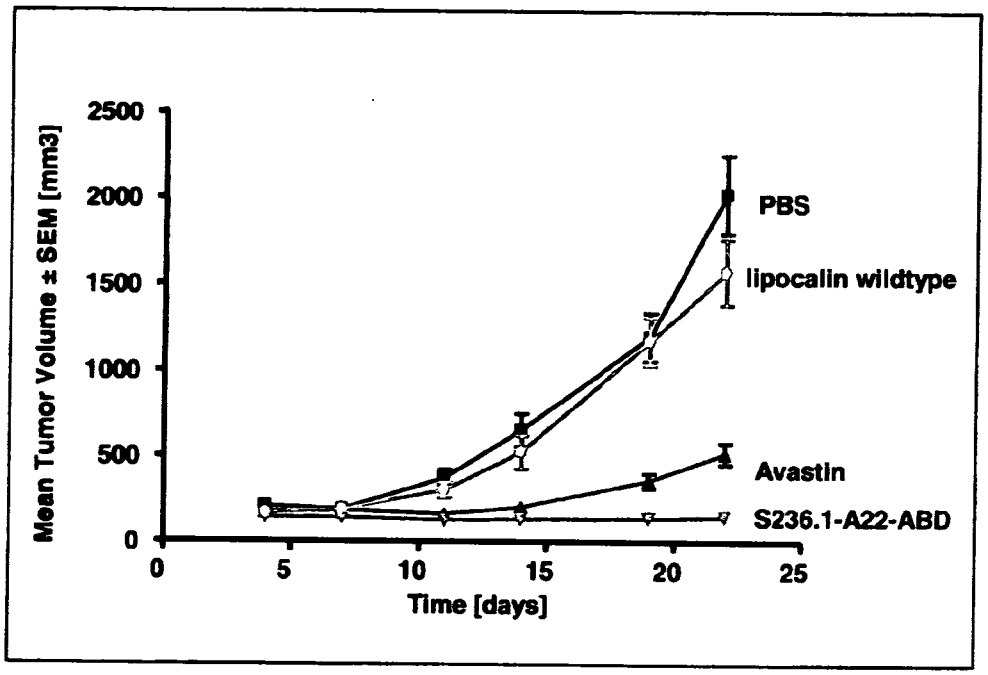

Mean Tumor Volume ± SEM [mm3]
2500
2000
1500
1000
500
0
0
5
10
15
20
25
Time [days]
PBS
lipocalin wildtype
Avastin
S236.1-A22-ABD

Fig. 36

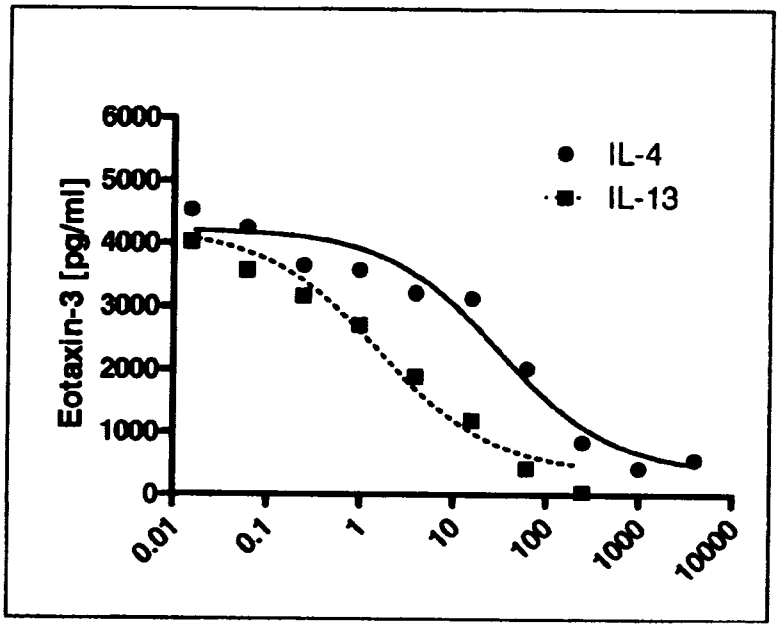

Eotaxin-3 [pg/ml]
6000
5000
4000
3000
2000
1000
0
0.01
0.1
1
10
100
1000
10000
IL-4
IL-13

Fig. 37

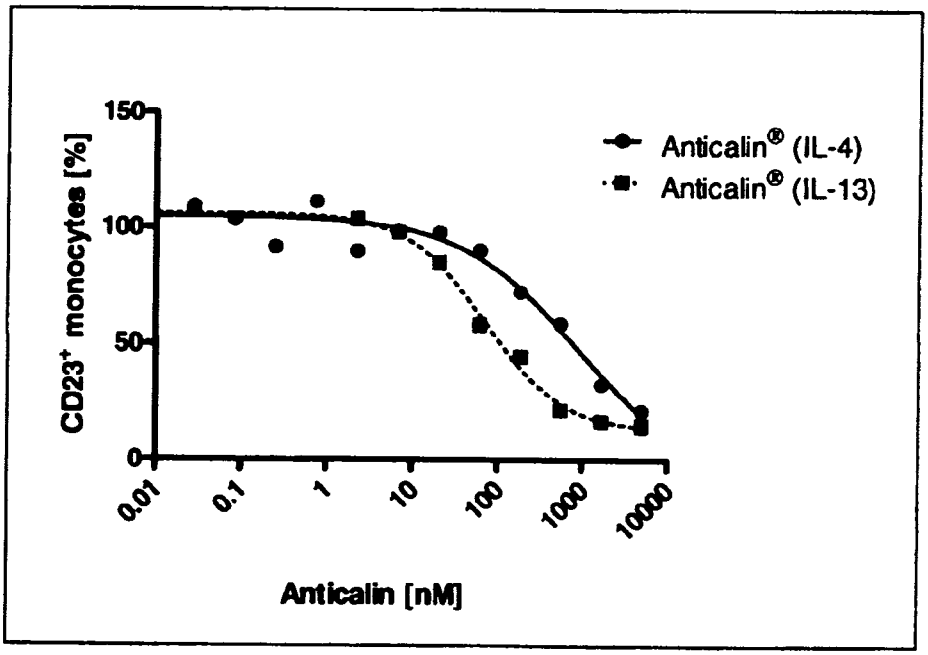

CD23+ monocytes [%]
150
100
50
0
0.01
0.1
1
10
100
1000
10000
Anticalin [nM]
Anticalin® (IL-4)
Anticalin® (IL-13)

Fig. 38

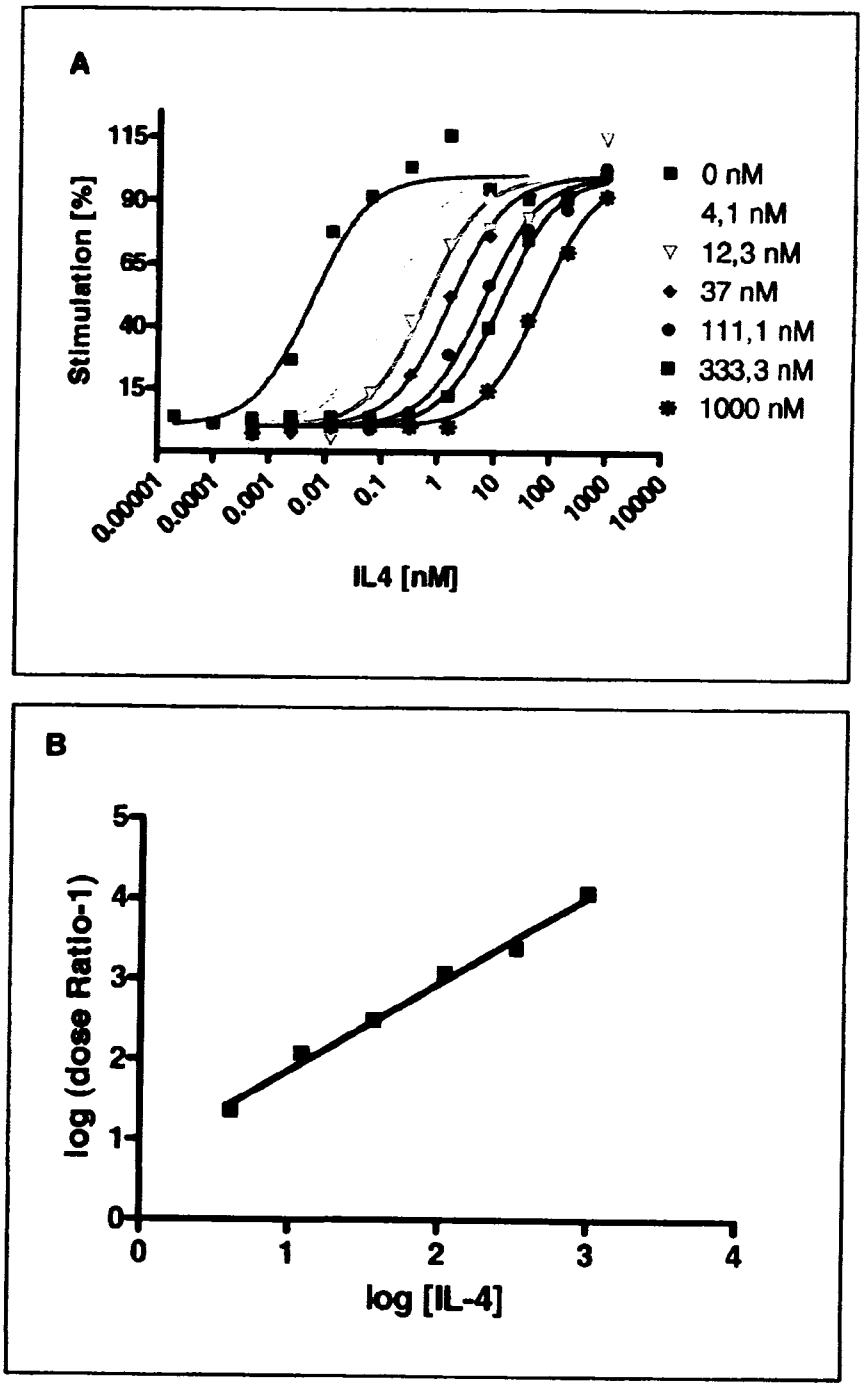

A
Stimulation [%]
115
90
65
40
15
0.00001
0.0001
0.001
0.01
0.1
1
10
100
1000
10000
IL4 [nM]
0 nM
4,1 nM
12,3 nM
37 nM
111,1 nM
333,3 nM
1000 nM
B
log (dose Ratio-1)
5
4
3
2
1
0
0
1
2
3
4
log [IL-4]

Fig. 39

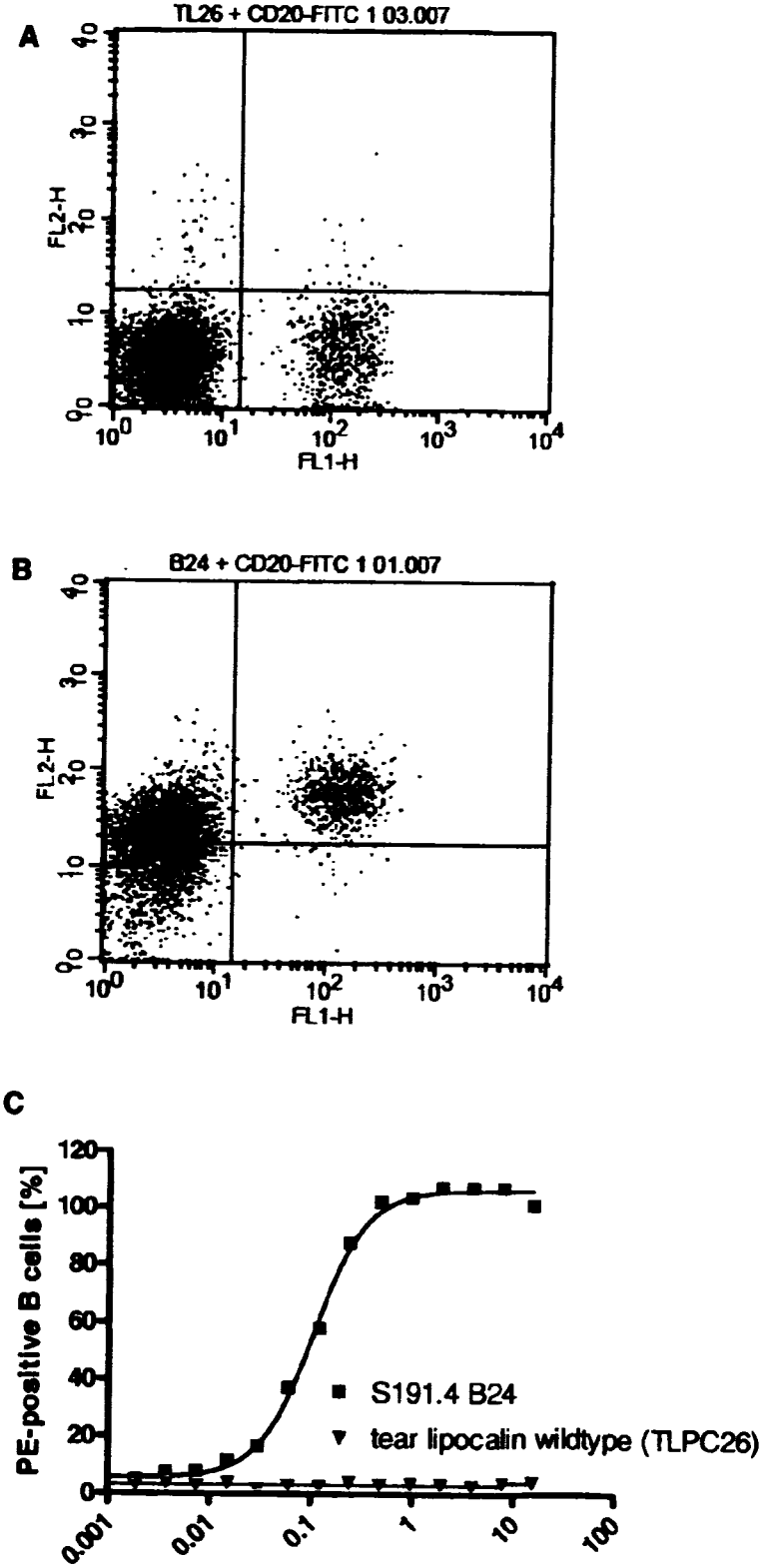

A
TL26 + CD20-FITC 1 03.007
FL2-H
FL1-H
B
B24 + CD20-FITC 1 01.007
FL2-H
FL1-H
C
PE-positive B cells [%]
S191.4 B24
tear lipocalin wildtype (TLPC26)

Fig. 40

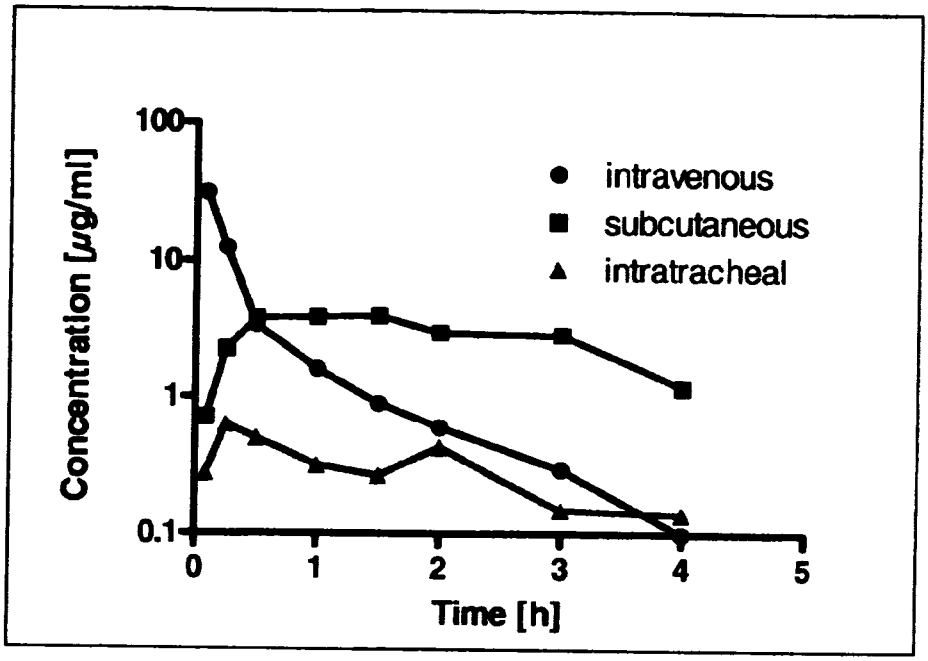
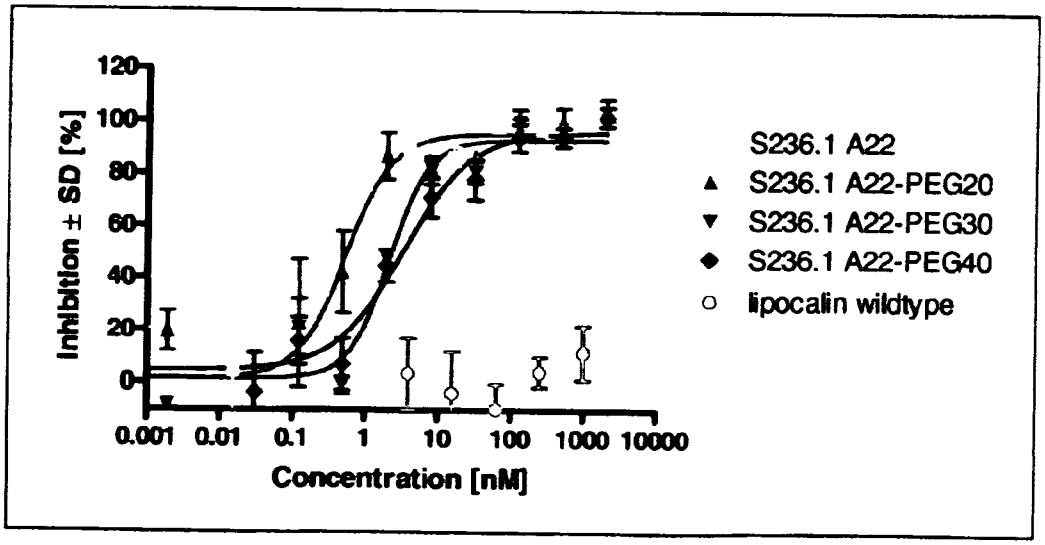

Concentration [µg/ml]
100
10
1
0.1
0
1
2
3
4
5
Time [h]
intravenous
subcutaneous
intratracheal

Fig. 41


Inhibition ± SD [%]
120
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
1000
10000
Concentration [nM]
S236.1 A22
S236.1 A22-PEG20
S236.1 A22-PEG30
S236.1 A22-PEG40
lipocalin wildtype