DDD1:

SHIQIPPGLTELLQGYTVEVLRQQPPDL
VEFAVEYFTRLREARA (SEQ ID NO:1)

DDD2:

CGHIQIPPGLTELLQGYTVEVLRQQPPDLVEFA
VEYFTRLREARA (SEQ ID NO:2)

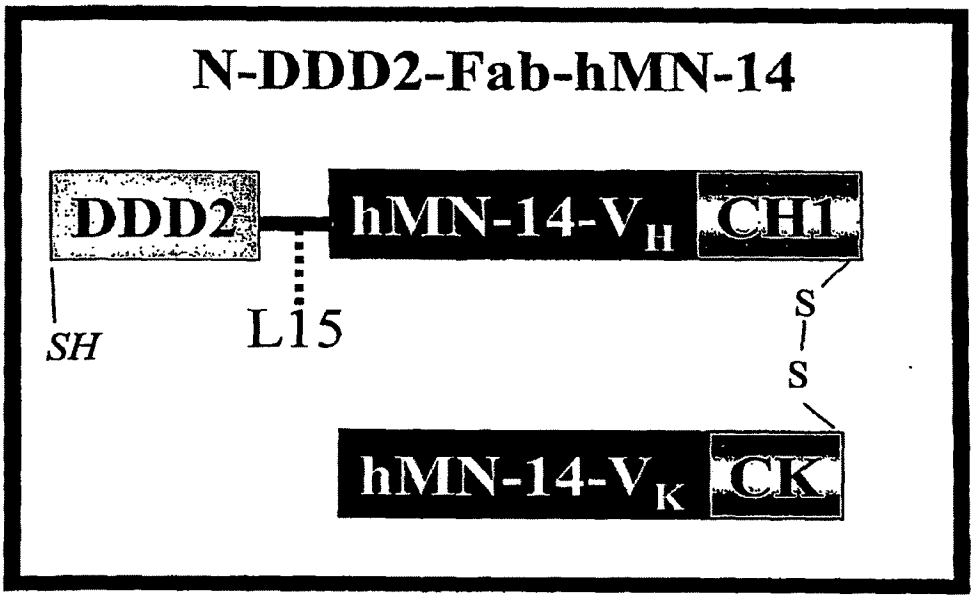
Figure 1

AD1:QIEYLAKQIVDNAIQQA (SEQ ID NO:3)

AD2: CGQIEYLAKQIVDNAIQQAGC (SEQ ID NO:4)

Figure 2

A


N-DDD2-Fab-hMN-14
DDD2
hMN-14-V H
CH1
SH
L15
S
S
hMN-14-V K
CK

B

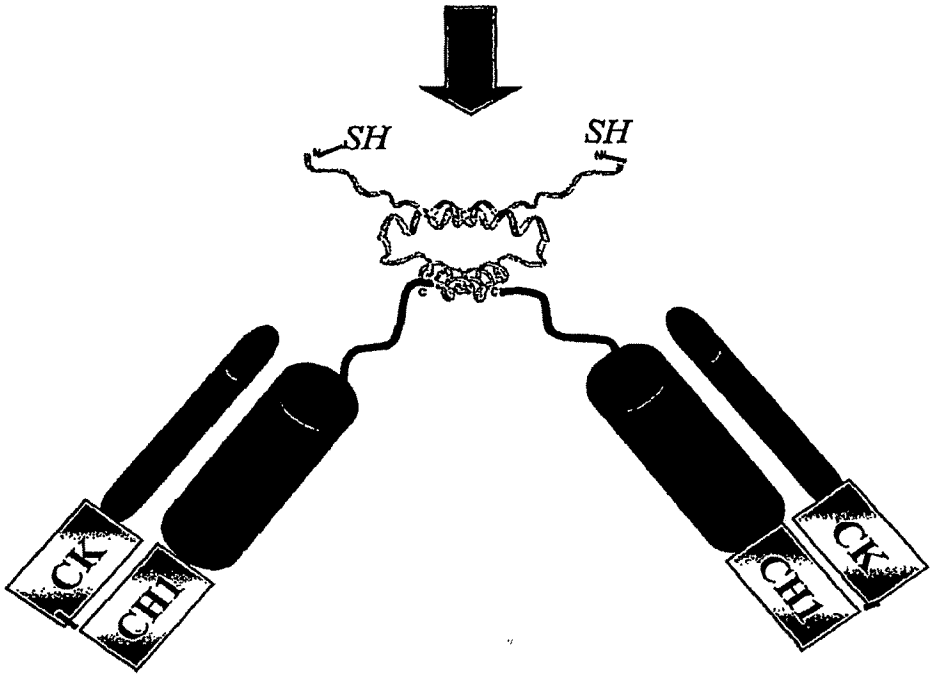

SH
SH
CK
CH1
CK
CH1

Figure 3

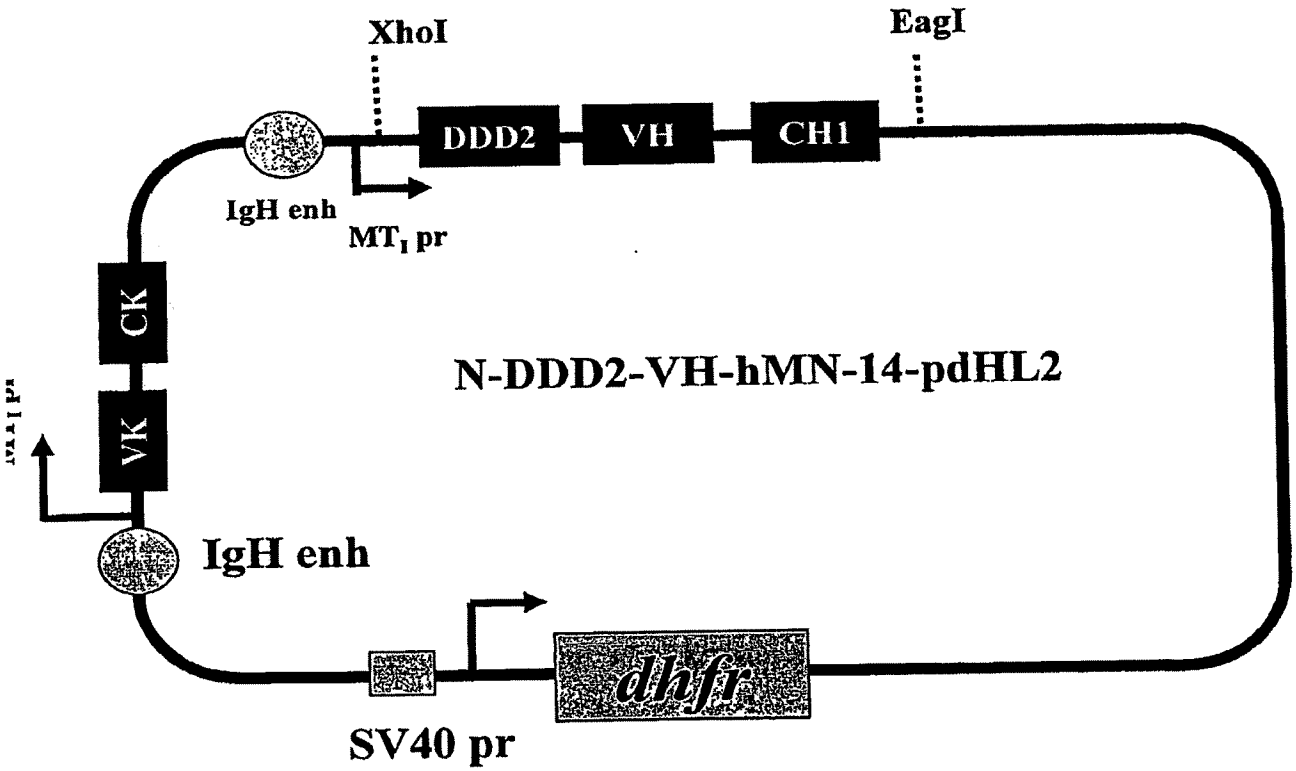
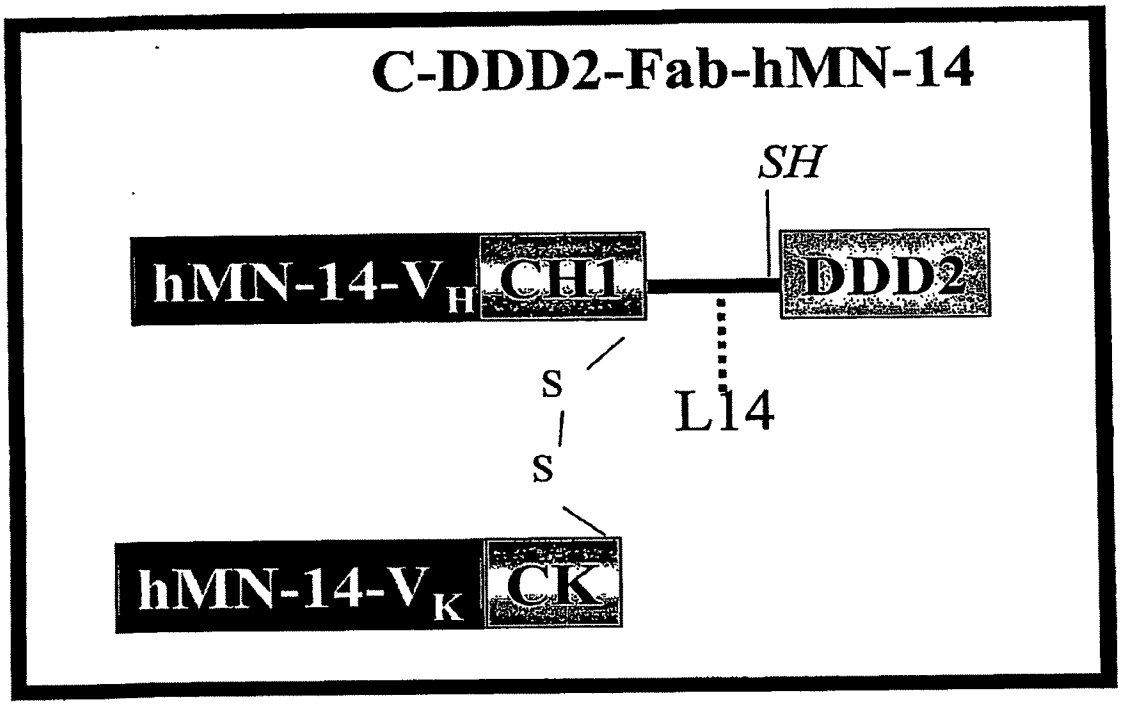

XhoI
EagI
DDD2
VH
CH1
IgH enh
MT1 pr
CK
VK
N-DDD2-VH-hMN-14-pdHL2
IgH enh
dhfr
SV40 pr

Figure 4

A


C-DDD2-Fab-hMN-14
SH
hMN-14-V H
CH1
DDD2
S
S
L14
hMN-14-V K
CK

B

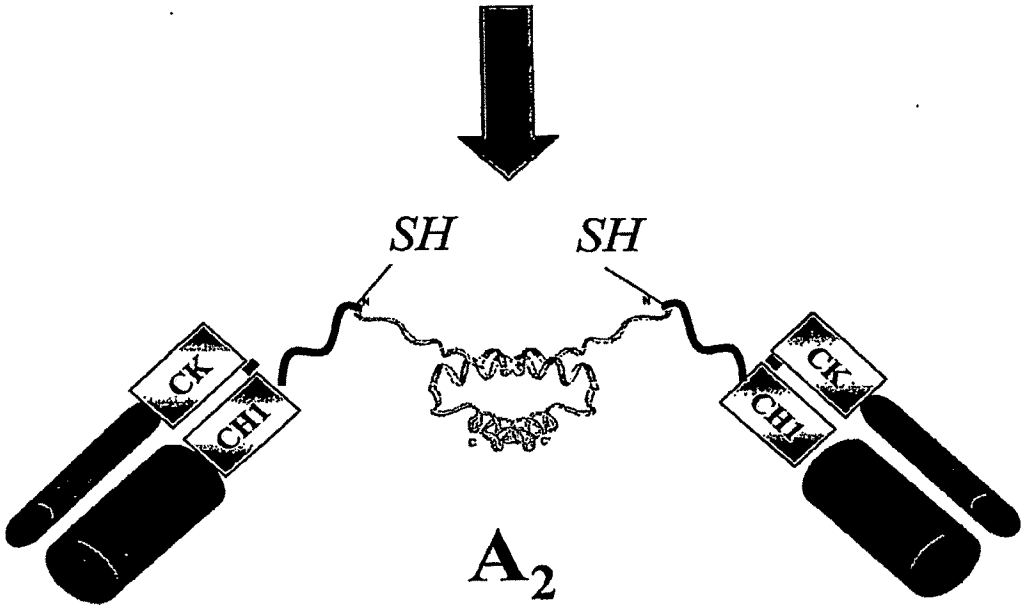

SH
SH
CK
CH1
CK
CH1
A2

Figure 5

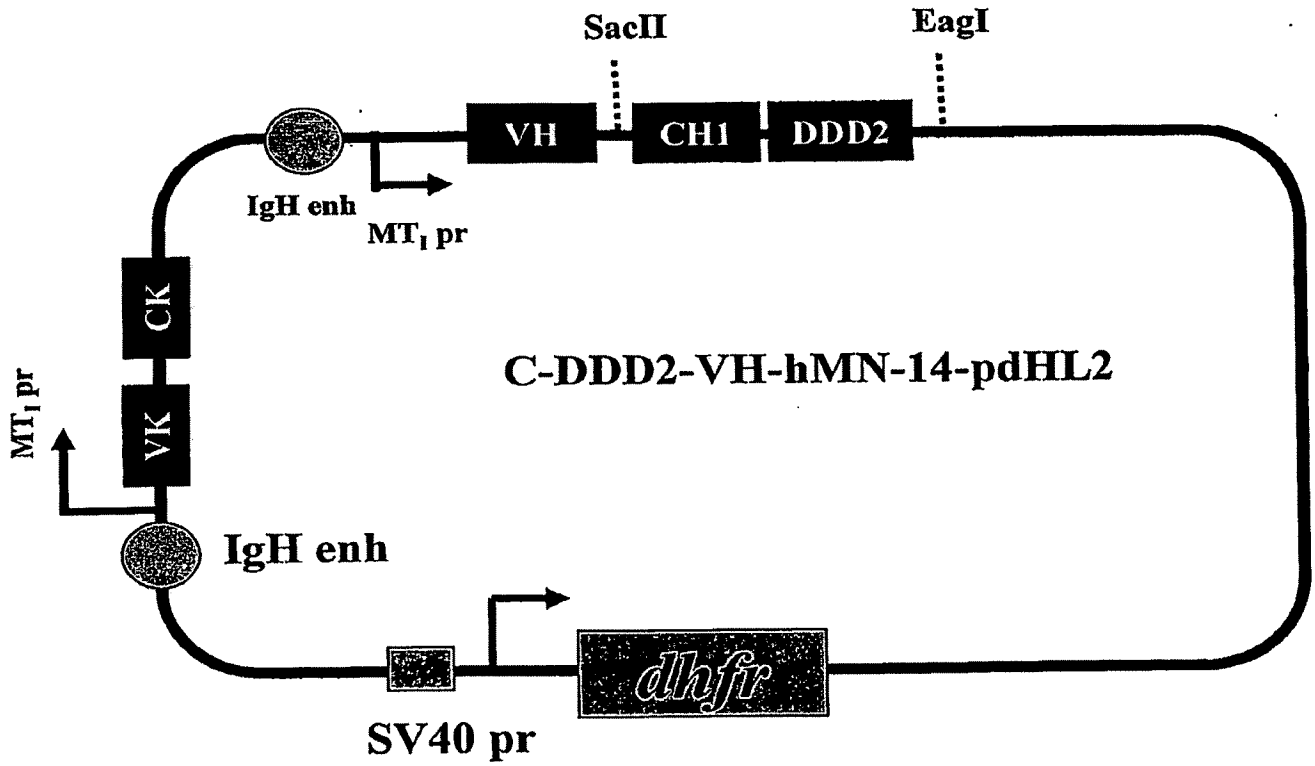
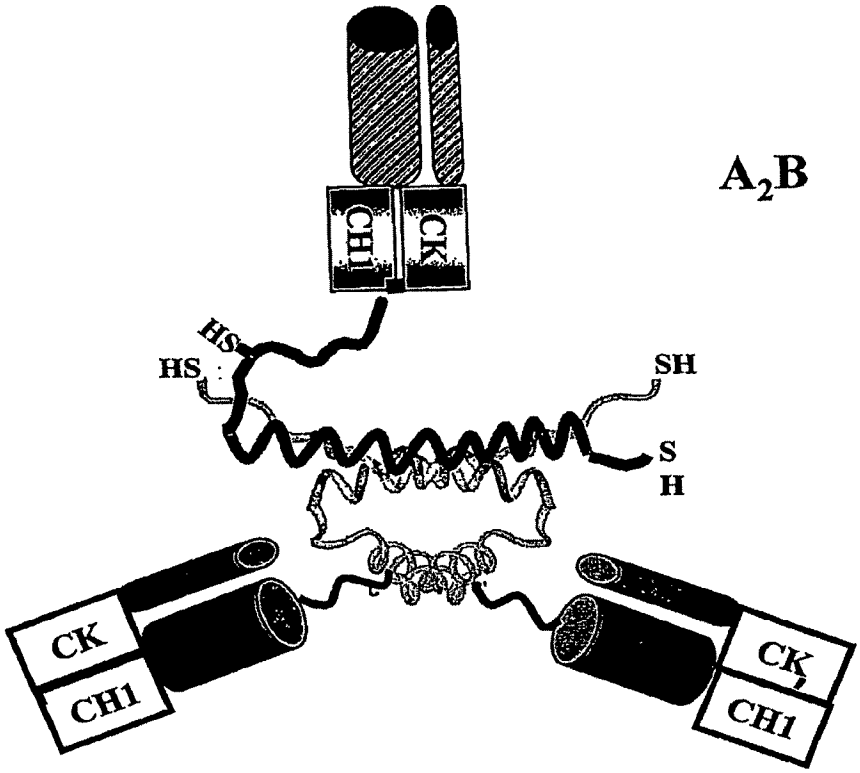

SacII
EagI
VH
CH1
DDD2
IgH enh
MT1 pr
CK
VK
MT1 pr
C-DDD2-VH-hMN-14-pdHL2
IgH enh
dhfr
SV40 pr

Figure 6

A



B

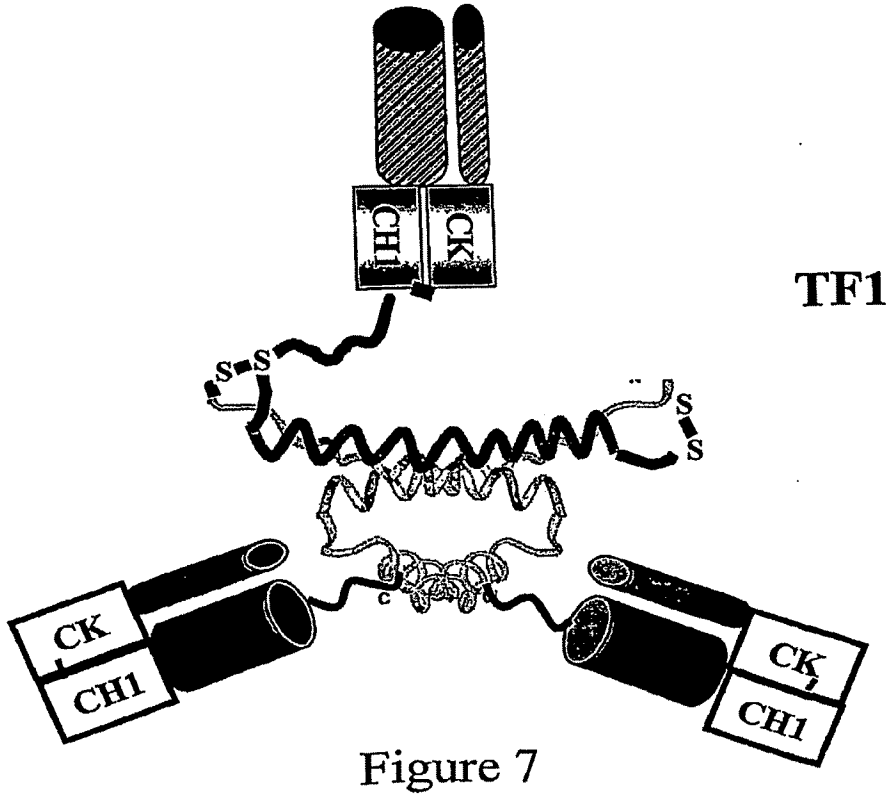


Figure 7

TF2

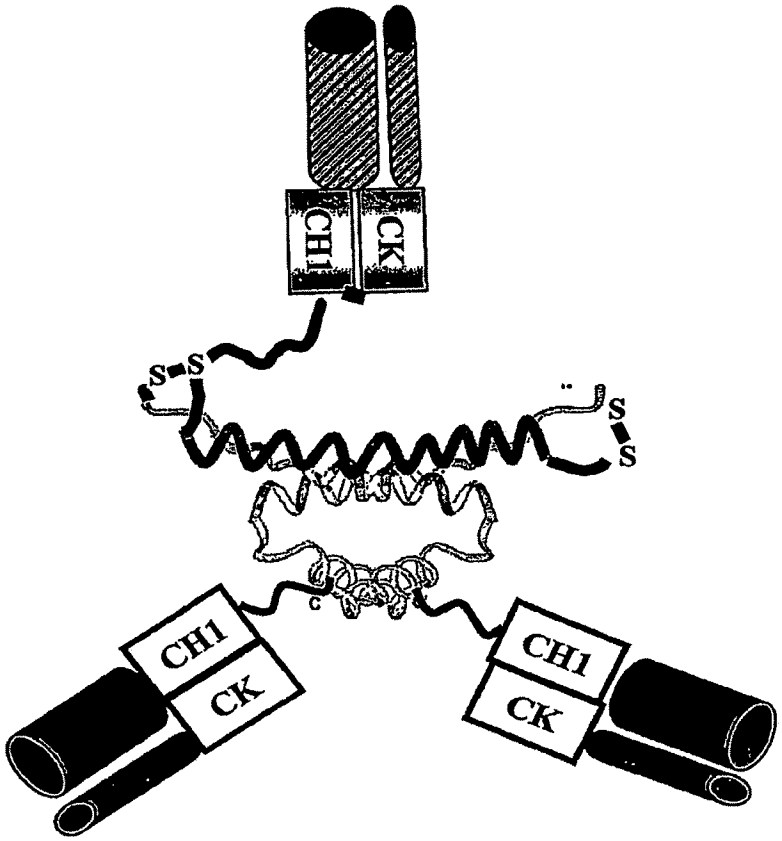
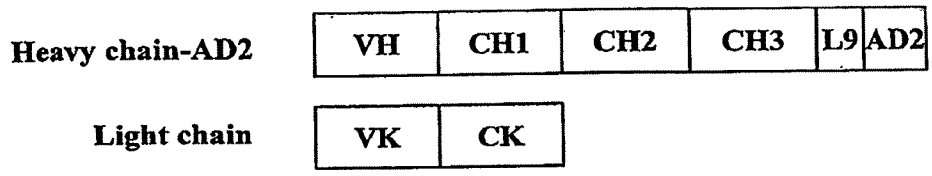


Figure 8

A


Heavy chain-AD2
VH
CH1
CH2
CH3
L9
AD2
Light chain
VK
CK

B

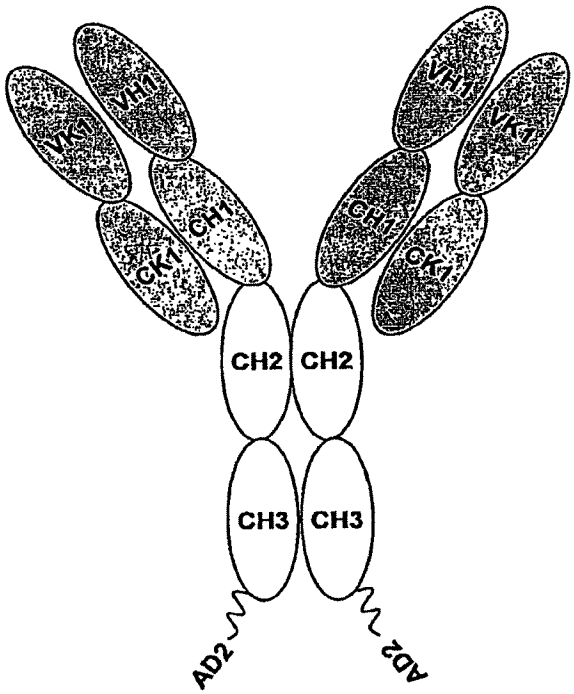

VH1
VK1
CH1
CK1
VH1
VK1
CH1
CK1
CH2
CH2
CH3
CH3
AD2
AD2

Figure 9

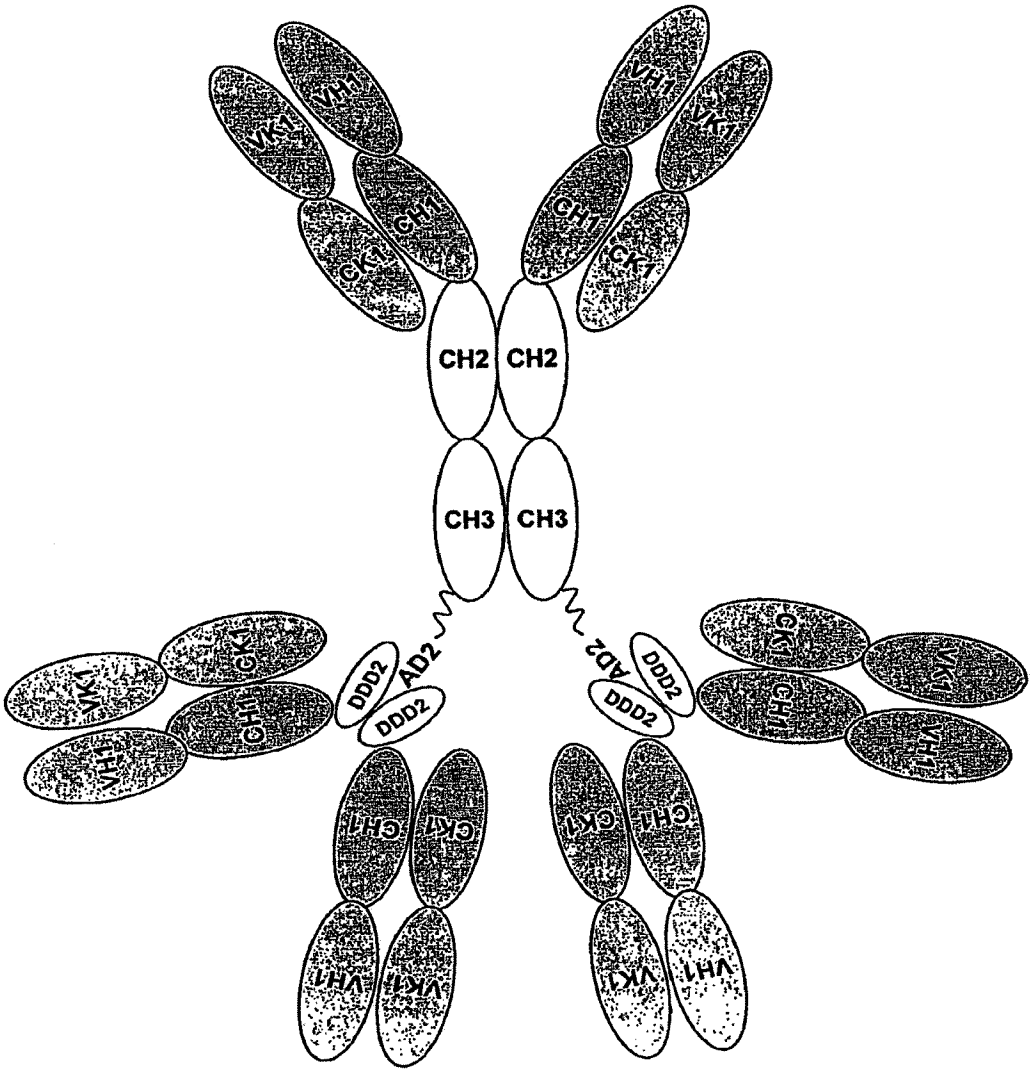

VH1
VK1
CH1
CK1
VH1
VK1
CH1
CK1
CH2
CH2
CH3
CH3
AD2
DDD2
DDD2
AD2
DDD2
DDD2
VK1
CK1
CH1
VH1
CK1
VK1
CH1
VH1
CH1
CK1
VH1
VK1
CK1
CH1
VK1
VH1

Figure 10

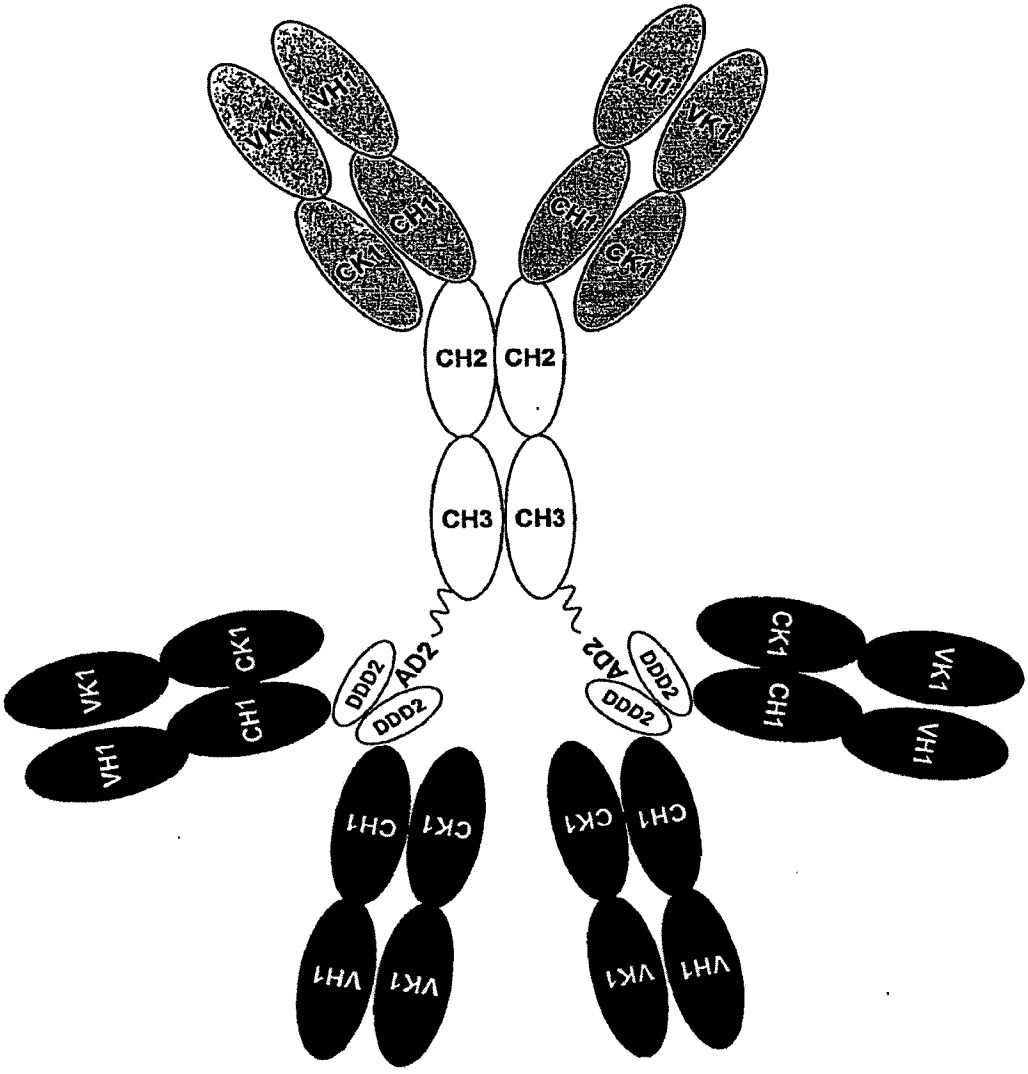
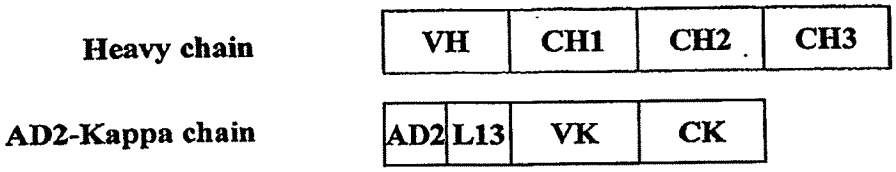

VH1
VK1
CH1
CK1
CH2
CH2
CH3
CH3
AD2
DDD2
DDD2
AD2
DDD2
DDD2
VK1
CK1
CH1
VH1
CK1
CH1
VH1
VK1
CK1
CH1
VH1
VK1

Figure 11

A


Heavy chain
VH
CH1
CH2
CH3
AD2-Kappa chain
AD2
L13
VK
CK

B

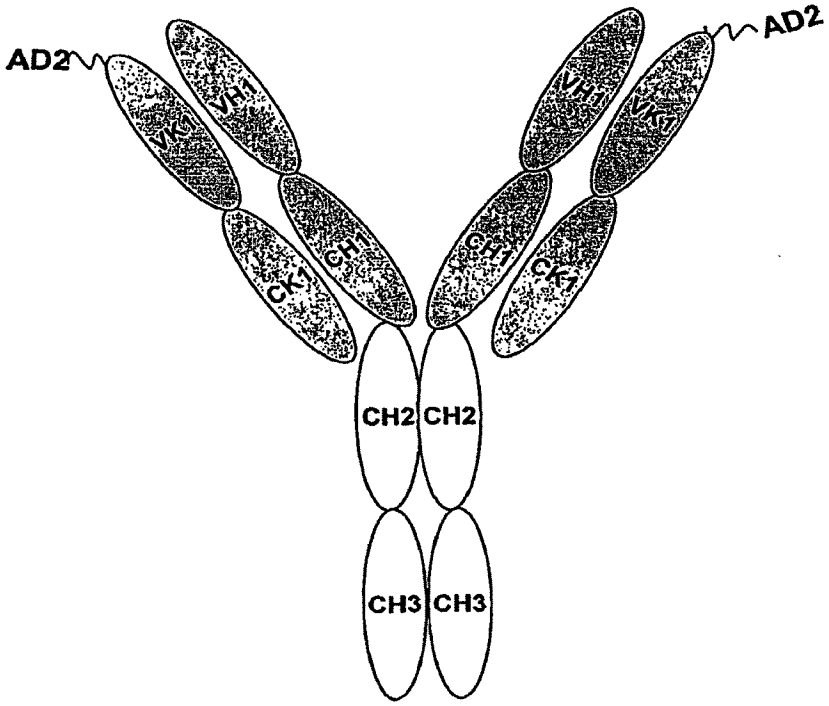

AD2
VK1
VH1
CK1
CH1
CH2
CH2
CH3
CH3
VH1
VK1
CH1
CK1
AD2

Figure 12

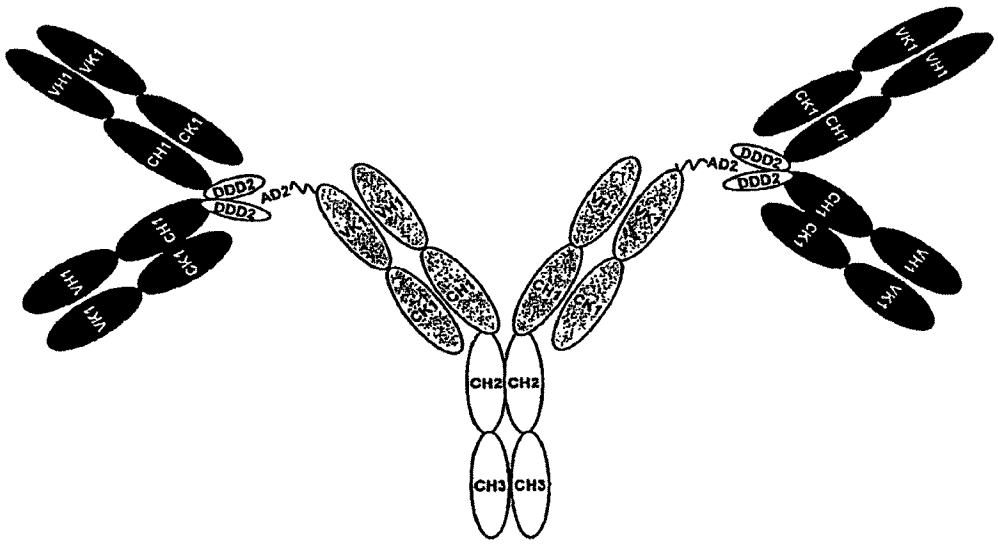

VH1
VK1
CH1
CK1
DDD2
DDD2
AD2
VHH
VKH
CHH
CKH
CH2
CH2
CH3
CH3
AD2
DDD2
DDD2
VK1
VH1
CK1
CH1
CHH
CKH
VHH
VKH

Figure 13

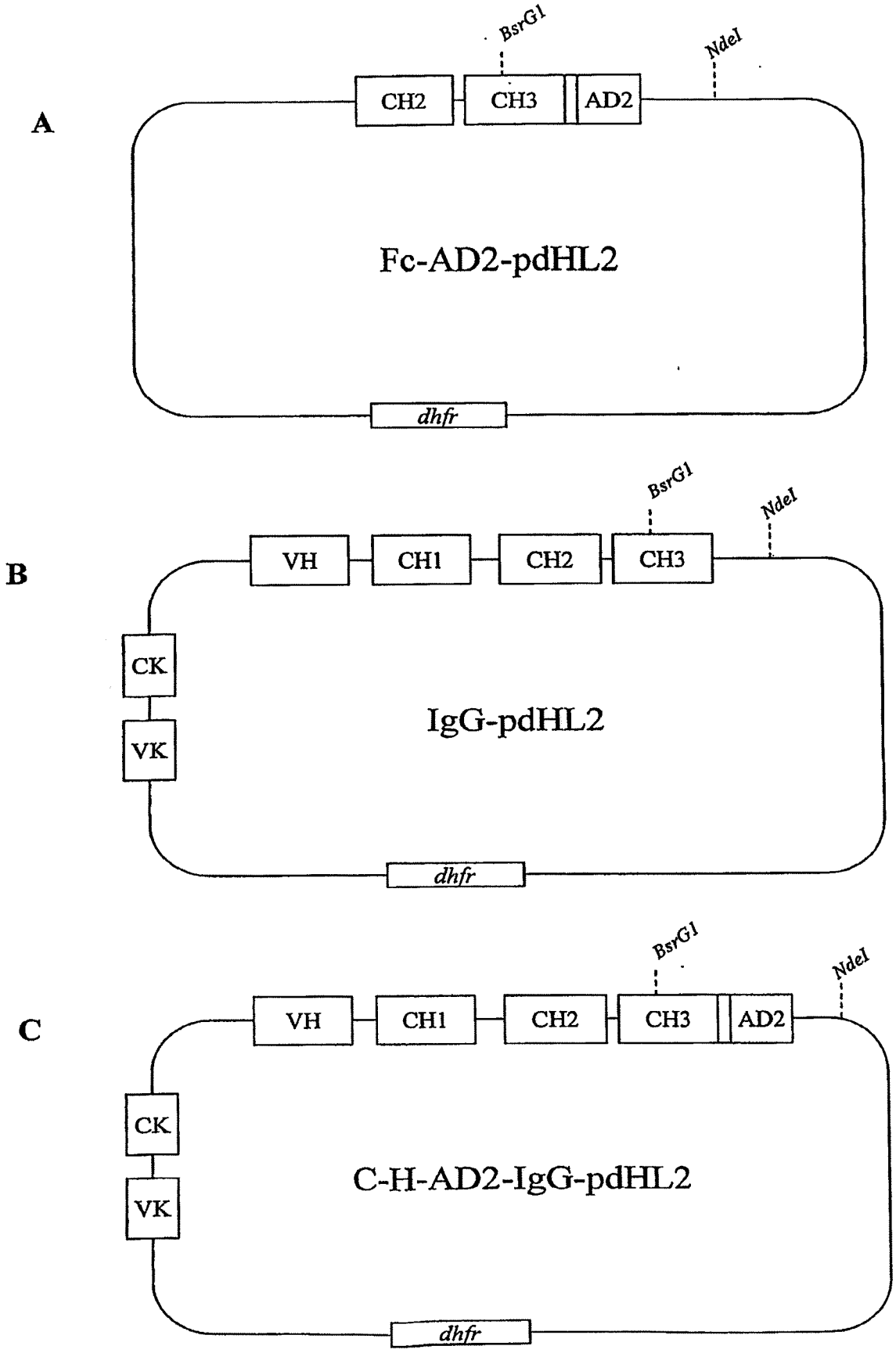

A
BsrG1
NdeI
CH2
CH3
AD2
Fc-AD2-pdHL2
dhfr
B
BsrG1
NdeI
VH
CH1
CH2
CH3
CK
VK
IgG-pdHL2
dhfr
C
BsrG1
NdeI
VH
CH1
CH2
CH3
AD2
CK
VK
C-H-AD2-IgG-pdHL2
dhfr

Figure 14

SE-HPLC Analysis of C-H-AD2-hLL2-IgG

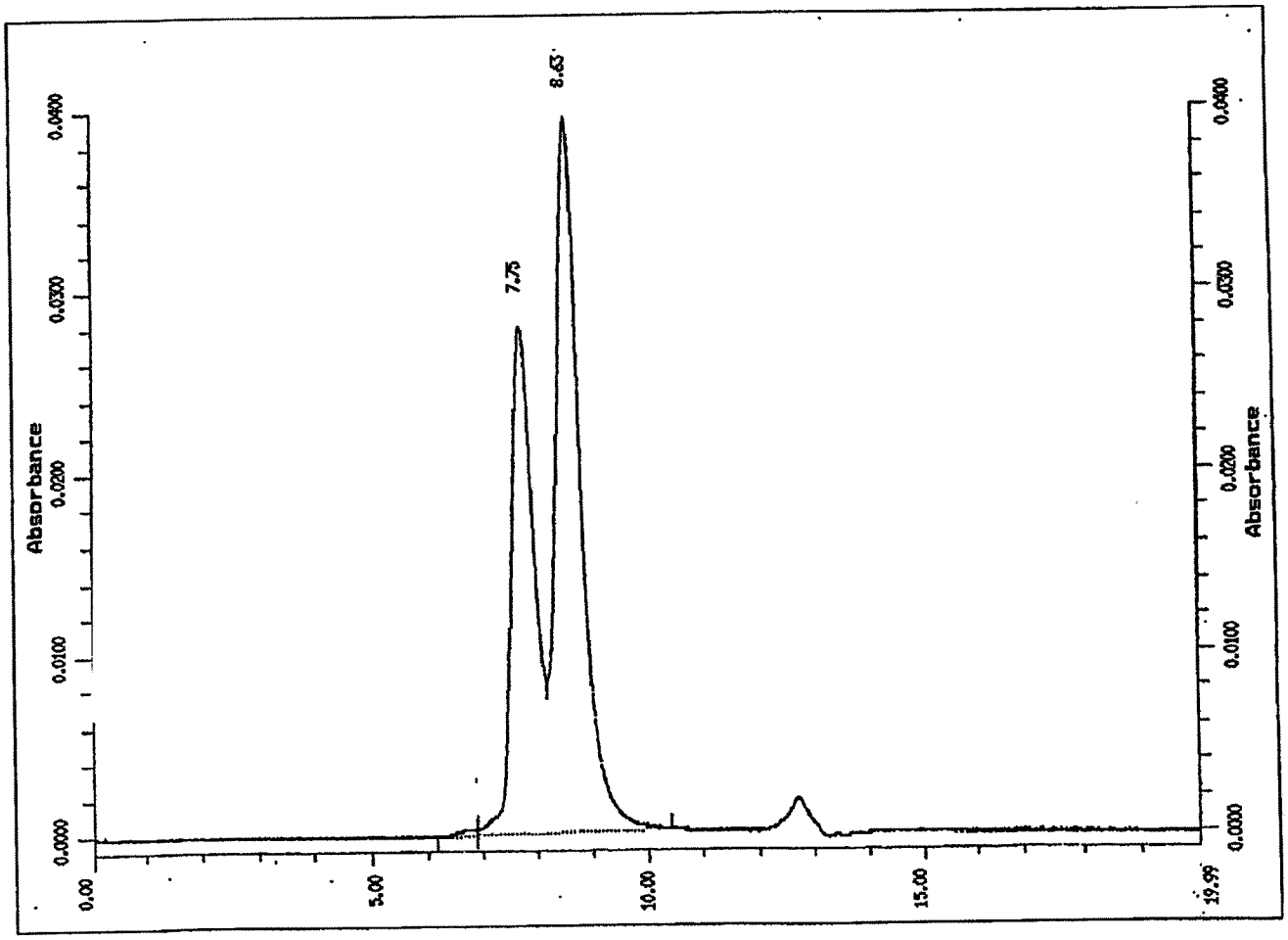


Figure 15

SDS-PAGE Analysis of C-H-AD2-hLL2

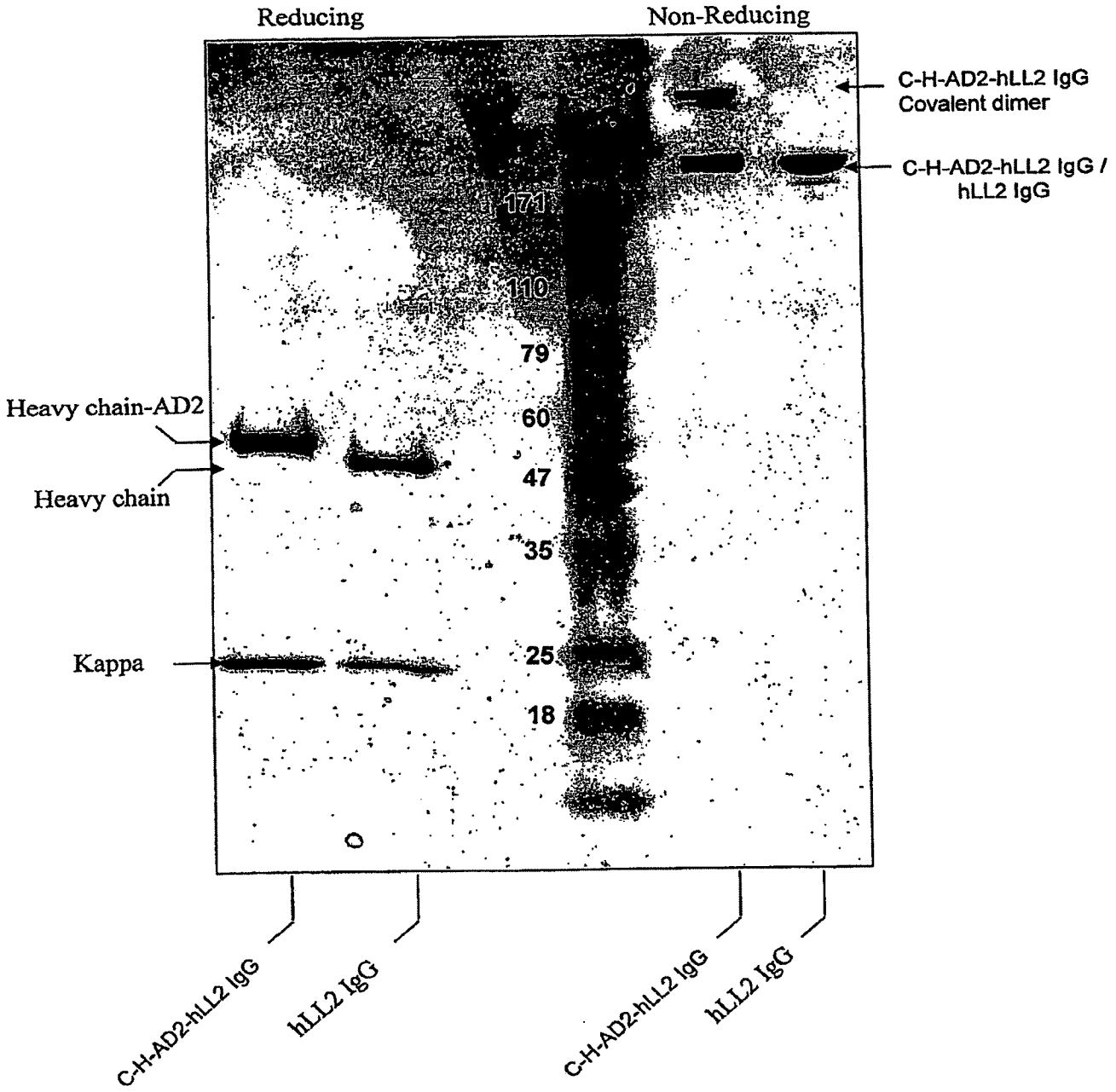
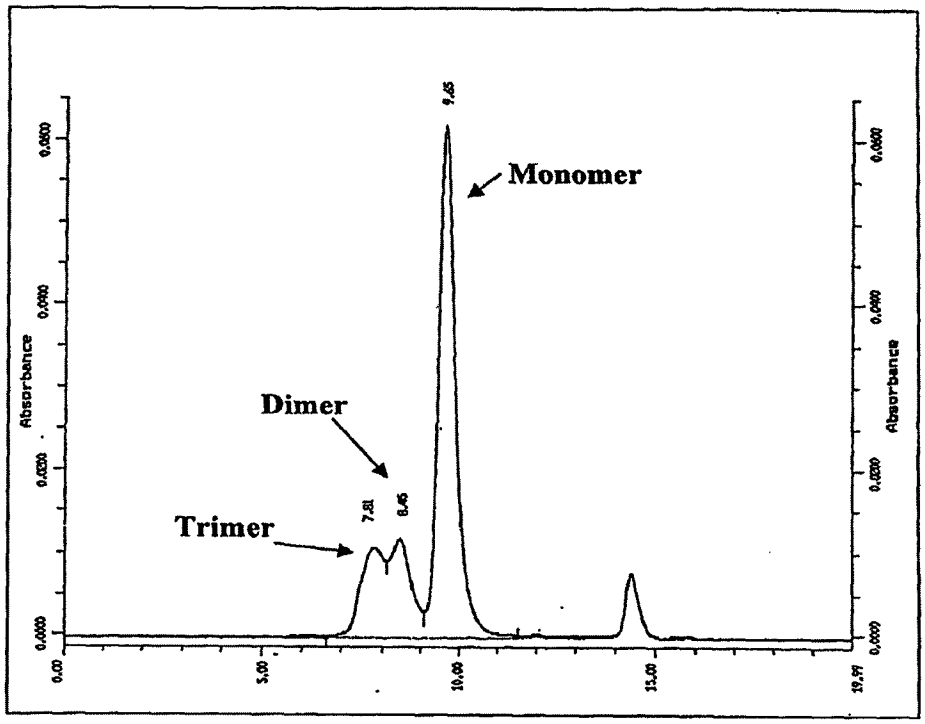


Figure 16

SE-HPLC analysis of N-K-AD2-hA20 IgG

A



B

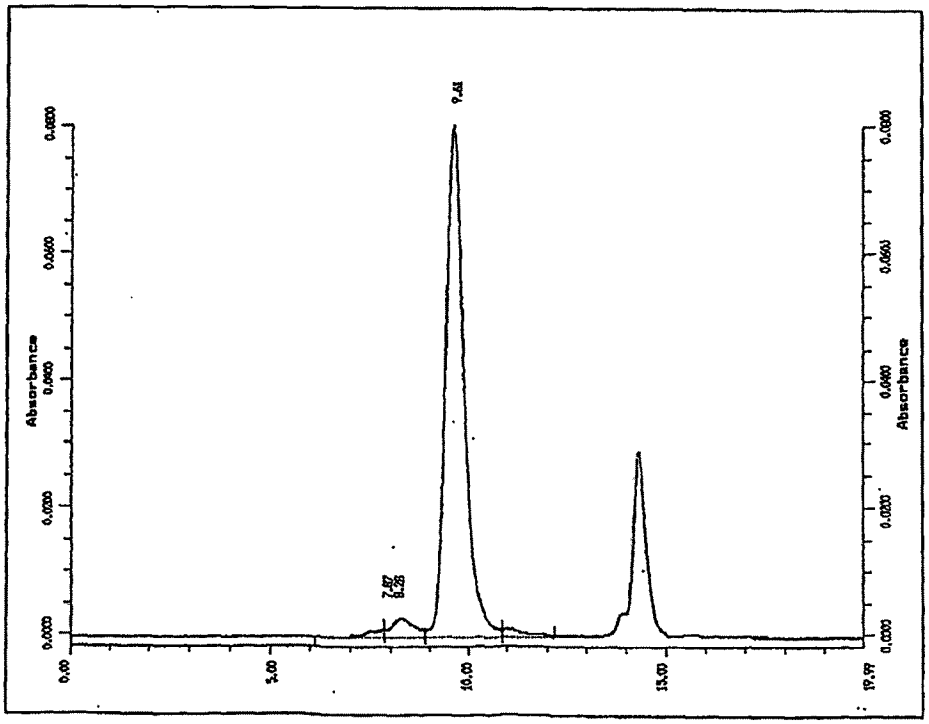


Figure 17

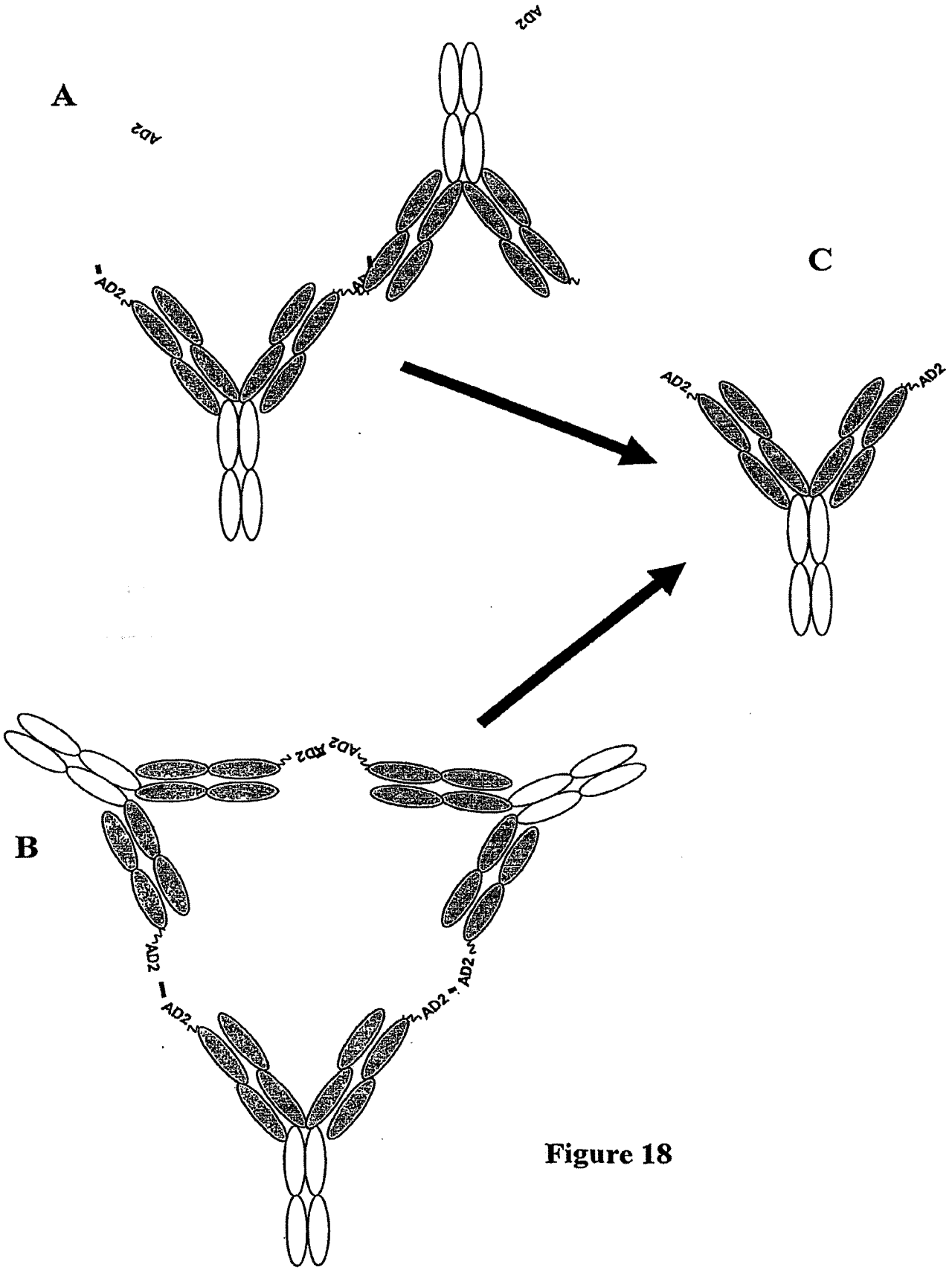


Figure 18

SE-HPLC Analysis of Hex-hA20

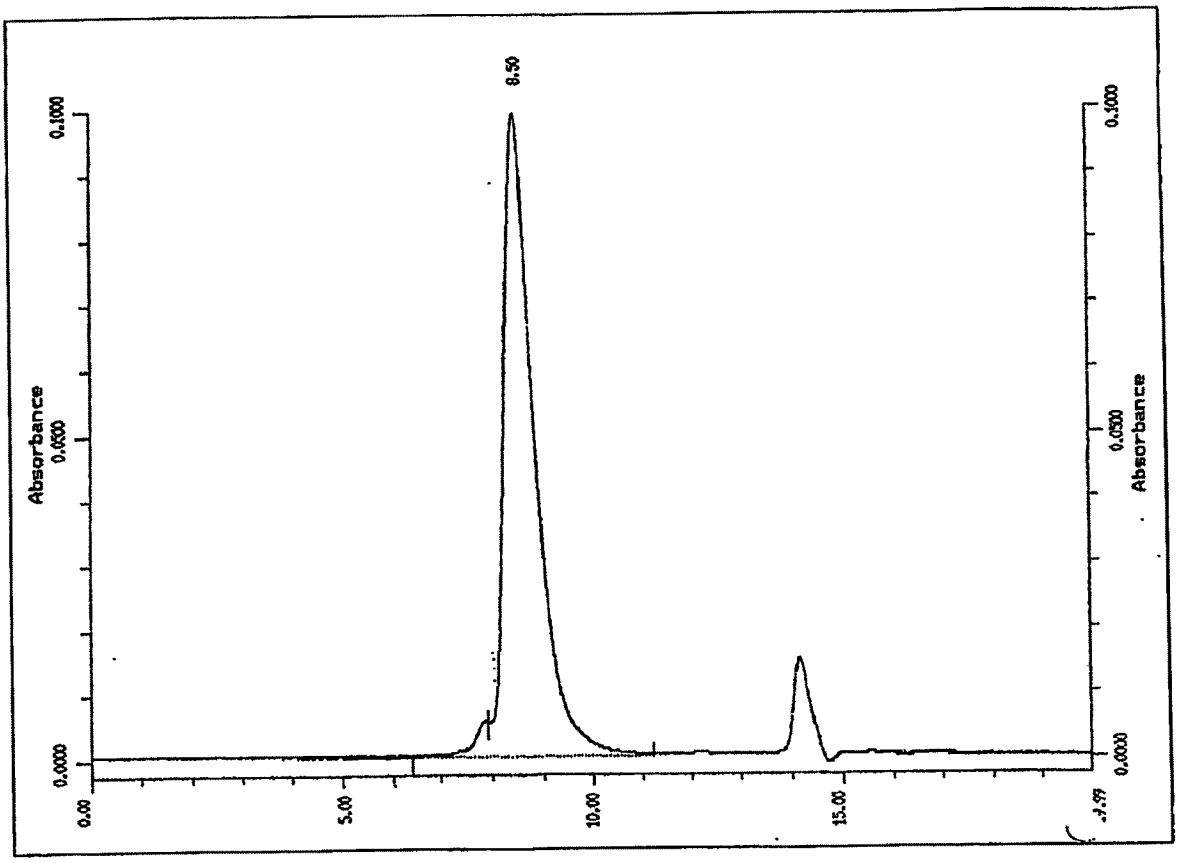
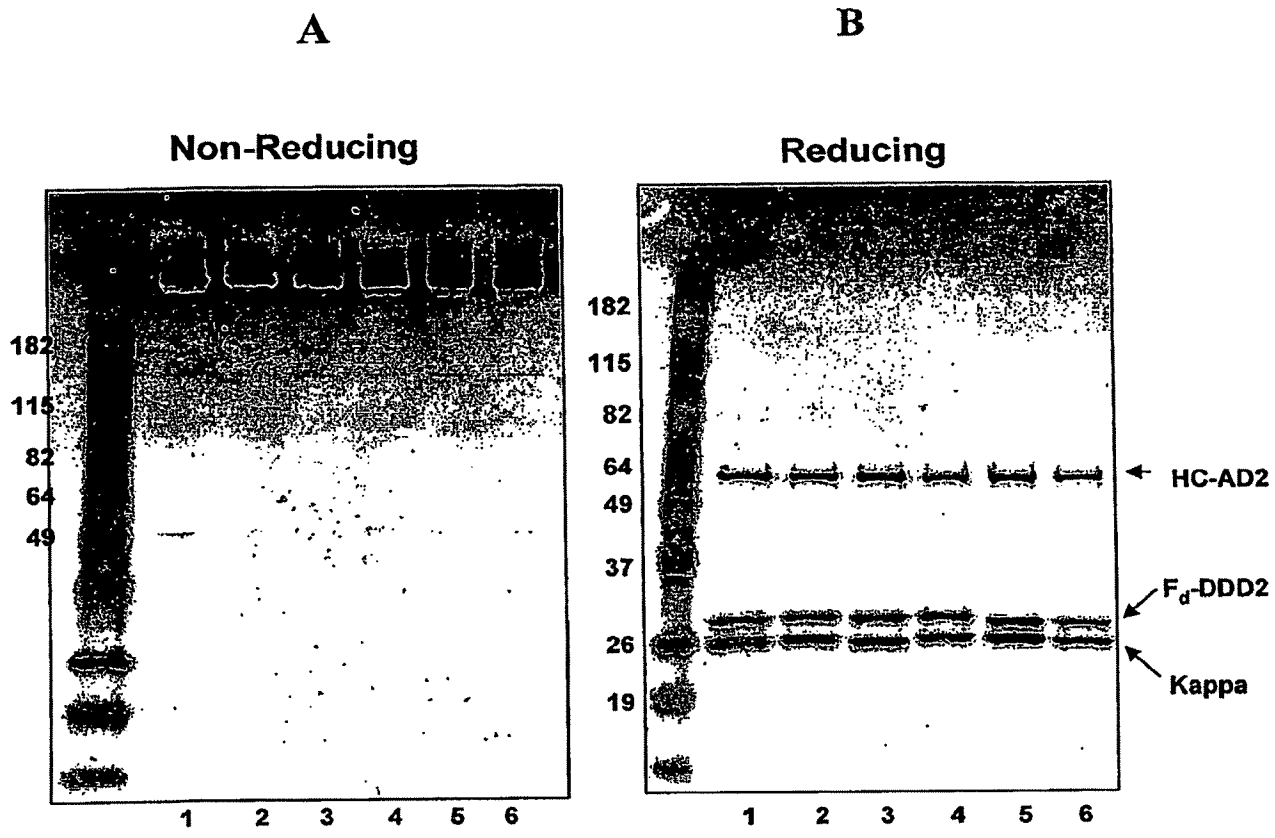


Figure 19



1. DNL1
2. DNL2
3. Hex-hA20
4. Hex-hLL2
5. DNL1C
6. DNL2C

Figure 20

SE-HPLC Analysis of Hex-hLL2

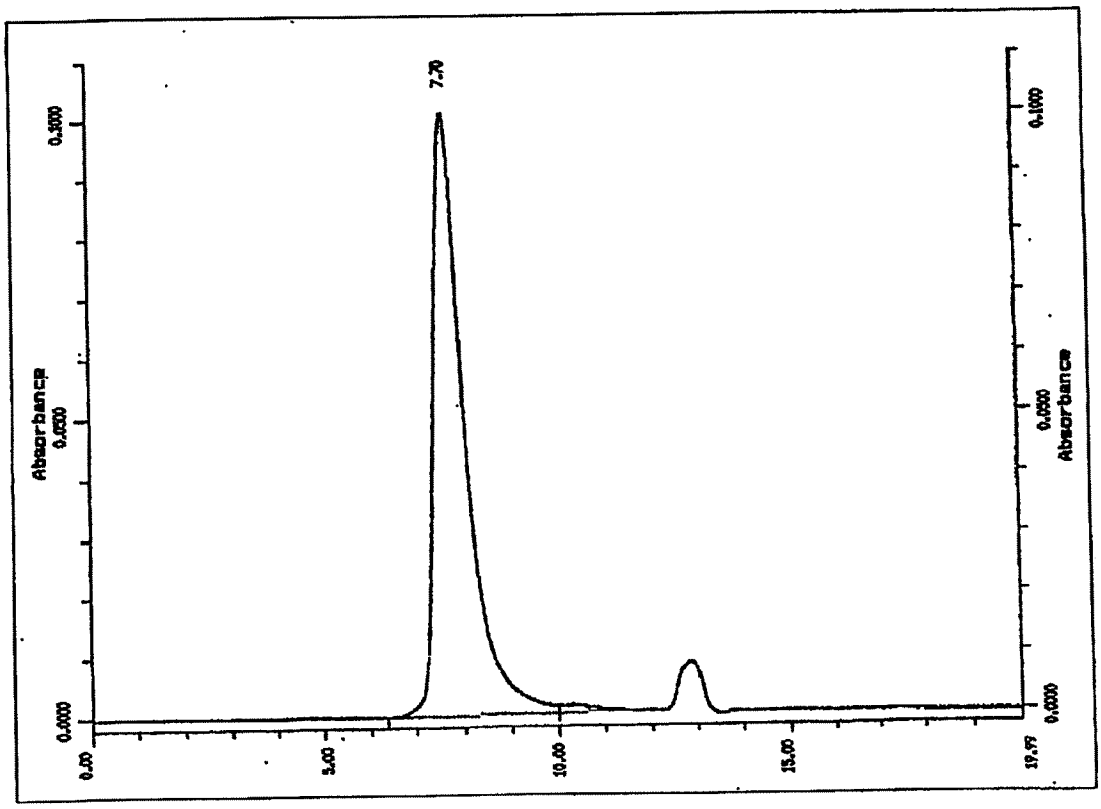
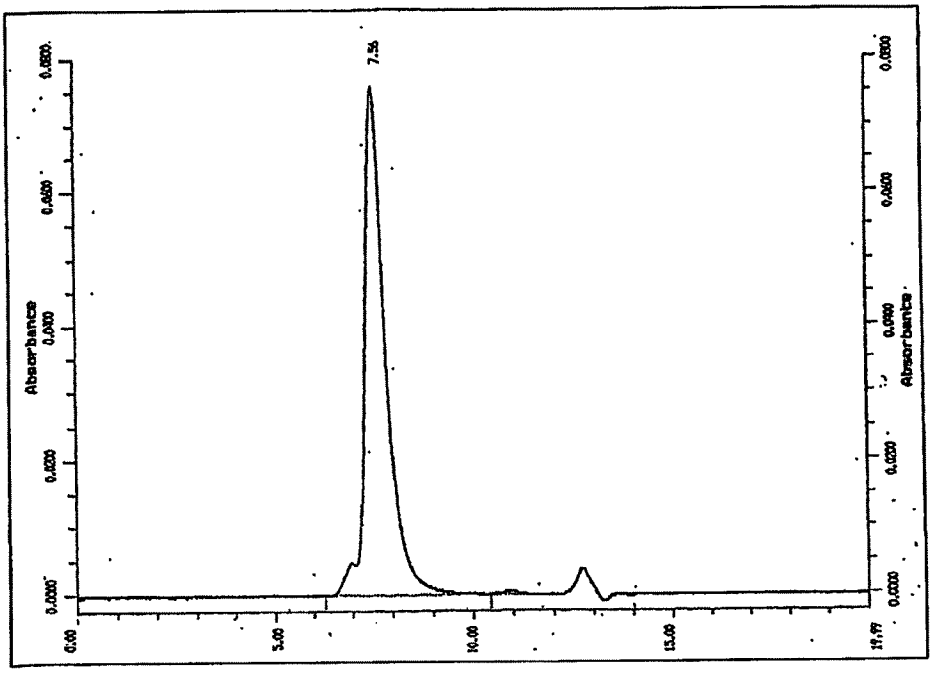


Figure 21

SE-HPLC Analysis of DNL1 and DNL1C

A



B

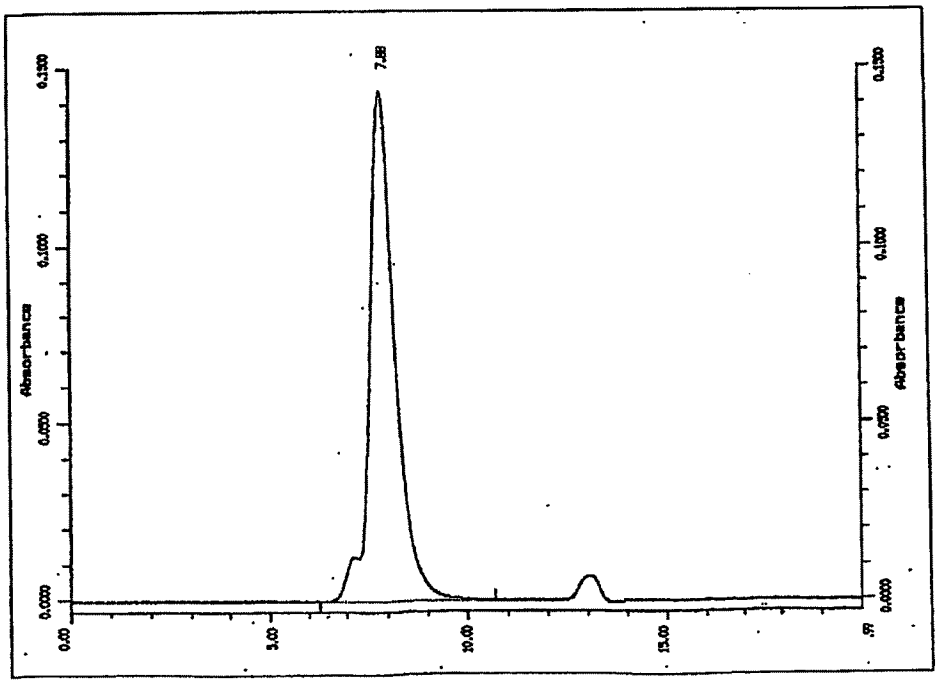


Figure 22

SE-HPLC Analysis of DNL2

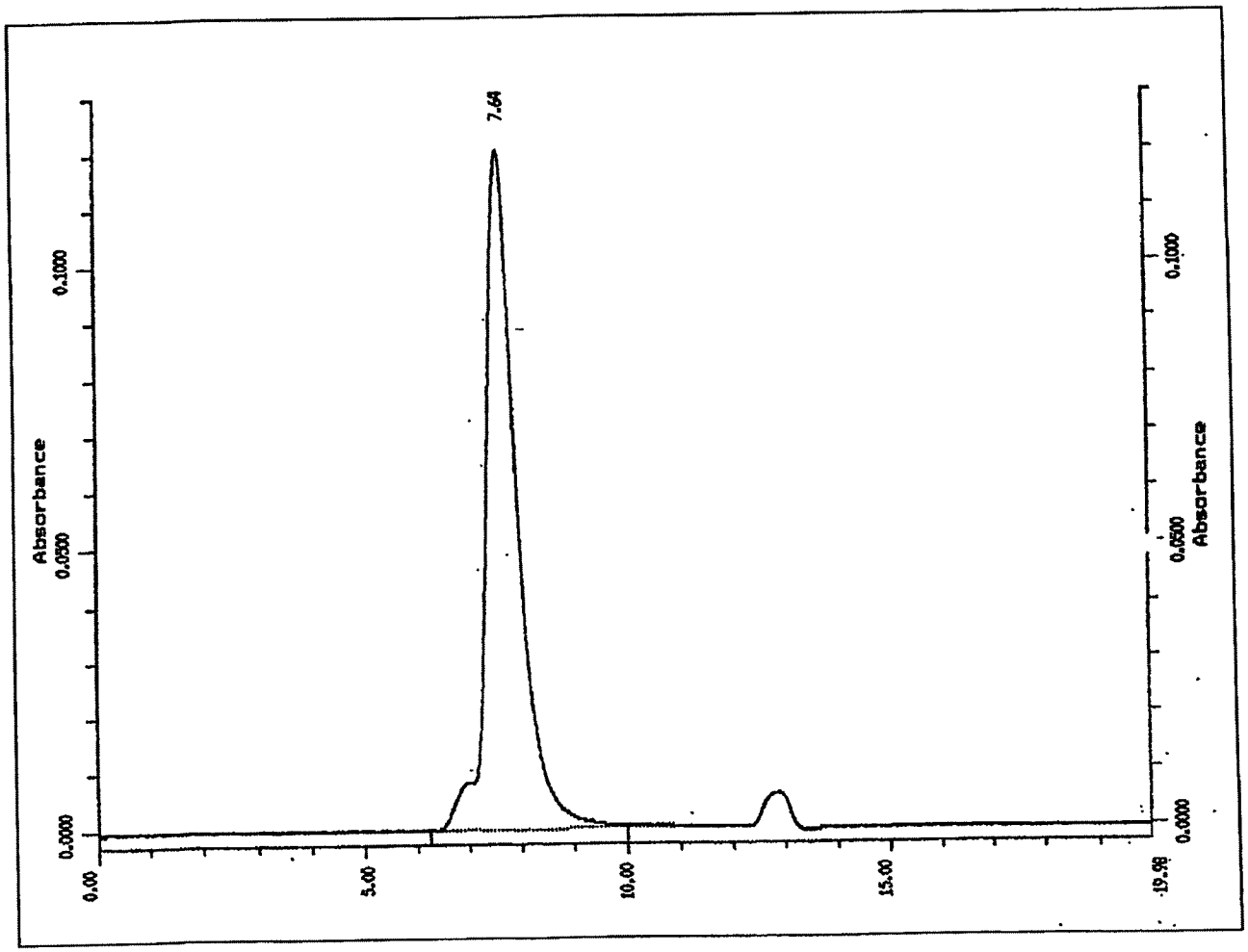
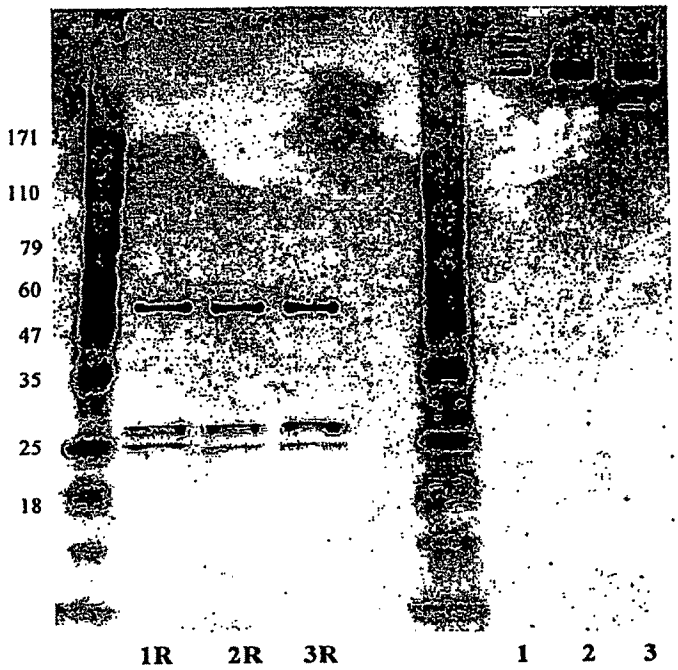


Figure 23



1. DNL3
2. K-Hex-hA20, prep 1
3. K-Hex-hA20, prep 2
1R. DNL3 reduced
2R. Khex-hA20 reduced
3R. K-Hex-hA20 reduced

Figure 24

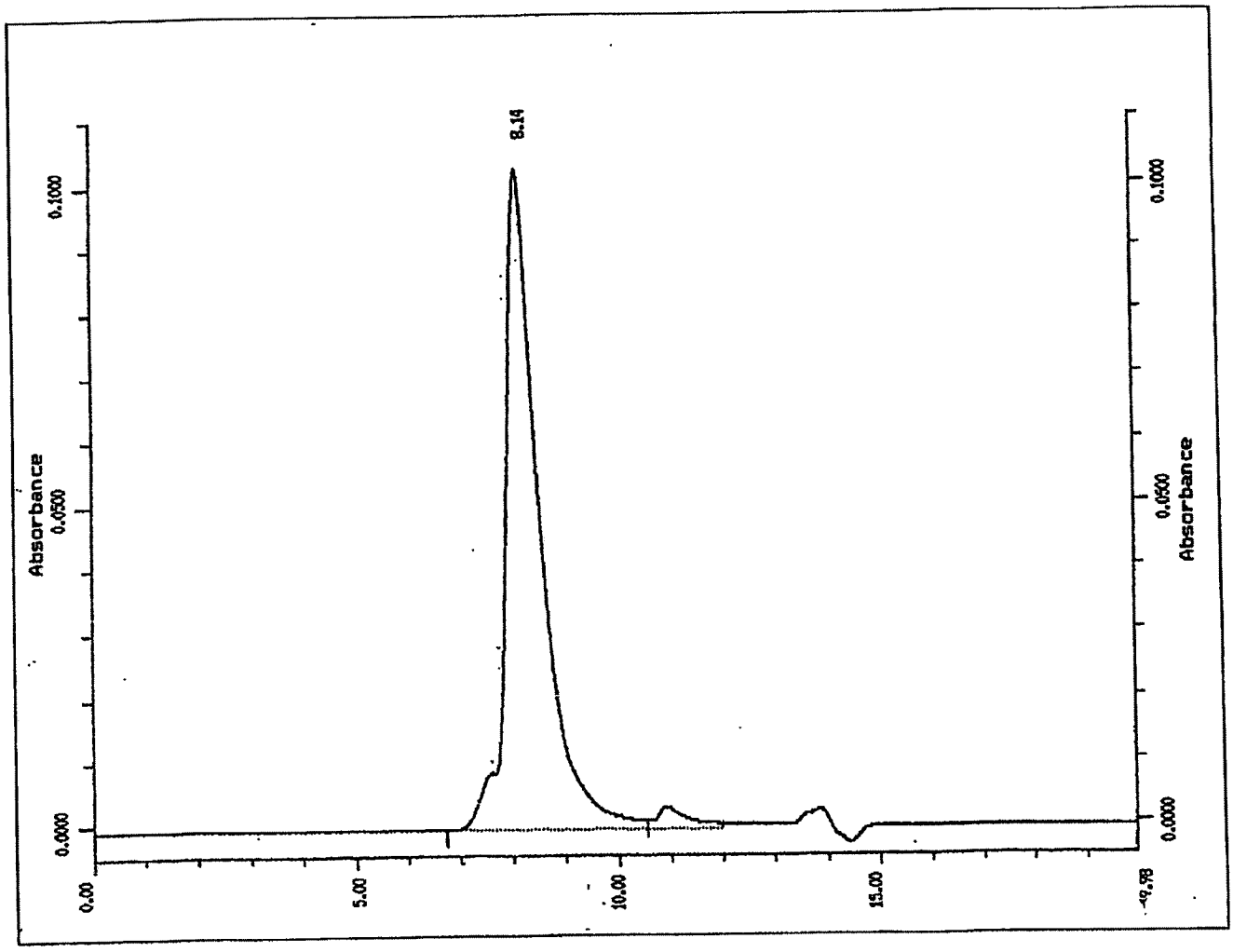
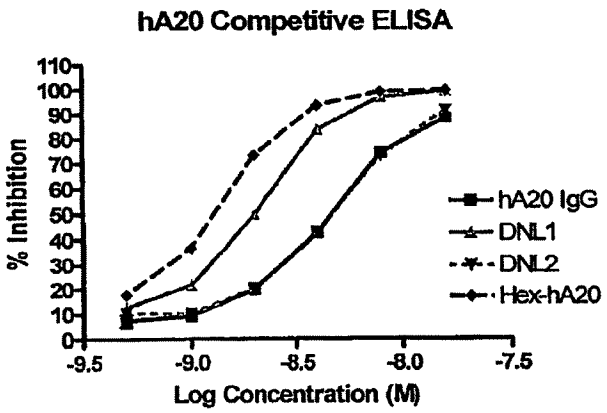


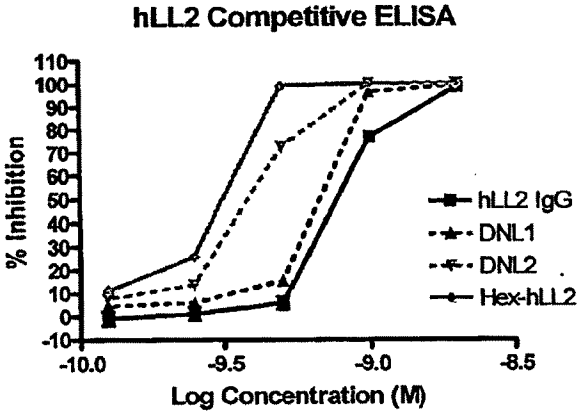
Figure 25

A



	EC50
hA20 IgG	5.155e-009
DNL1	1.355e-009
DNL2	5.791e-009
Hex-hA20	5.062e-010

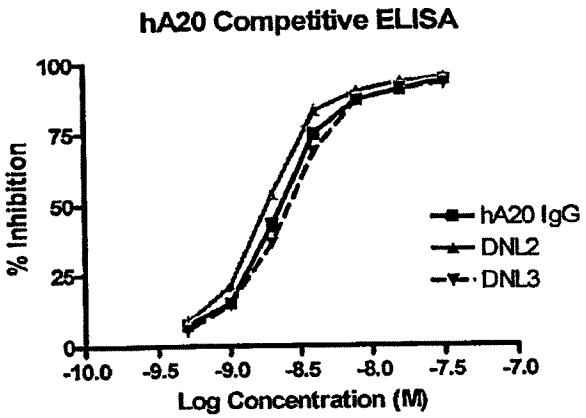
B



	EC50
hLL2 IgG	2.393e-009
DNL1	1.276e-009
DNL2	2.850e-010
Hex-hLL2	1.102e-010

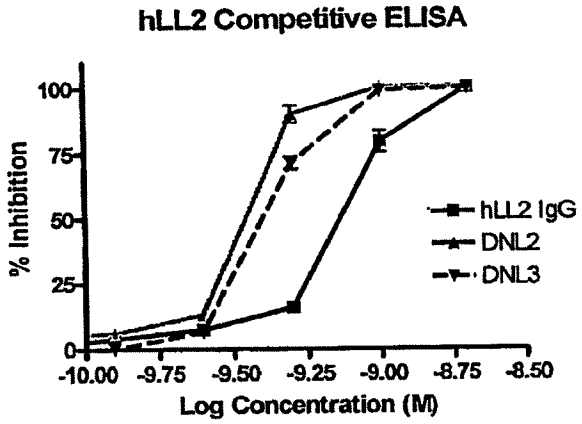
Figure 26

A



	EC50
hA20 IgG	1.482e-009
DNL2	9.605e-010
DNL3	1.820e-009

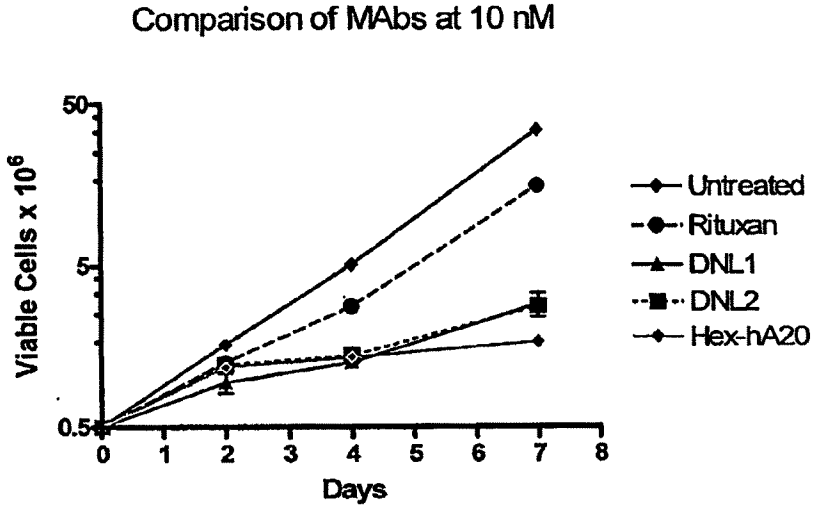
B



	EC50
hLL2 IgG	1.026e-009
DNL2	2.716e-010
DNL3	3.493e-010

Figure 27

A


Comparison of MAbs at 10 nM
Viable Cells x 10^6
50
5
0.5
0 1 2 3 4 5 6 7 8
Days
Untreated
Rituxan
DNL1
DNL2
Hex-hA20

B

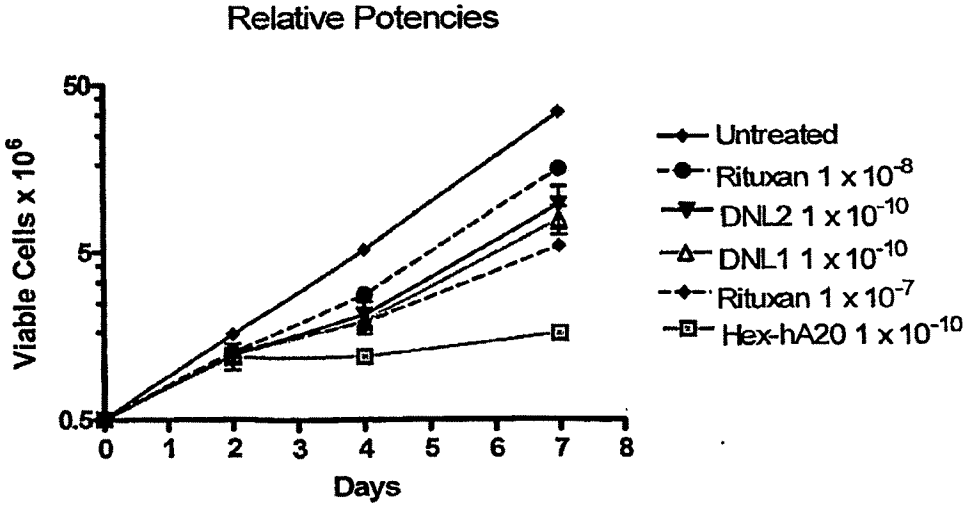
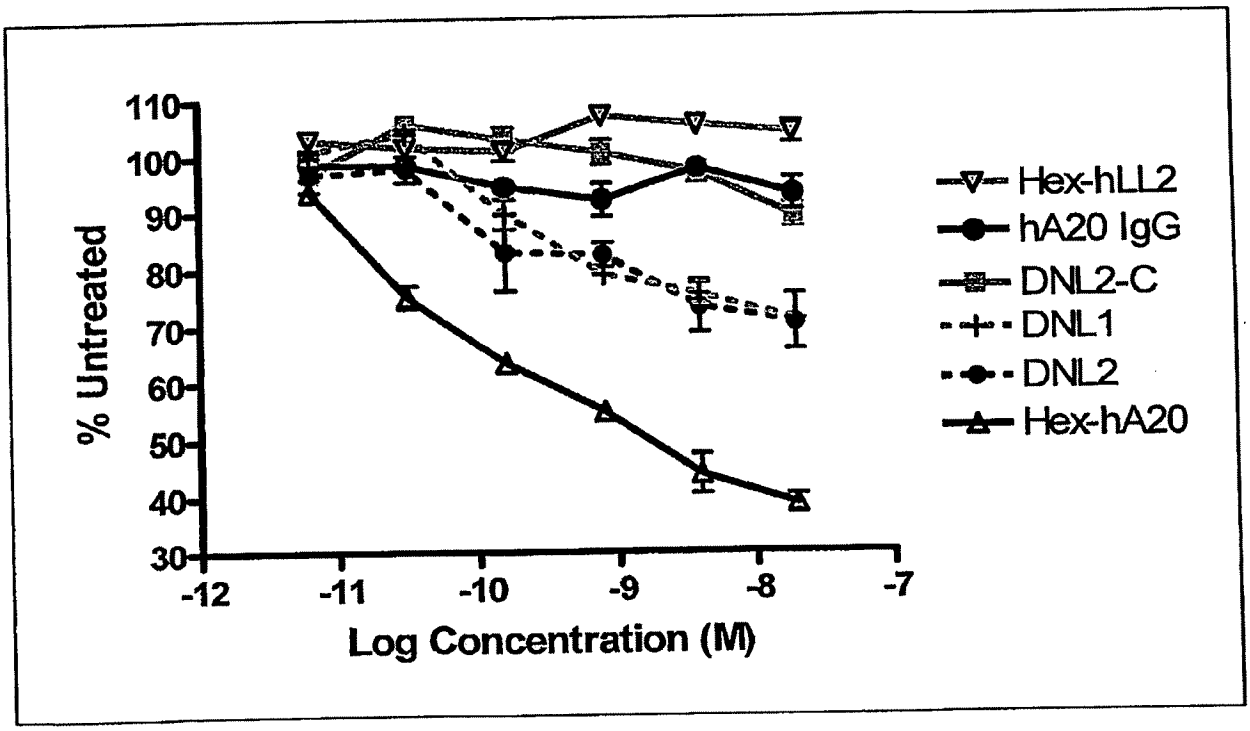
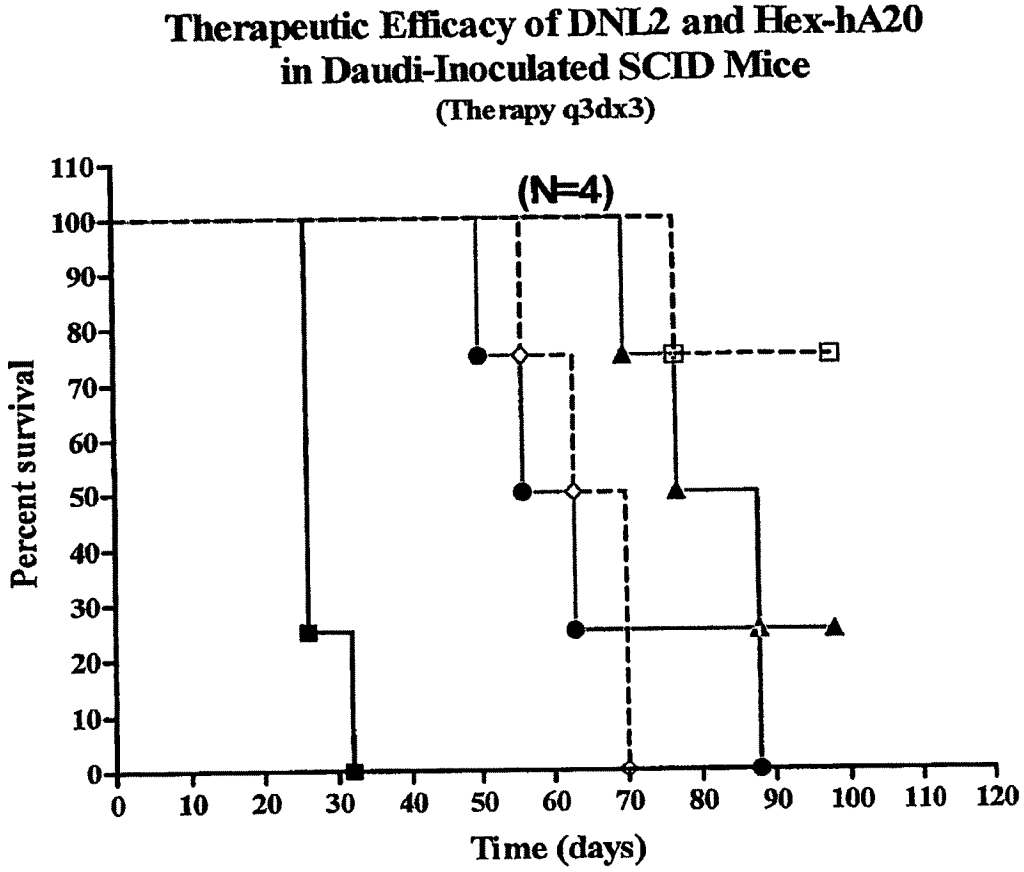

Relative Potencies
Viable Cells x 10^6
50
5
0.5
0 1 2 3 4 5 6 7 8
Days
Untreated
Rituxan 1 x 10^-8
DNL2 1 x 10^-10
DNL1 1 x 10^-10
Rituxan 1 x 10^-7
Hex-hA20 1 x 10^-10

Figure 28



	EC_{40}	Potency vs. Rituxan
Rituxan	> 2 x 10^{-7} M	-------
DNL1 & DNL2	2 x 10^{-9} M	>100 X
Hex-hA20	2 x 10^{-11} M	>10,000 X

Figure 29



	Median Survival	Survivors/ Total
—▲— DNL-2 (20 μg i.p., q3dx3)	82.5 days	1/4
—●— DNL-2 (4 μg i.p., q3dx3)	59.5 days	0/4
—⊟— hA20-hex (20 μg i.p., q3dx3)	>98 days	3/4
—◇— hA20-hex (4 μg i.p., q3dx3)	66.5 days	0/4
—■— Saline (100 μL i.p., q3dx3	26 days	0/4

P = 0.0058 for all treatments vs. saline control

Figure 30

Figure 31

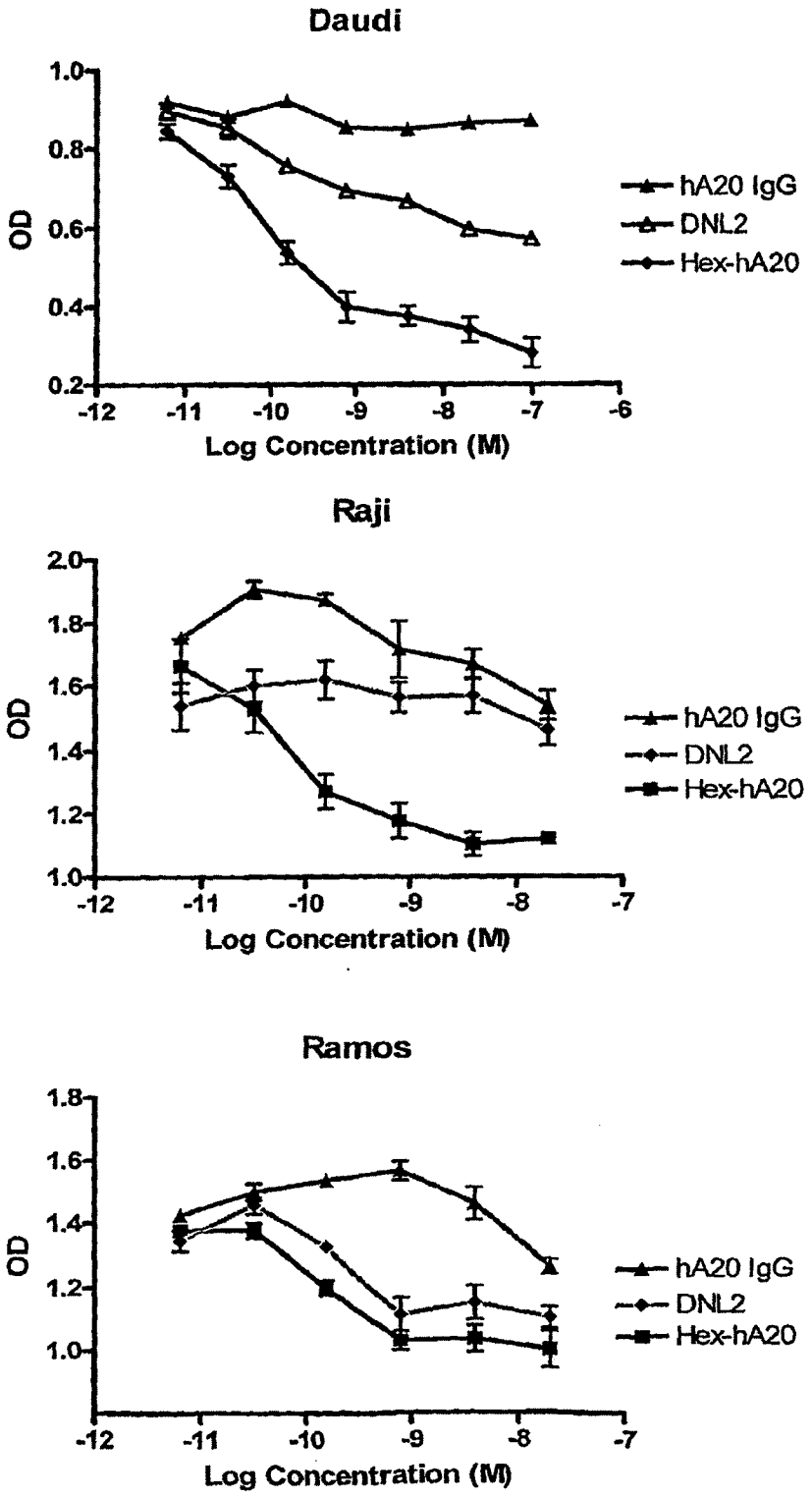

Daudi
OD
1.0
0.8
0.6
0.4
0.2
-12
-11
-10
-9
-8
-7
-6
Log Concentration (M)
hA20 IgG
DNL2
Hex-hA20
Raji
OD
2.0
1.8
1.6
1.4
1.2
1.0
-12
-11
-10
-9
-8
-7
Log Concentration (M)
hA20 IgG
DNL2
Hex-hA20
Ramos
OD
1.8
1.6
1.4
1.2
1.0
-12
-11
-10
-9
-8
-7
Log Concentration (M)
hA20 IgG
DNL2
Hex-hA20

Figure 32

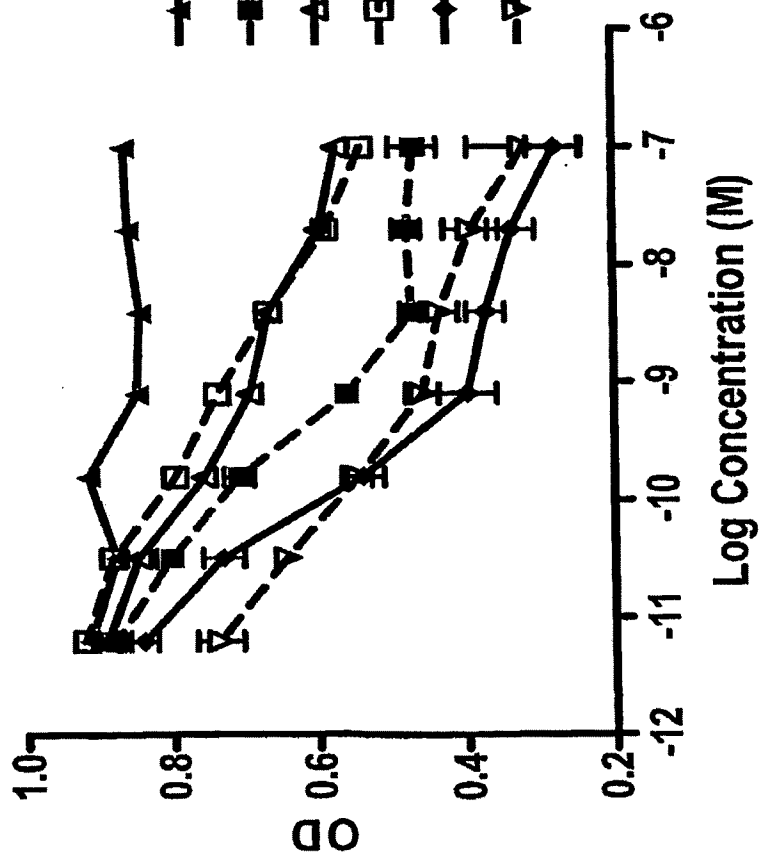

Daudi
hA20 IgG
hA20 IgG + cross-linker
DNL2
DNL2 + cross-linker
Hex-hA20
Hex-hA20 + cross-linker
OD
1.0
0.8
0.6
0.4
0.2
-12
-11
-10
-9
-8
-7
-6
Log Concentration (M)

Figure 33

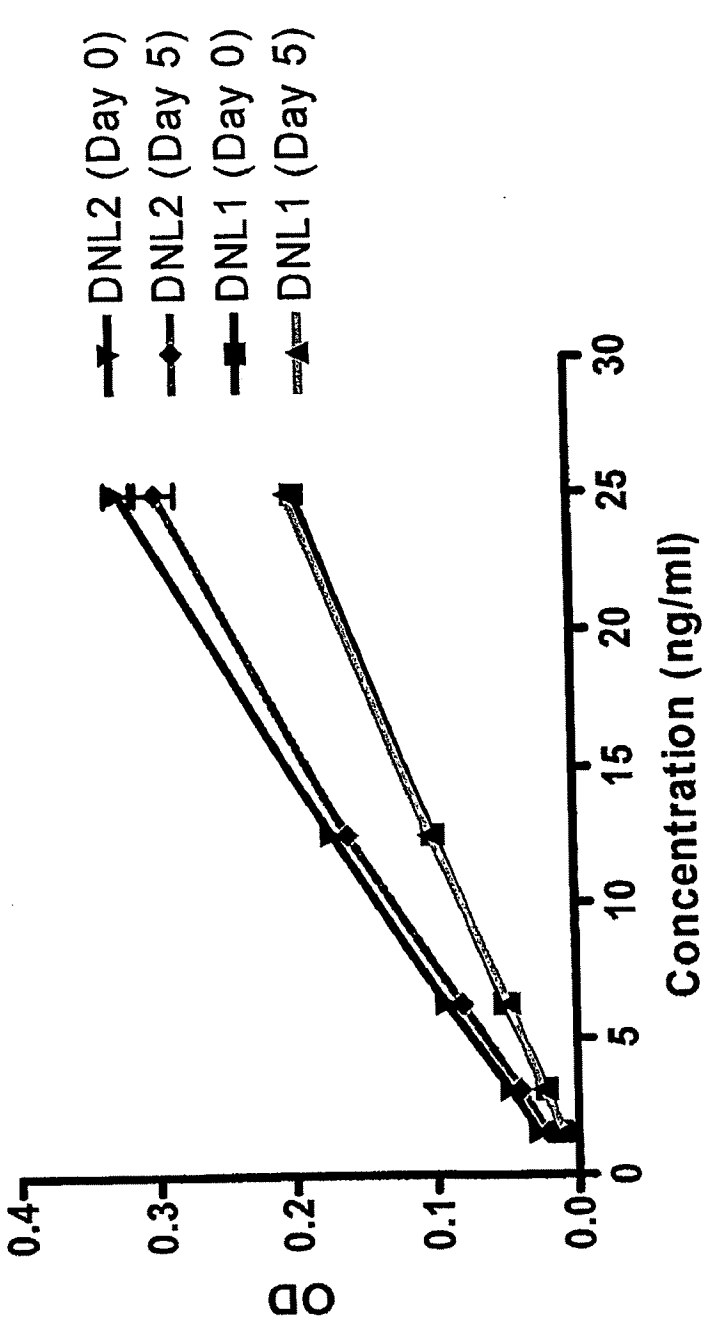

Serum Stability of DNL1 and DNL2
DNL2 (Day 0)
DNL2 (Day 5)
DNL1 (Day 0)
DNL1 (Day 5)
OD
0.4
0.3
0.2
0.1
0.0
0
5
10
15
20
25
30
Concentration (ng/ml)

Figure 34

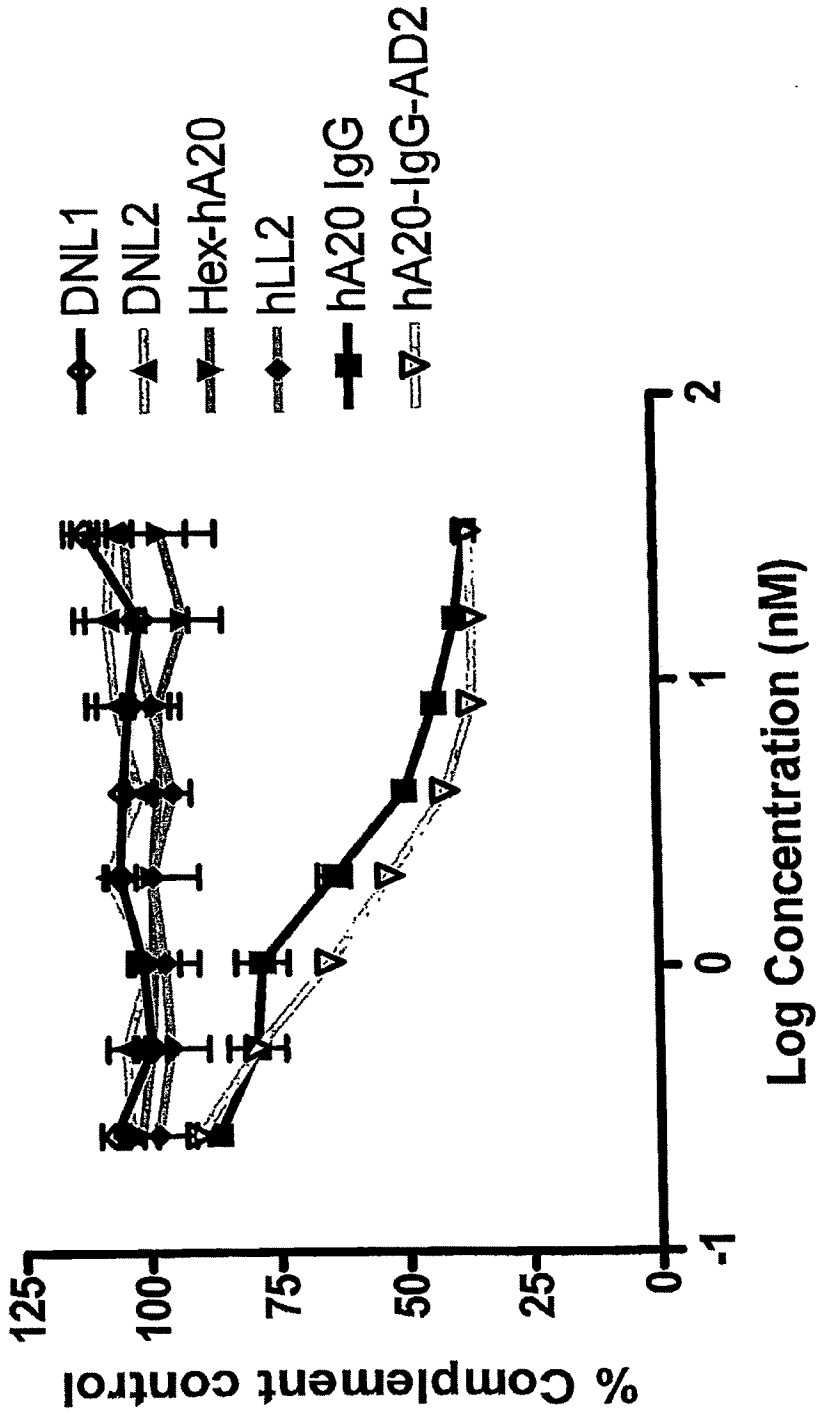
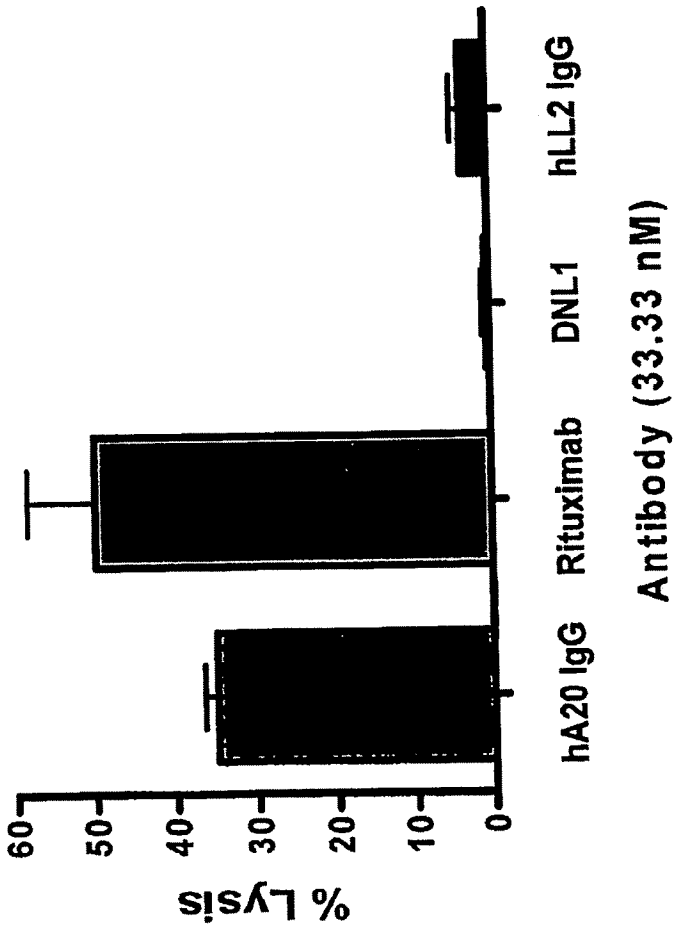

CDC Activity on Daudi Cells
DNL1
DNL2
Hex-hA20
hLL2
hA20 IgG
hA20-IgG-AD2
% Complement control
125
100
75
50
25
0
-1
0
1
2
Log Concentration (nM)

Figure 35


ADCC on Daudi
(40:1 Effector-to-Target Ratio)
% Lysis
60
50
40
30
20
10
0
hA20 IgG
Rituximab
DNL1
hLL2 IgG
Antibody (33.33 nM)

SEQUENCE LISTING

<110> IBC Pharmaceuticals, Inc.
Chang, Chien Hsing
Goldenberg, David M.
Rossi, Edmund A.

<120> MULTIVALENT IMMUNOGLOBULIN-BASED BIOACTIVE ASSEMBLIES

<130> 345588

<150> PCT/US2006/01076
<151> 2006-03-24

<150> PCT/US2006/012084
<151> 2006-03-29

<150> PCT/US2006/025499
<151> 2006-06-29

<150> 11/389,358
<151> 2006-03-24

<150> 11/391,584
<151> 2006-03-28

<150> 11/478,021
<151> 2006-06-29

<150> 60/782,332
<151> 2006-03-14

<150> 60/728,292
<151> 2005-10-19

<150> 60/751,196
<151> 2005-12-16

<150> 60/864,530
<151> 2006-11-06

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1 5 10 15

Thr Val Glu Val Leu Arg Gln Gln Pro Pro Asp Leu Val Glu Phe Ala
20 25 30

Val Glu Tyr Phe Thr Arg Leu Arg Glu Ala Arg Ala
35 40

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Tyr Thr Val Glu Val Leu Arg Gln Gln Pro Pro Asp Leu Val Glu Phe
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Ala Val Glu Tyr Phe Thr Arg Leu Arg Glu Ala Arg Ala
35 40 45

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Ala

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Pro Pro Gly Leu Thr Glu Leu Leu Gln Gly Tyr Thr Val Glu Val Leu
20 25 30

Arg Gln Gln Pro Pro Asp Leu Val Glu Phe Ala Val Glu Tyr Phe Thr
35 40 45

Arg Leu Arg Glu Ala Arg Ala
50 55

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tgctgtcgca gcacctccac cgtgtagccc tg 92

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Leu Ala Lys Gln Ile Val Asp Asn Ala Ile Gln Gln Ala
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Ile Gln Lys Ala Leu Tyr Glu Ile Gln Lys
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cggagctgct gca 73

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Phe Arg Glu Leu Asn Glu Ala Leu Glu Leu Lys Asp Ala Gln Ala

20 25 30

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Gln Ala Lys Gly Cys
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