1/11

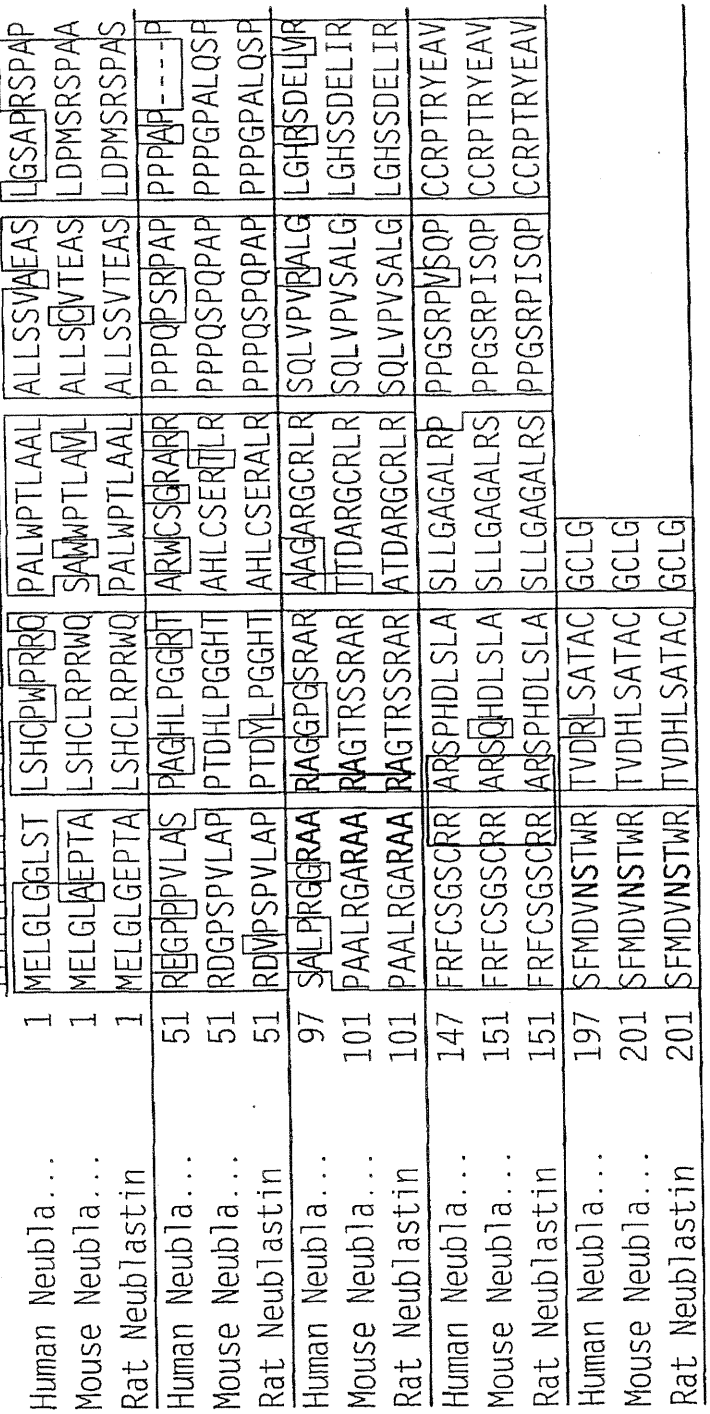

10
20
30
40
50
Human Neubla... 1 MELGLGGLST LSHCPWPRRQ PALWPTLAAL ALLSSVAEAS LGSAPRSPAP
Mouse Neubla... 1 MELGLAEPTA LSHCLRPRWQ SAMWPTLAVL ALLSCVTEAS LDPMSRSPAA
Rat Neublastin 1 MELGLGEPTA LSHCLRPRWQ PALWPTLAAL ALLSSVTEAS LDPMSRSPAS
Human Neubla... 51 REGPPPVLAS PAGHLPGGRT ARWCSGRARR PPPQPSRPAP PPPAP---P
Mouse Neubla... 51 RDGPSPVLAP PTDHLPGGHT AHLCSERTLR PPPQSPQPAP PPPGPALQSP
Rat Neublastin 51 RDVPSPVLAP PTDYLPGGHT AHLCSERALR PPPQSPQPAP PPPGPALQSP
Human Neubla... 97 SALPRGGRAA RAGGPGSRAR AAGARGCRLR SQLVPVRALG LGHRSDELVR
Mouse Neubla... 101 PAALRGARAA RAGTRSSRAR TTDARGCRLR SQLVPVSALG LGHSSDELIR
Rat Neublastin 101 PAALRGARAA RAGTRSSRAR ATDARGCRLR SQLVPVSALG LGHSSDELIR
Human Neubla... 147 FRFCSGSCRR ARSPHDLSLA SLLGAGALRP PPGSRPVSQP CCRPTRYEAV
Mouse Neubla... 151 FRFCSGSCRR ARSQHDLSLA SLLGAGALRS PPGSRPISQP CCRPTRYEAV
Rat Neublastin 151 FRFCSGSCRR ARSPHDLSLA SLLGAGALRS PPGSRPISQP CCRPTRYEAV
Human Neubla... 197 SFMDVNSTWR TVDRLSATAC GCLG
Mouse Neubla... 201 SFMDVNSTWR TVDHLSATAC GCLG
Rat Neublastin 201 SFMDVNSTWR TVDHLSATAC GCLG

FIG. 1

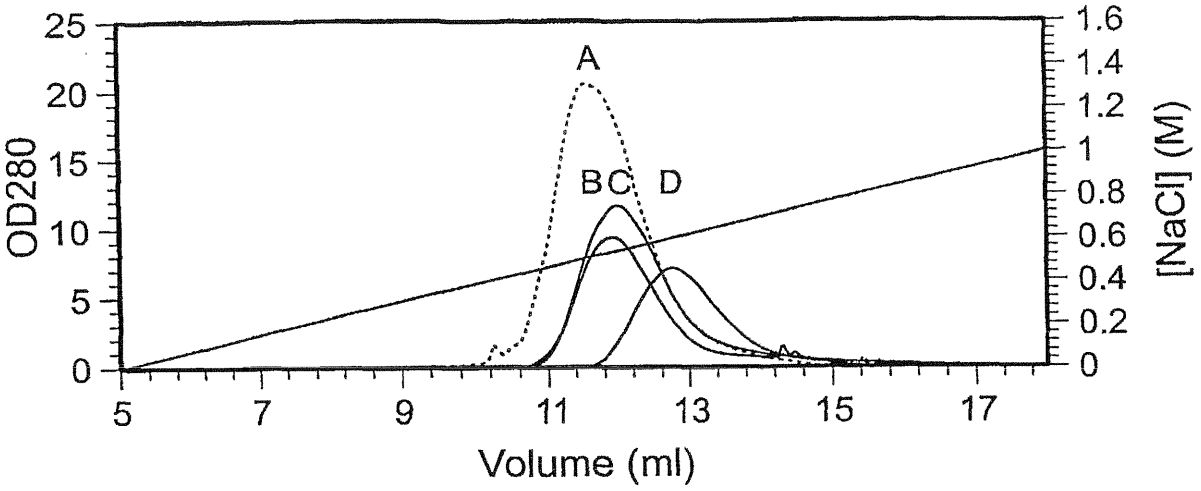

25
20
15
10
5
0
OD280
A
B C D
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
[NaCl] (M)
5
7
9
11
13
15
17
Volume (ml)

FIG. 2A

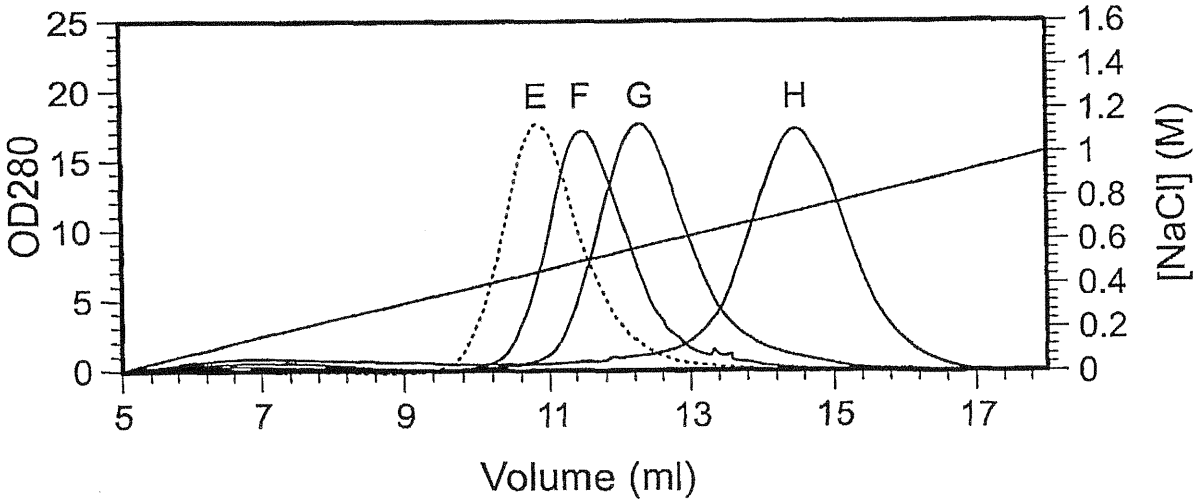

25
20
15
10
5
0
OD280
E F G
H
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
[NaCl] (M)
5
7
9
11
13
15
17
Volume (ml)

FIG. 2B

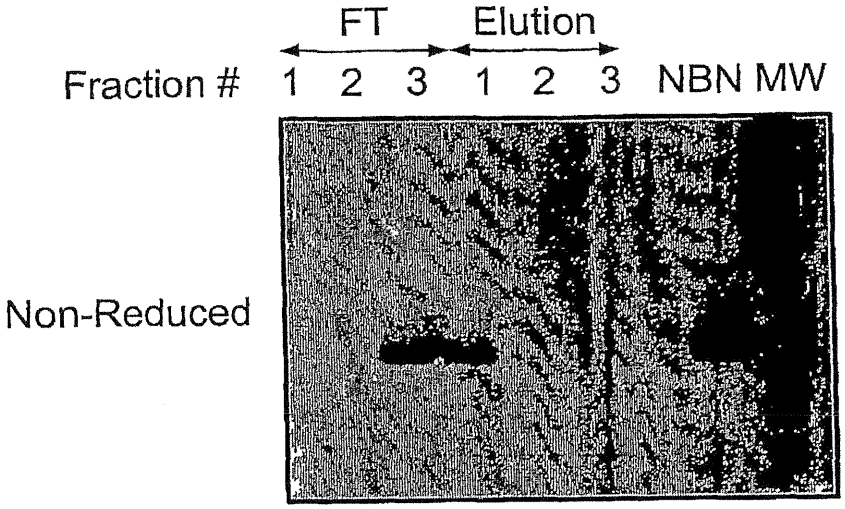

FT
Elution
Fraction # 1 2 3 1 2 3 NBN MW
Non-Reduced

FIG. 3A

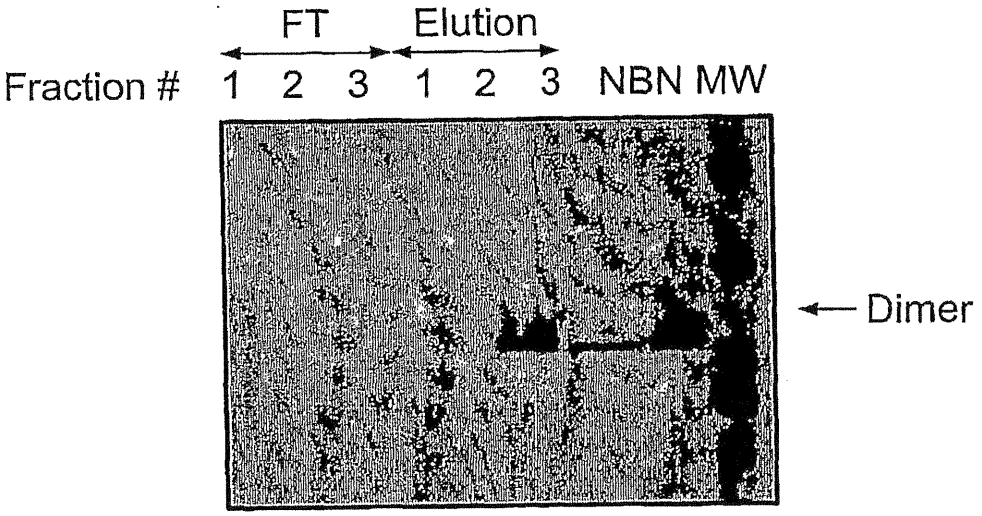

FT
Elution
Fraction # 1 2 3 1 2 3 NBN MW
Dimer

FIG. 3B

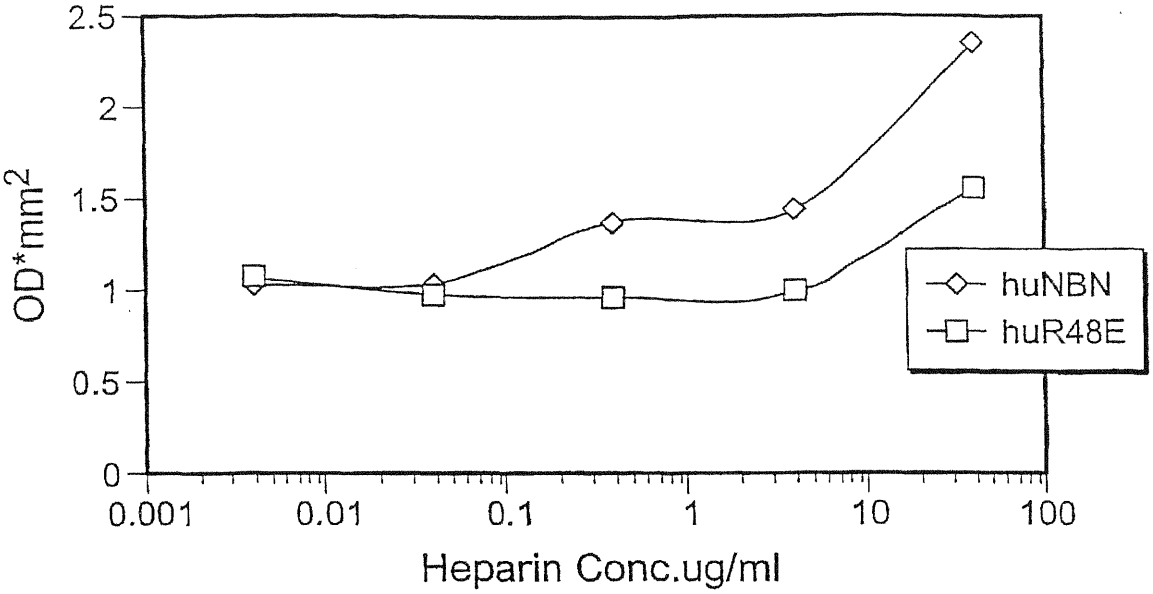

2.5
2
1.5
1
0.5
0
OD*mm2
0.001
0.01
0.1
1
10
100
Heparin Conc.ug/ml
huNBN
huR48E

FIG. 4

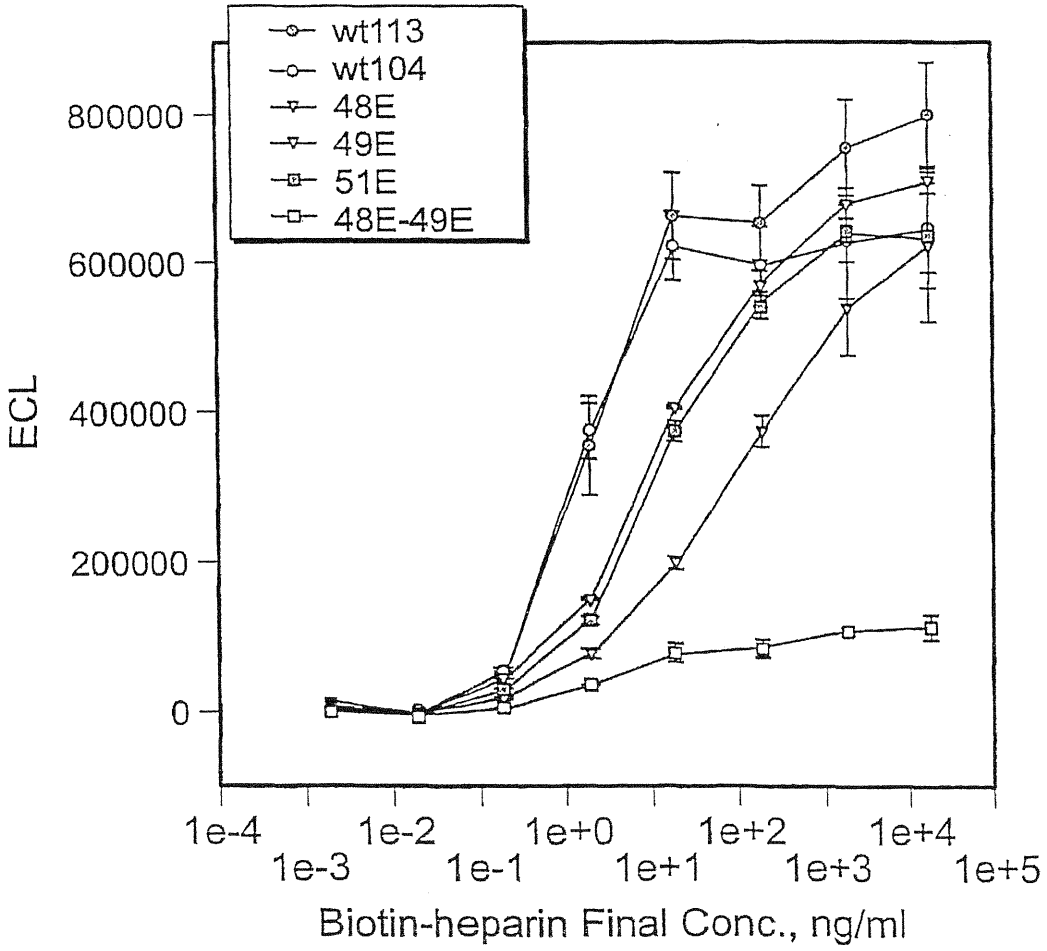

wt113
wt104
48E
49E
51E
48E-49E
800000
600000
400000
200000
0
ECL
1e-4
1e-3
1e-2
1e-1
1e+0
1e+1
1e+2
1e+3
1e+4
1e+5
Biotin-heparin Final Conc., ng/ml

FIG. 5

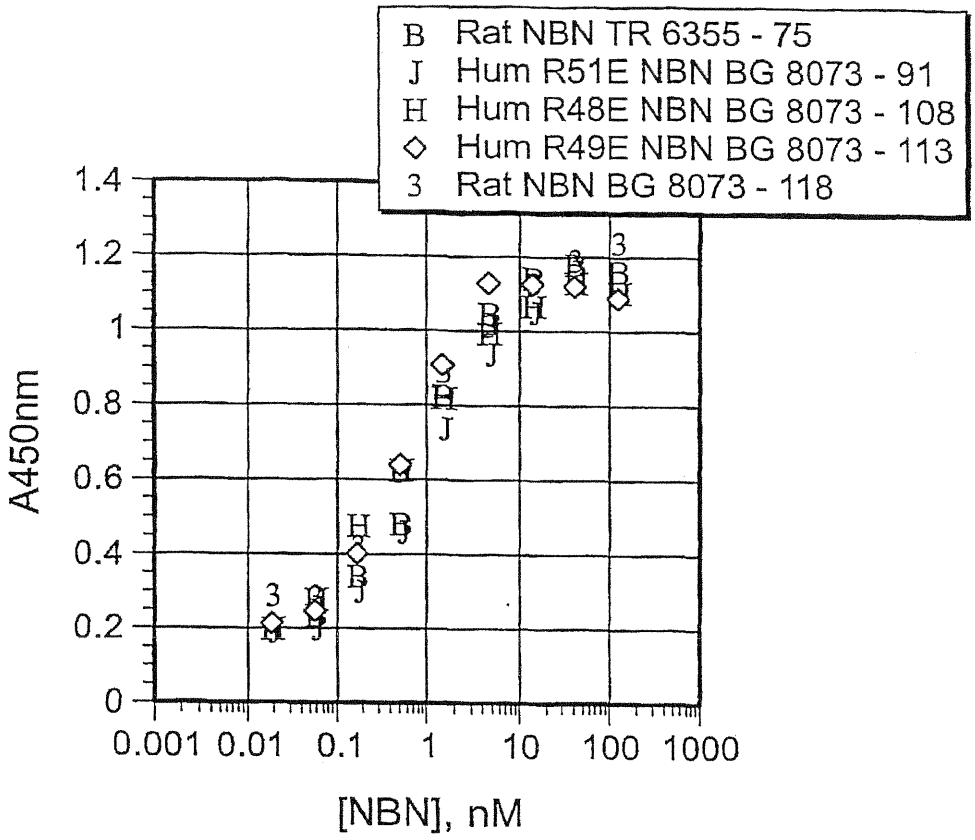

B Rat NBN TR 6355 - 75
J Hum R51E NBN BG 8073 - 91
H Hum R48E NBN BG 8073 - 108
◇ Hum R49E NBN BG 8073 - 113
3 Rat NBN BG 8073 - 118
A450nm
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0.001 0.01 0.1 1 10 100 1000
[NBN], nM

FIG. 6

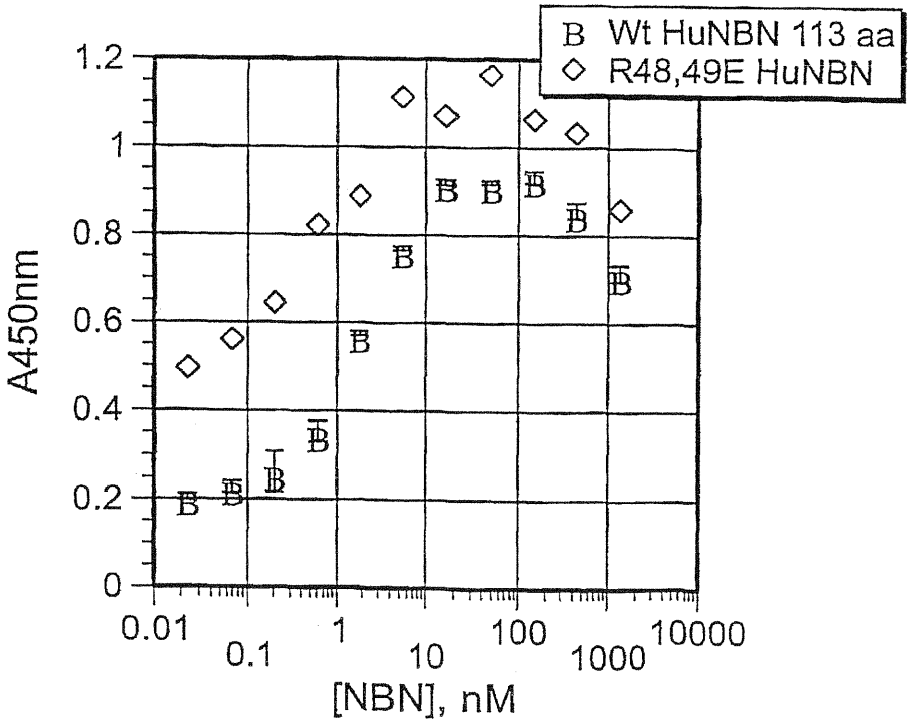

B Wt HuNBN 113 aa
◇ R48,49E HuNBN
A450nm
1.2
1
0.8
0.6
0.4
0.2
0
0.01 0.1 1 10 100 1000 10000
[NBN], nM

FIG. 7A

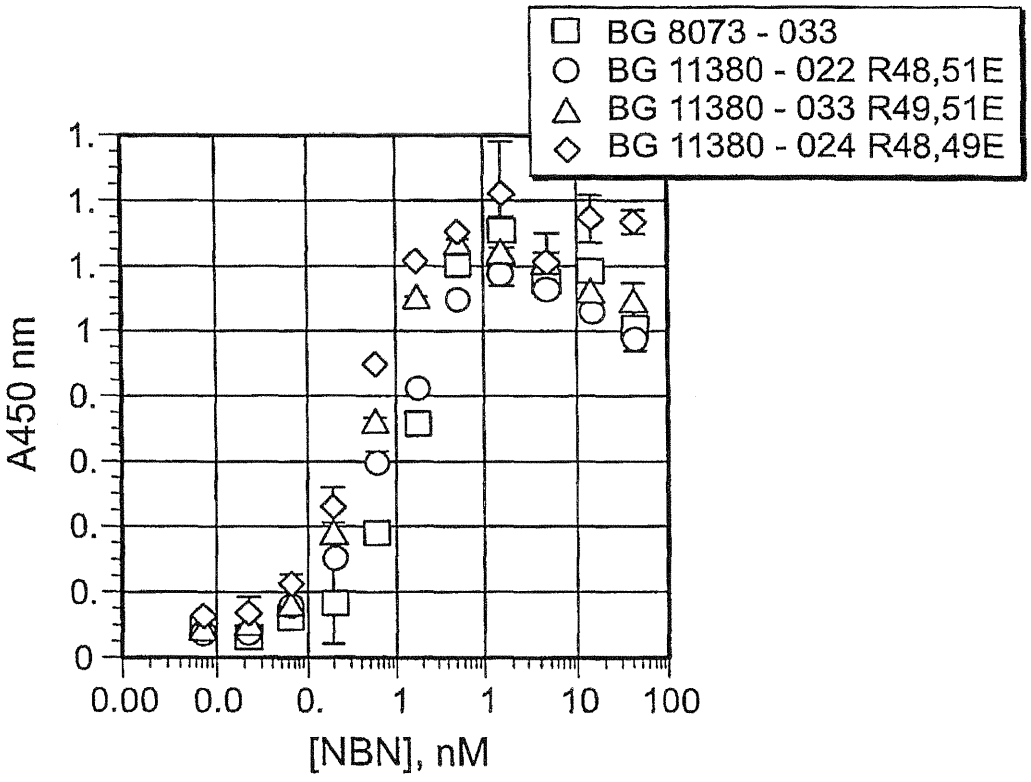

BG 8073 - 033
BG 11380 - 022 R48,51E
BG 11380 - 033 R49,51E
BG 11380 - 024 R48,49E
A450 nm
1.
1.
1.
1
0.
0.
0.
0.
0
0.00
0.0
0.
1
1
10
100
[NBN], nM

FIG. 7B

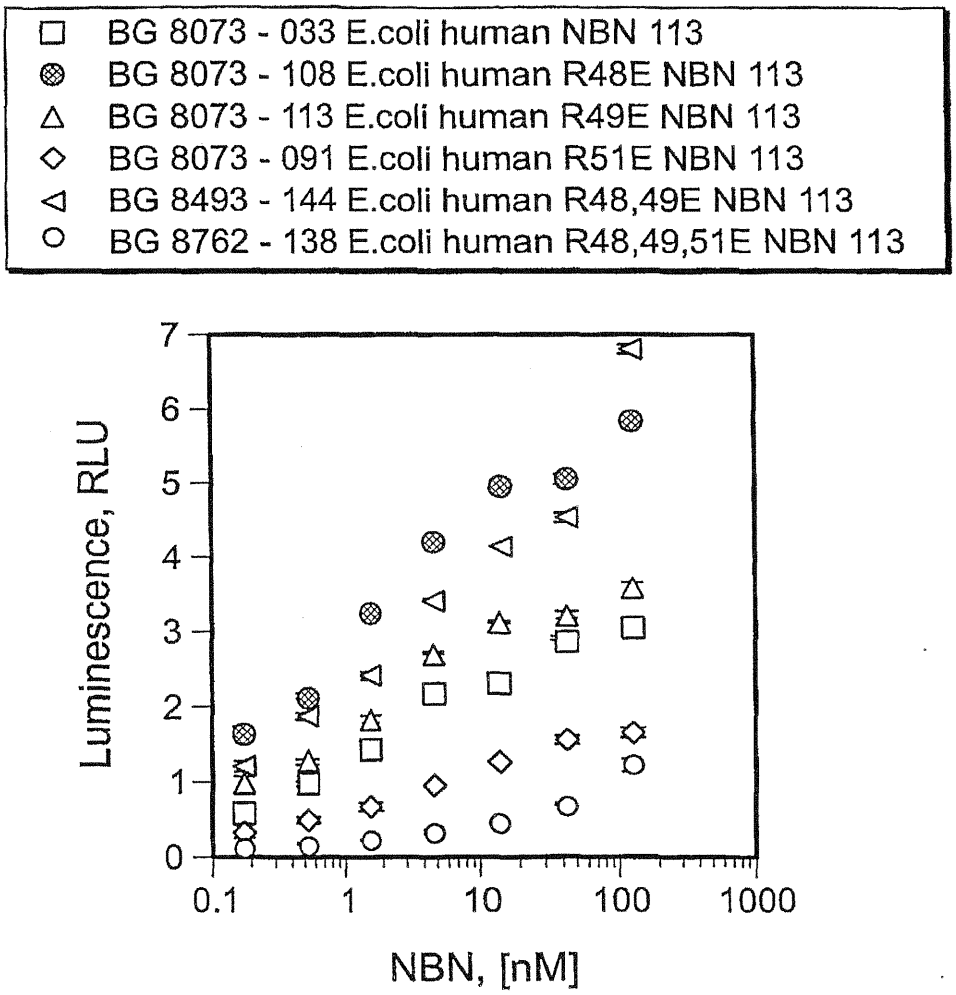

BG 8073 - 033 E.coli human NBN 113
BG 8073 - 108 E.coli human R48E NBN 113
BG 8073 - 113 E.coli human R49E NBN 113
BG 8073 - 091 E.coli human R51E NBN 113
BG 8493 - 144 E.coli human R48,49E NBN 113
BG 8762 - 138 E.coli human R48,49,51E NBN 113
Luminescence, RLU
0
1
2
3
4
5
6
7
0.1
1
10
100
1000
NBN, [nM]

FIG. 8

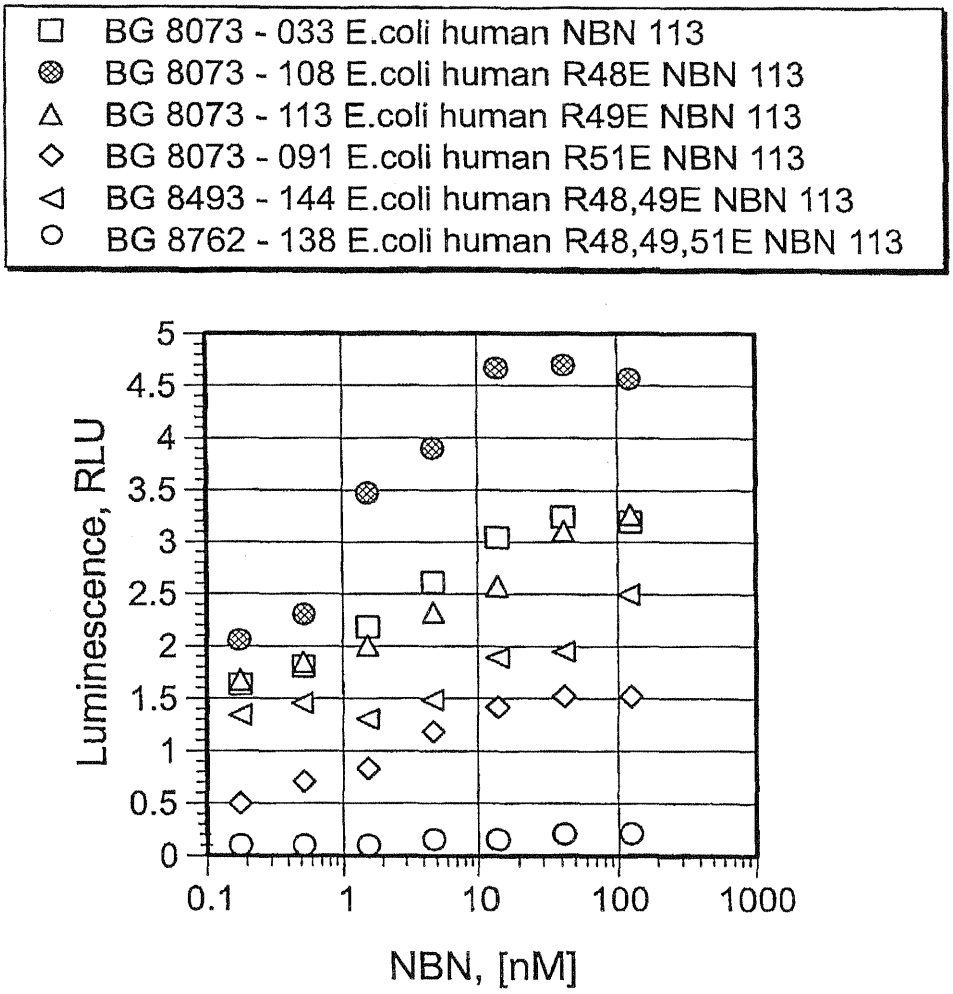

BG 8073 - 033 E.coli human NBN 113
BG 8073 - 108 E.coli human R48E NBN 113
BG 8073 - 113 E.coli human R49E NBN 113
BG 8073 - 091 E.coli human R51E NBN 113
BG 8493 - 144 E.coli human R48,49E NBN 113
BG 8762 - 138 E.coli human R48,49,51E NBN 113
Luminescence, RLU
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
0.1
1
10
100
1000
NBN, [nM]

FIG. 9

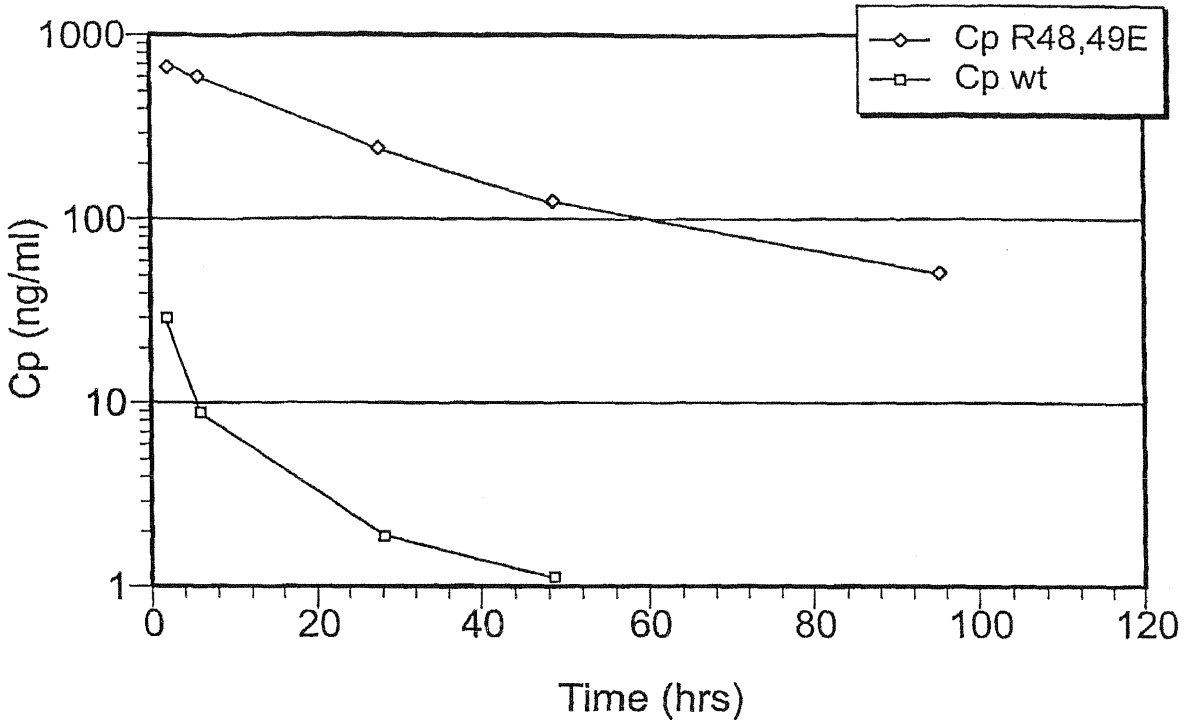

Cp R48,49E
Cp wt
1000
100
10
1
Cp (ng/ml)
0
20
40
60
80
100
120
Time (hrs)

FIG. 10

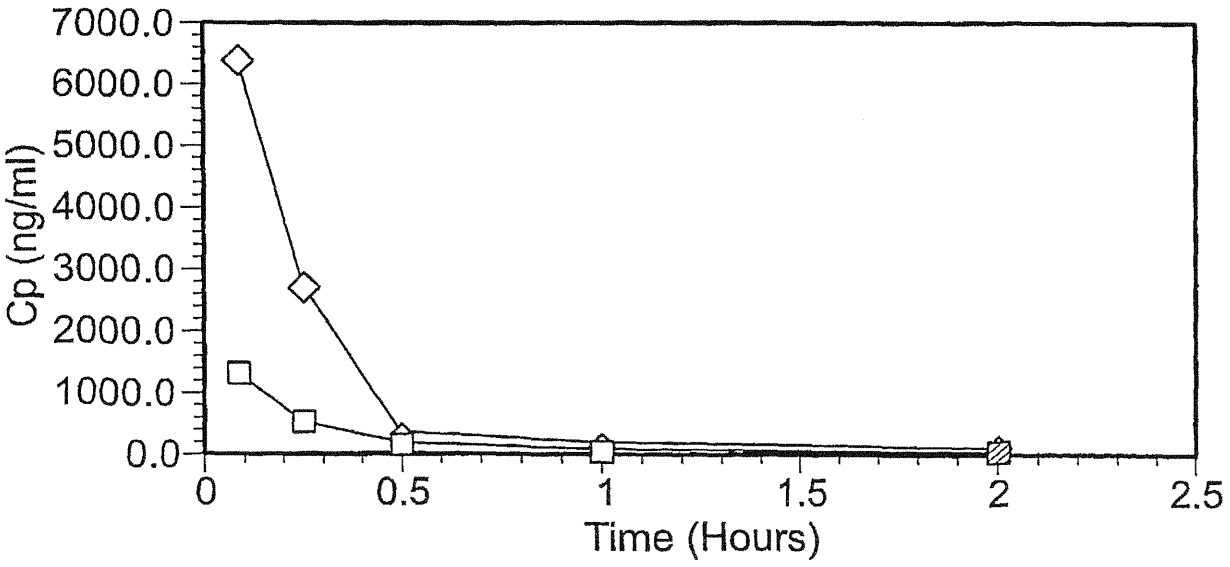

7000.0
6000.0
5000.0
4000.0
3000.0
2000.0
1000.0
0.0
Cp (ng/ml)
0
0.5
1
1.5
2
2.5
Time (Hours)

FIG. 11

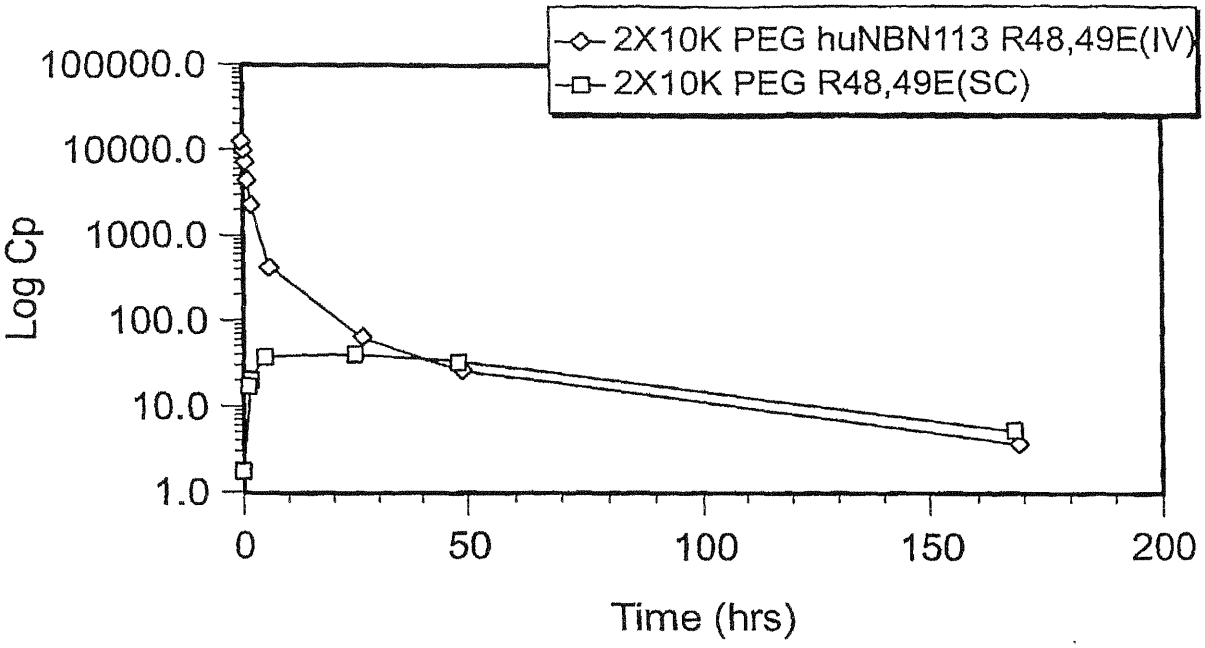

2X10K PEG huNBN113 R48,49E(IV)
2X10K PEG R48,49E(SC)
100000.0
10000.0
1000.0
100.0
10.0
1.0
Log Cp
0
50
100
150
200
Time (hrs)

FIG. 12

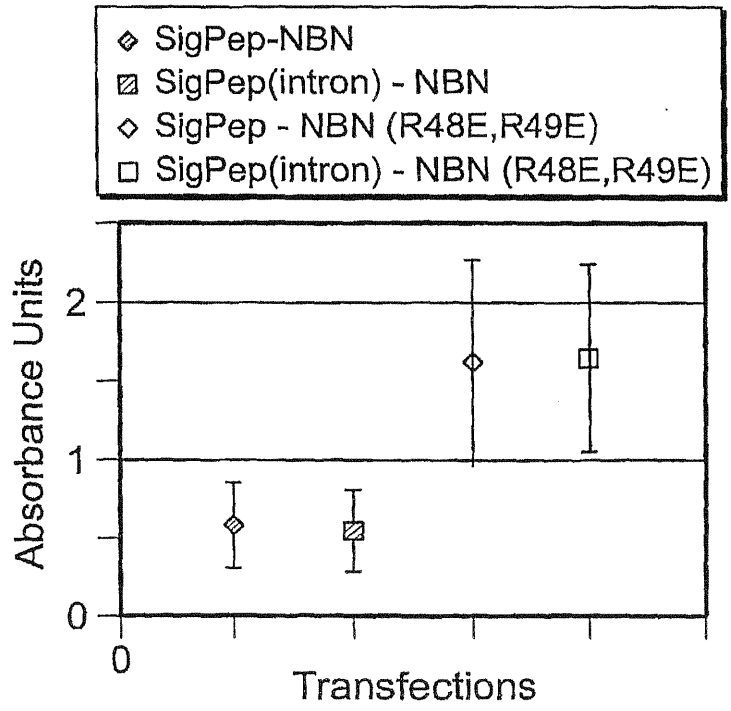

SigPep-NBN
SigPep(intron) - NBN
SigPep - NBN (R48E,R49E)
SigPep(intron) - NBN (R48E,R49E)
Absorbance Units
2
1
0
0
Transfections

FIG. 13

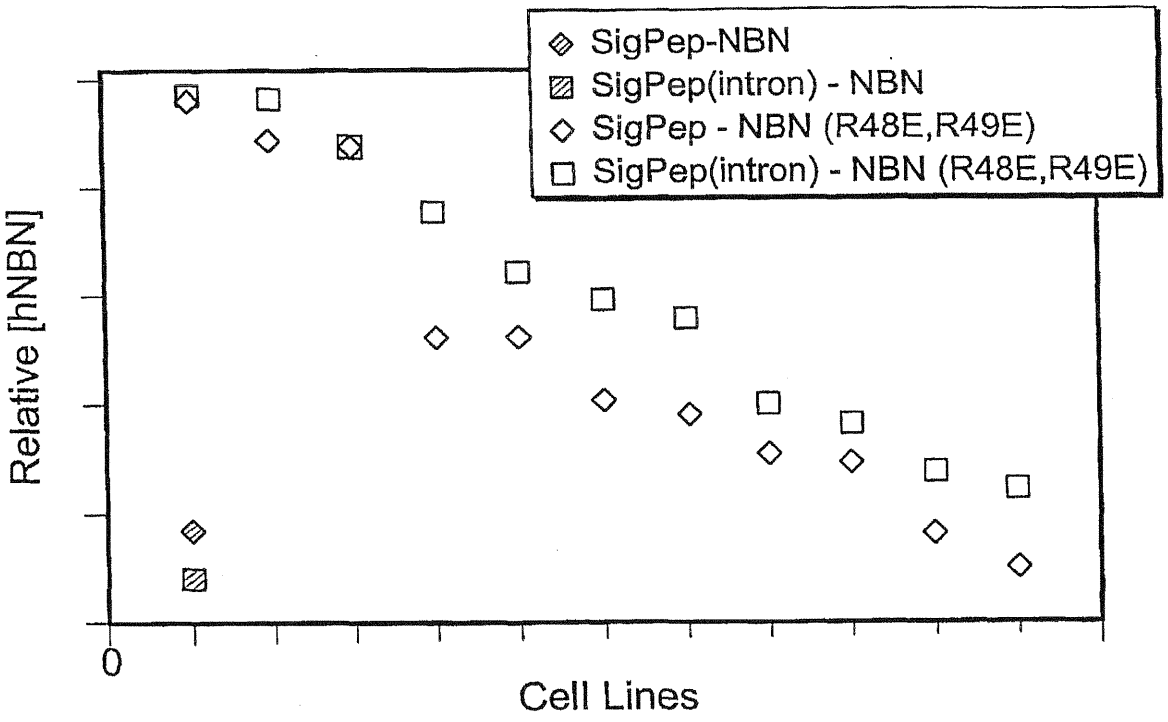

SigPep-NBN
SigPep(intron) - NBN
SigPep - NBN (R48E,R49E)
SigPep(intron) - NBN (R48E,R49E)
Relative [hNBN]
0
Cell Lines

FIG. 14