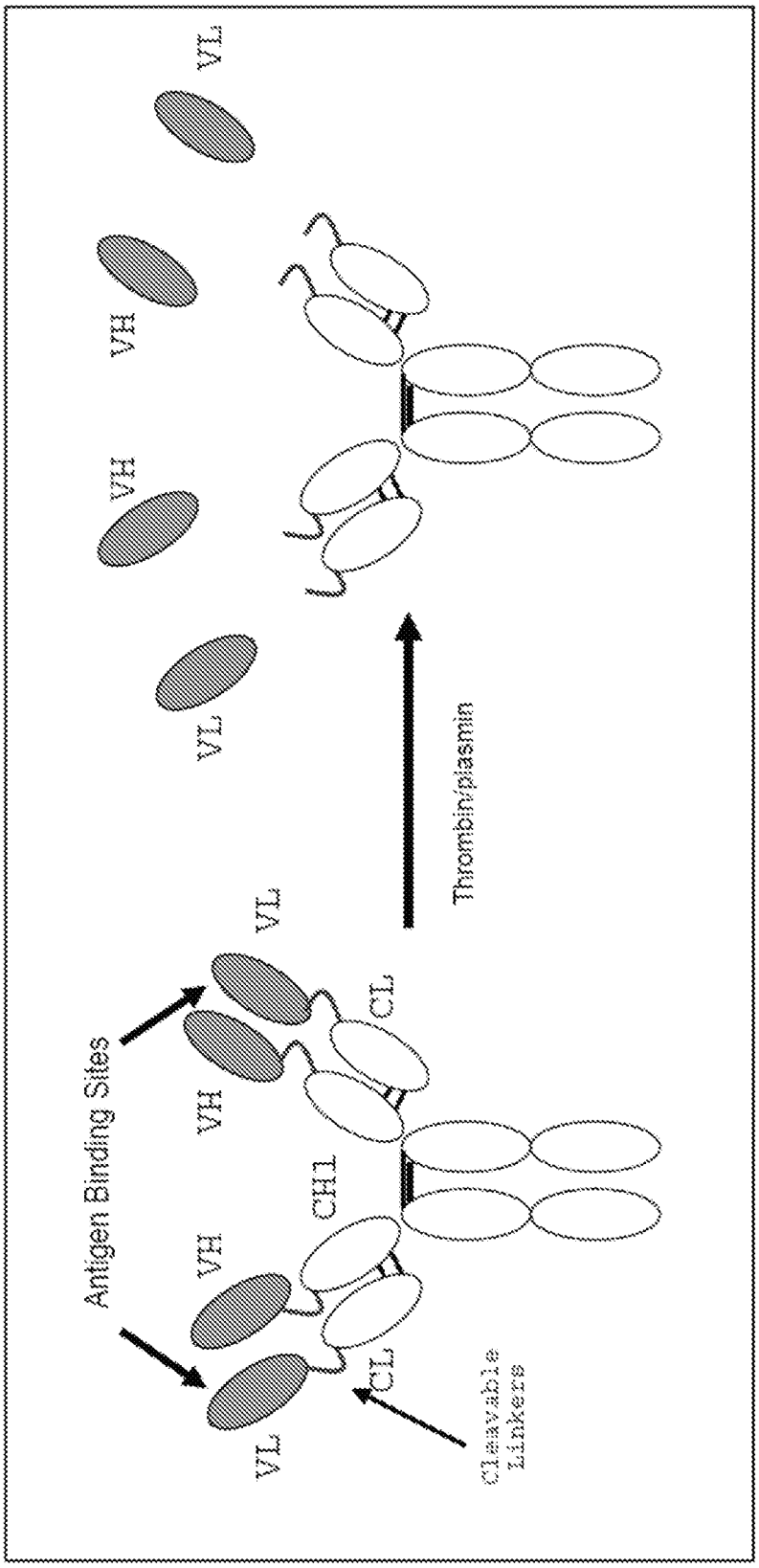

VL
VH
VH
VL
Thrombin/plasmin
VL
CL
VH
CH1
VH
CL
VL
Antigen Binding Sites
Cleavable Linkers

Fig. 1

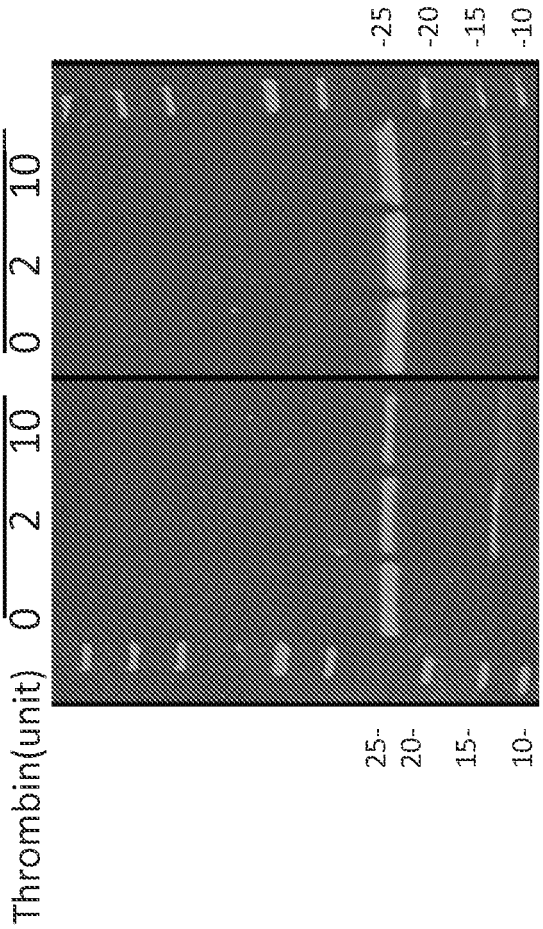
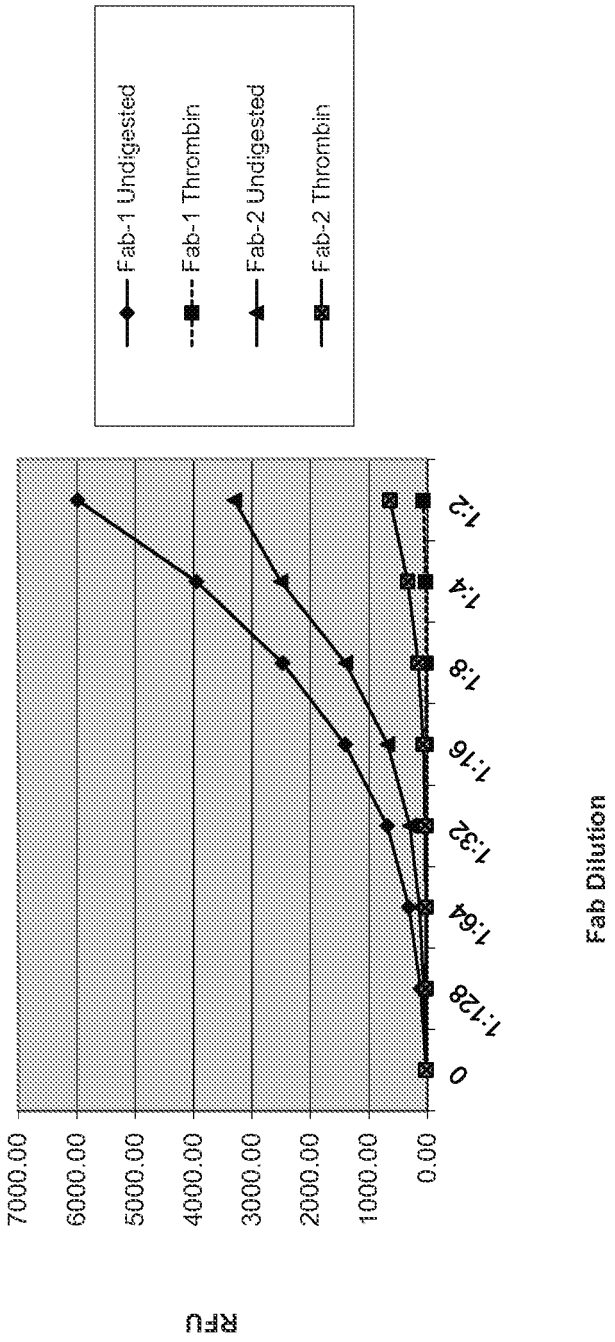


Figure 2

Figure 3


Loss of TFPI Binding of Thrombin Digested Fabs
Fab-1 Undigested
Fab-1 Thrombin
Fab-2 Undigested
Fab-2 Thrombin
RFU
7000.00
6000.00
5000.00
4000.00
3000.00
2000.00
1000.00
0.00
0
1:128
1:64
1:32
1:16
1:8
1:4
1:2
Fab Dilution

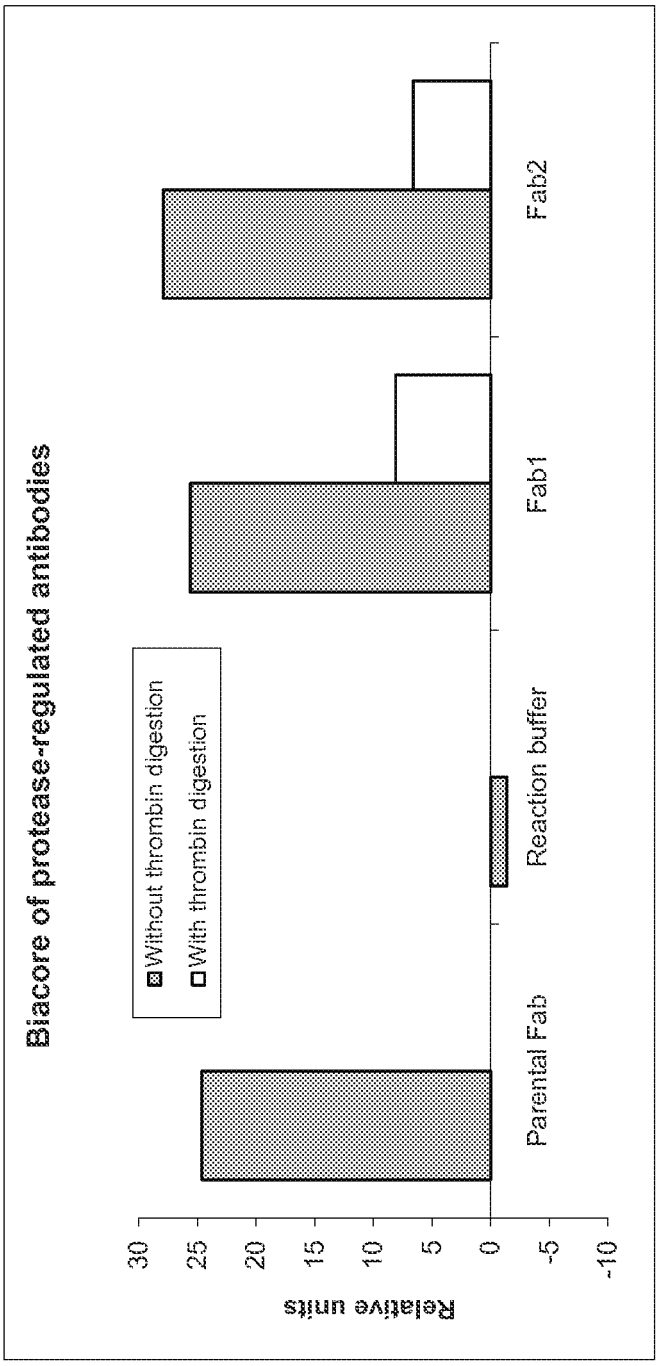

Biacore of protease-regulated antibodies
Without thrombin digestion
With thrombin digestion
Relative units
30
25
20
15
10
5
0
-5
-10
Parental Fab
Reaction buffer
Fab1
Fab2

Fig.4

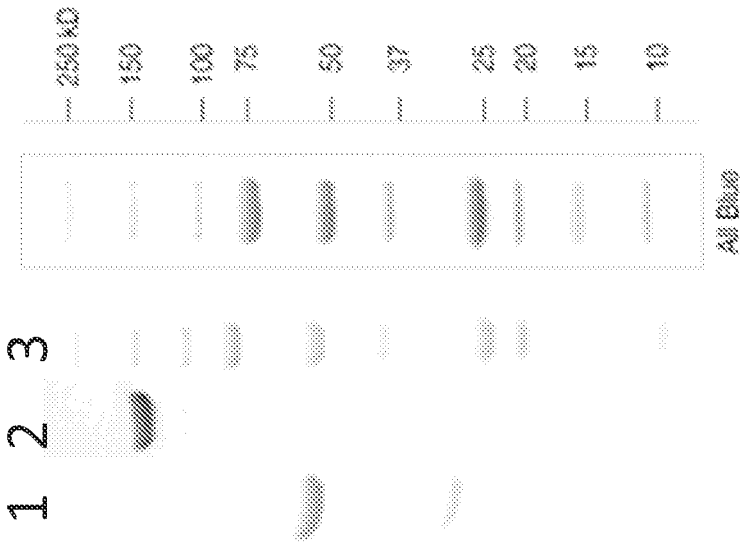
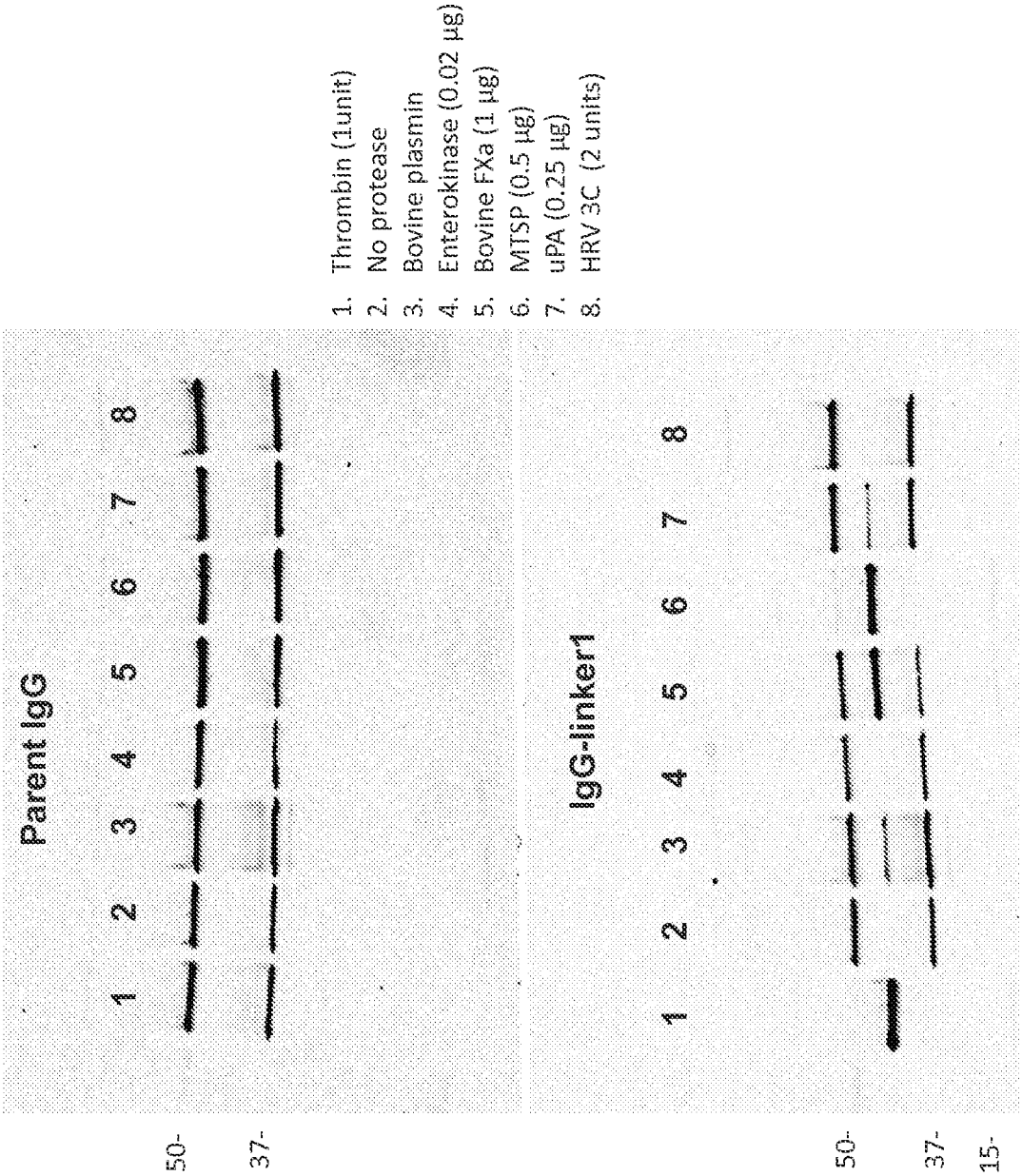


Fig. 5

Figure 6


Parent IgG
1 2 3 4 5 6 7 8
50-
37-
IgG-linker1
1 2 3 4 5 6 7 8
50-
37-
15-
1. Thrombin (1unit)
2. No protease
3. Bovine plasmin
4. Enterokinase (0.02 µg)
5. Bovine FXa (1 µg)
6. MTSP (0.5 µg)
7. uPA (0.25 µg)
8. HRV 3C (2 units)

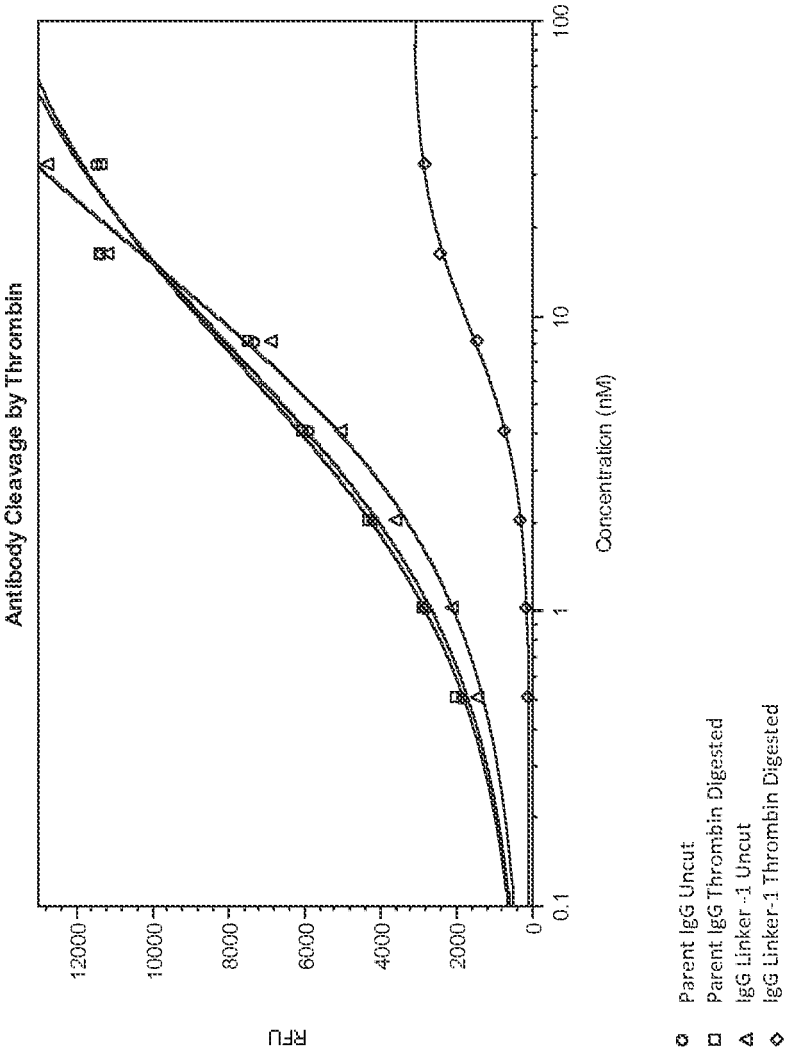

Antibody Cleavage by Thrombin
RFU
12000
10000
8000
6000
4000
2000
0
0.1
1
10
100
Concentration (nM)
Parent IgG Uncut
Parent IgG Thrombin Digested
IgG Linker -1 Uncut
IgG Linker-1 Thrombin Digested

Figure 7

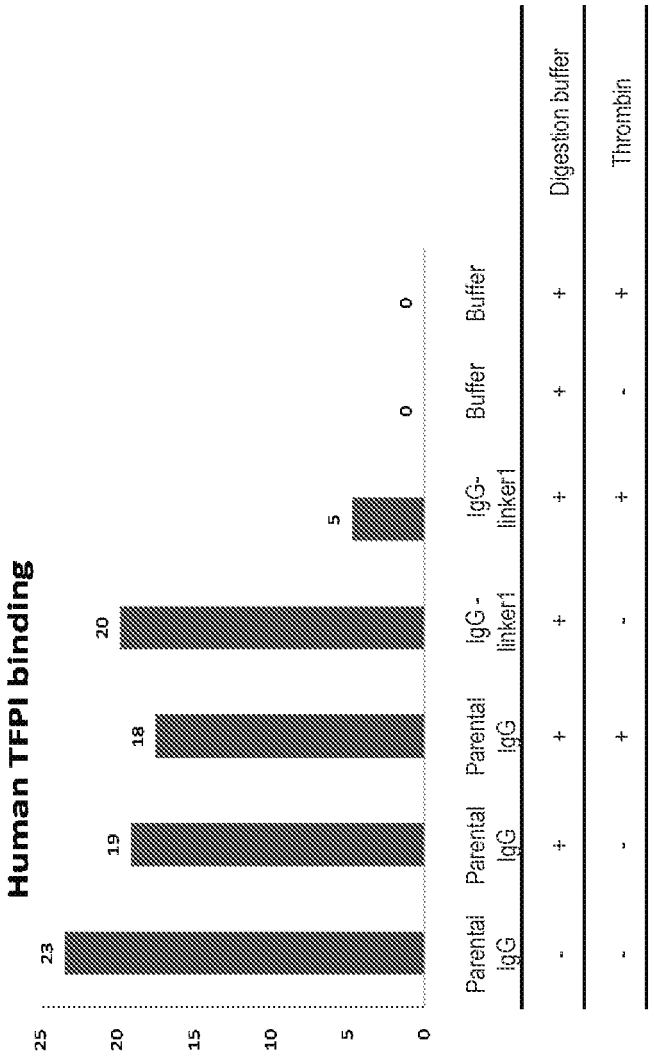
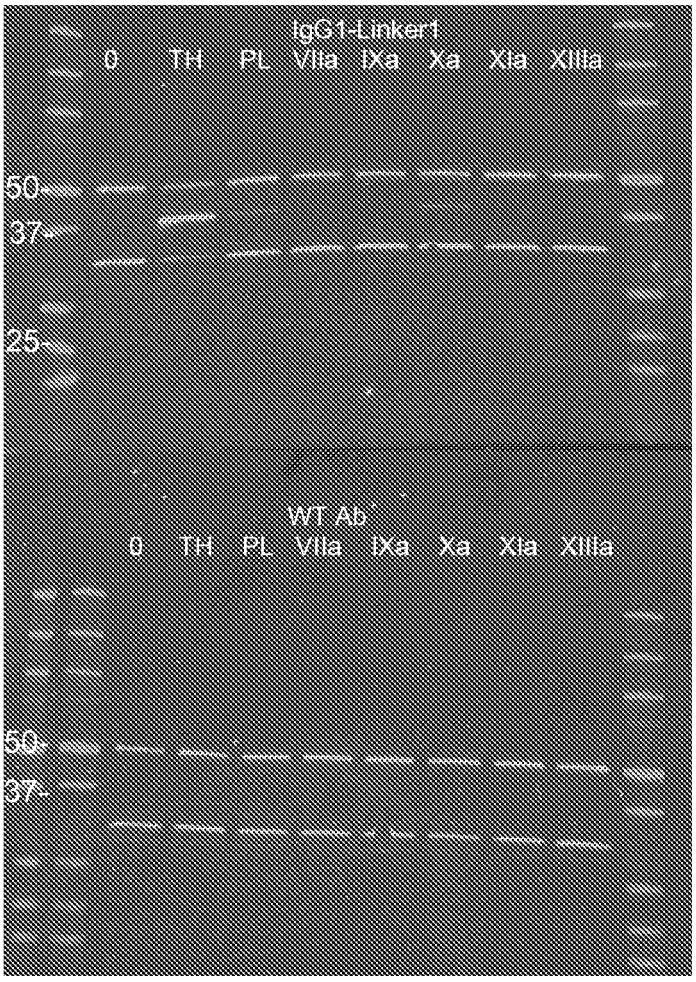

Human TFPI binding
25
20
15
10
5
0
23
19
18
20
5
0
0
Parental IgG
Parental IgG
Parental IgG
IgG-linker1
IgG-linker1
Buffer
Buffer
- + + + + + +
- - + - + - +
Digestion buffer
Thrombin

Fig. 8

Fig. 9


IgG1-Linker1
0
TH
PL
VIIa
IXa
Xa
XIa
XIIIa
50
37
25
WT Ab
0
TH
PL
VIIa
IXa
Xa
XIa
XIIIa
50
37