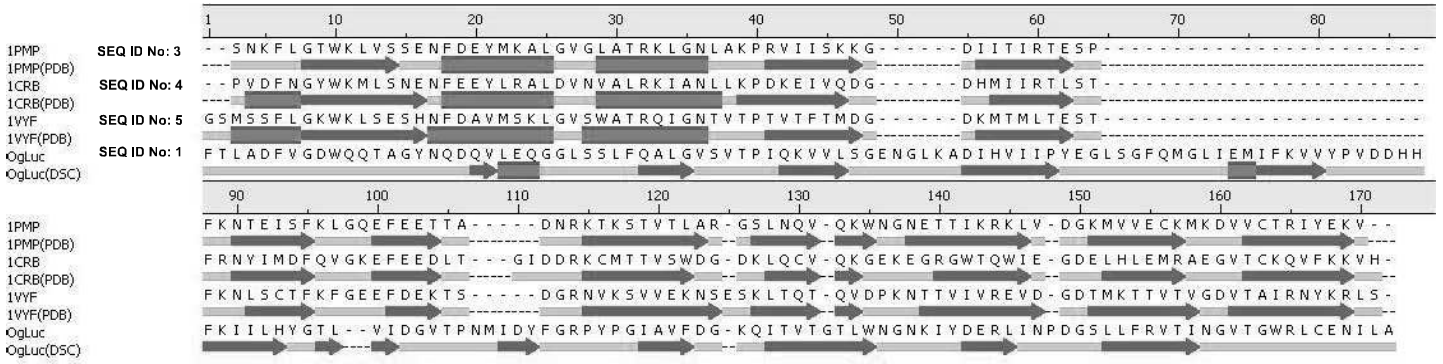
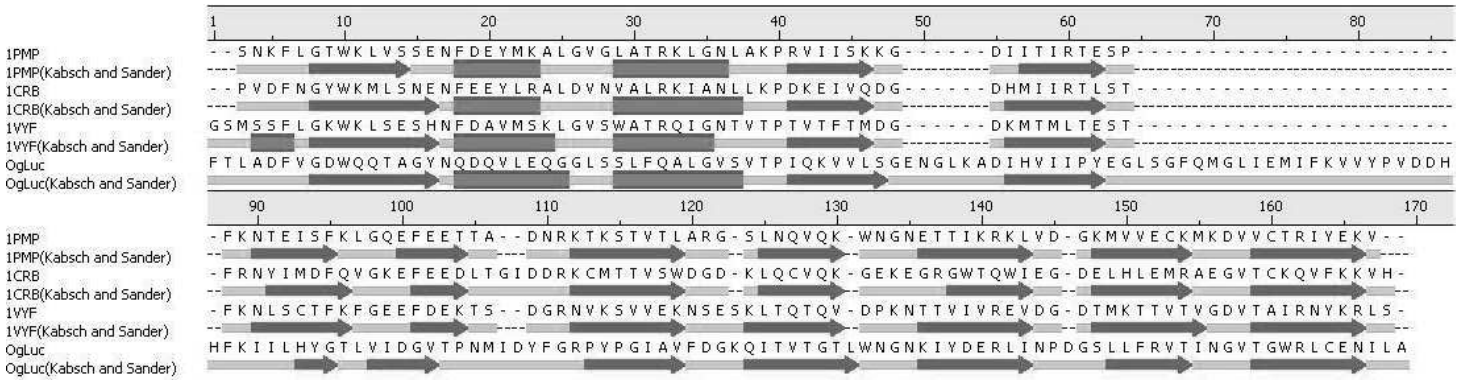
- approximate extent of helix secondary structure elements
- approximate extent of beta-sheet secondary structure elements
- secondary structure other than helix or beta-sheet

Secondary structure alignment #1:



Secondary structure alignment #2:



The sequence motifs centered on the conserved residues of the calycin structural signature can be seen in the alignments. The more highly conserved N-terminal MOTIF1 includes OgLuc residue Trp10, and the less well conserved C-terminal MOTIF2 includes OgLuc residue N166. For alignment #2, the approximate pair-wise percent protein sequence identities are: OgLuc-1PMP 14%, OgLuc-1CRB 9%, and OgLuc-1VYF 15%.

FIG. 1

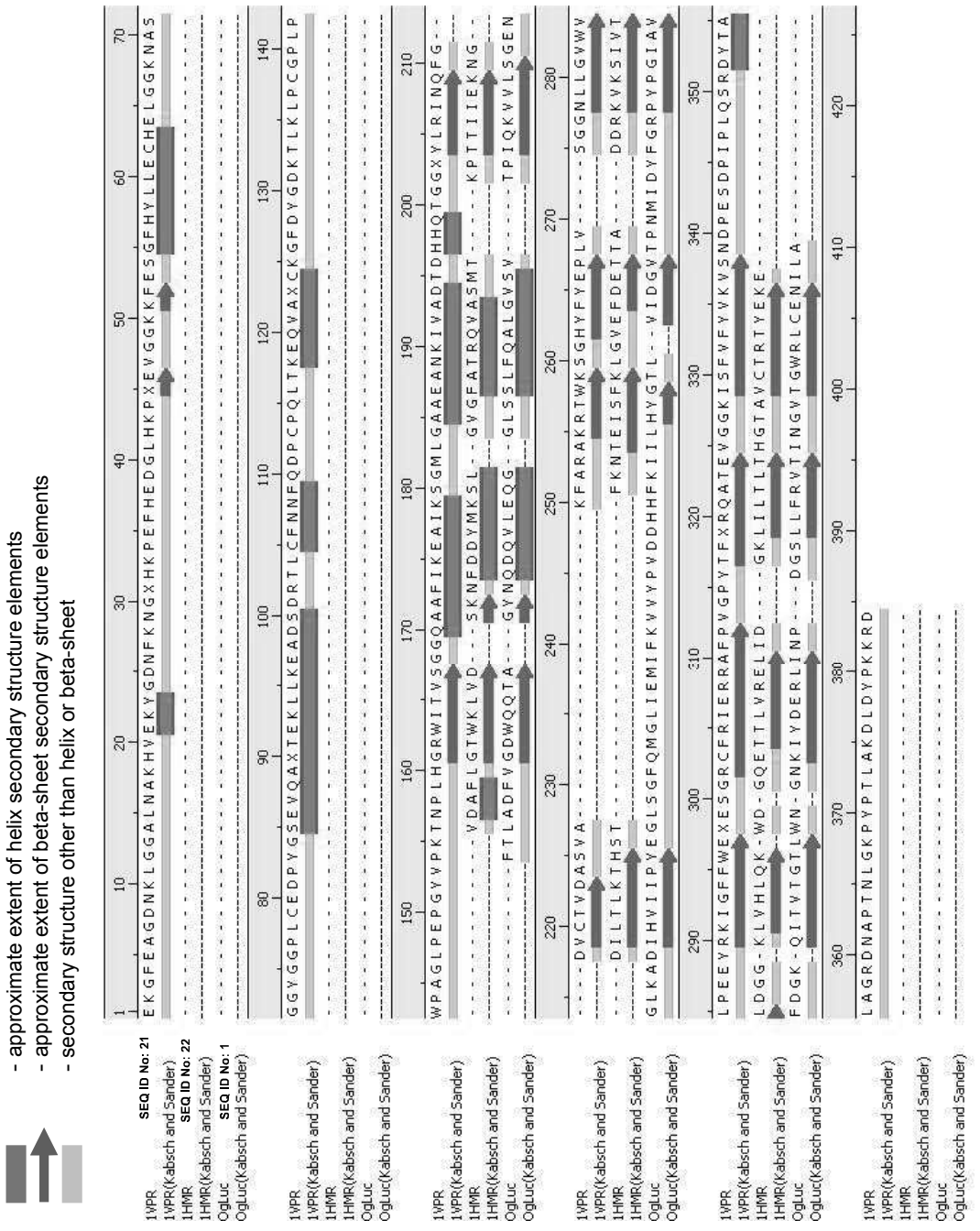

- approximate extent of helix secondary structure elements
- approximate extent of beta-sheet secondary structure elements
- secondary structure other than helix or beta-sheet
1WPR
1WPR(Kabsch and Sander)
1HMR
1HMR(Kabsch and Sander)
OgLuc
OgLuc(Kabsch and Sander)
SEQ ID No: 21
SEQ ID No: 22
SEQ ID No: 1

FIG. 2

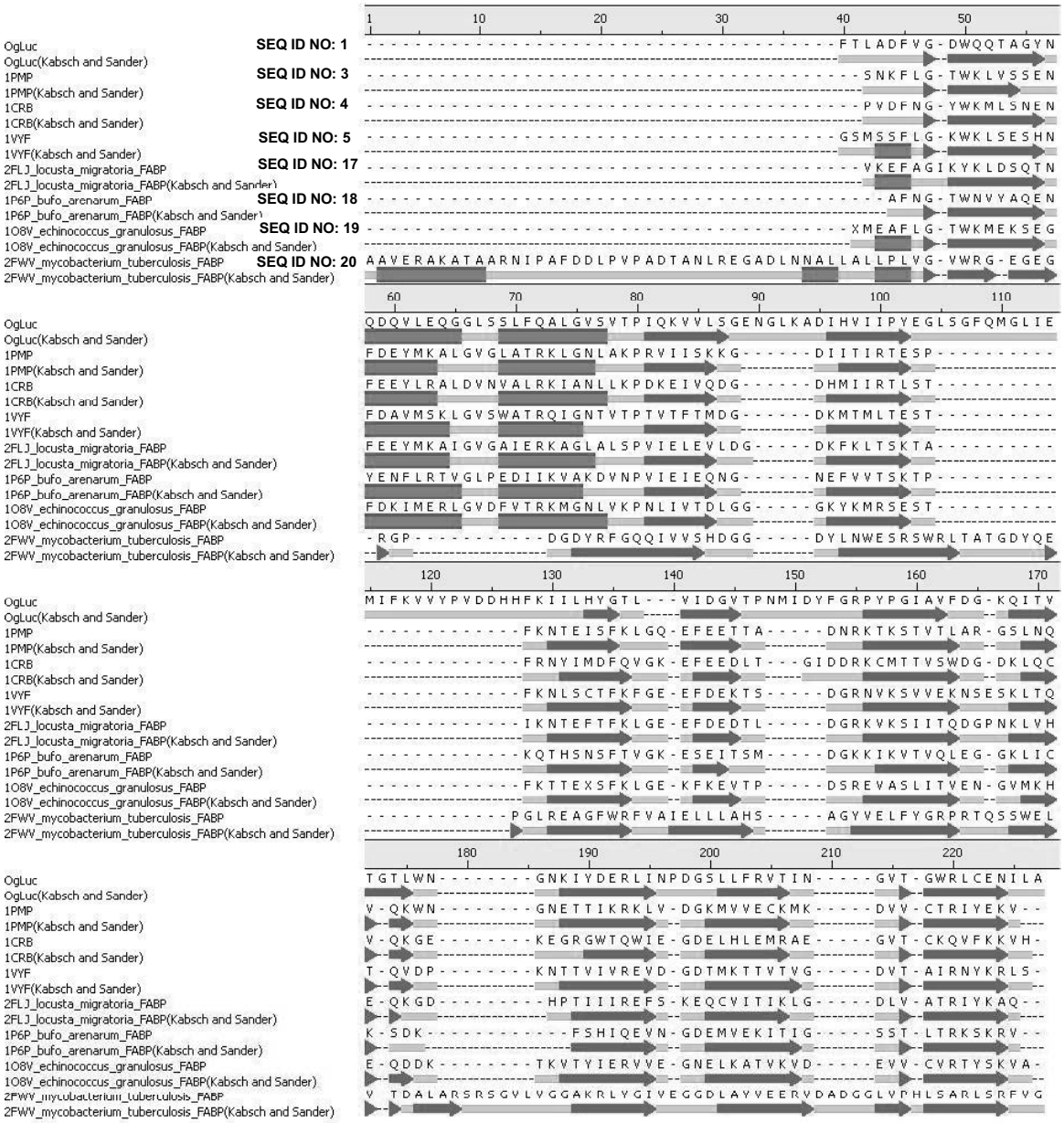

OgLuc
OgLuc(Kabsch and Sander)
1PMP
1PMP(Kabsch and Sander)
1CRB
1CRB(Kabsch and Sander)
1VYF
1VYF(Kabsch and Sander)
2FLJ_locusta_migratoria_FABP
2FLJ_locusta_migratoria_FABP(Kabsch and Sander)
1P6P_bufo_arenarum_FABP
1P6P_bufo_arenarum_FABP(Kabsch and Sander)
1O8V_echinococcus_granulosus_FABP
1O8V_echinococcus_granulosus_FABP(Kabsch and Sander)
2FWV_mycobacterium_tuberculosis_FABP
2FWV_mycobacterium_tuberculosis_FABP(Kabsch and Sander)
SEQ ID NO: 1
SEQ ID NO: 3
SEQ ID NO: 4
SEQ ID NO: 5
SEQ ID NO: 17
SEQ ID NO: 18
SEQ ID NO: 19
SEQ ID NO: 20

FIG. 3

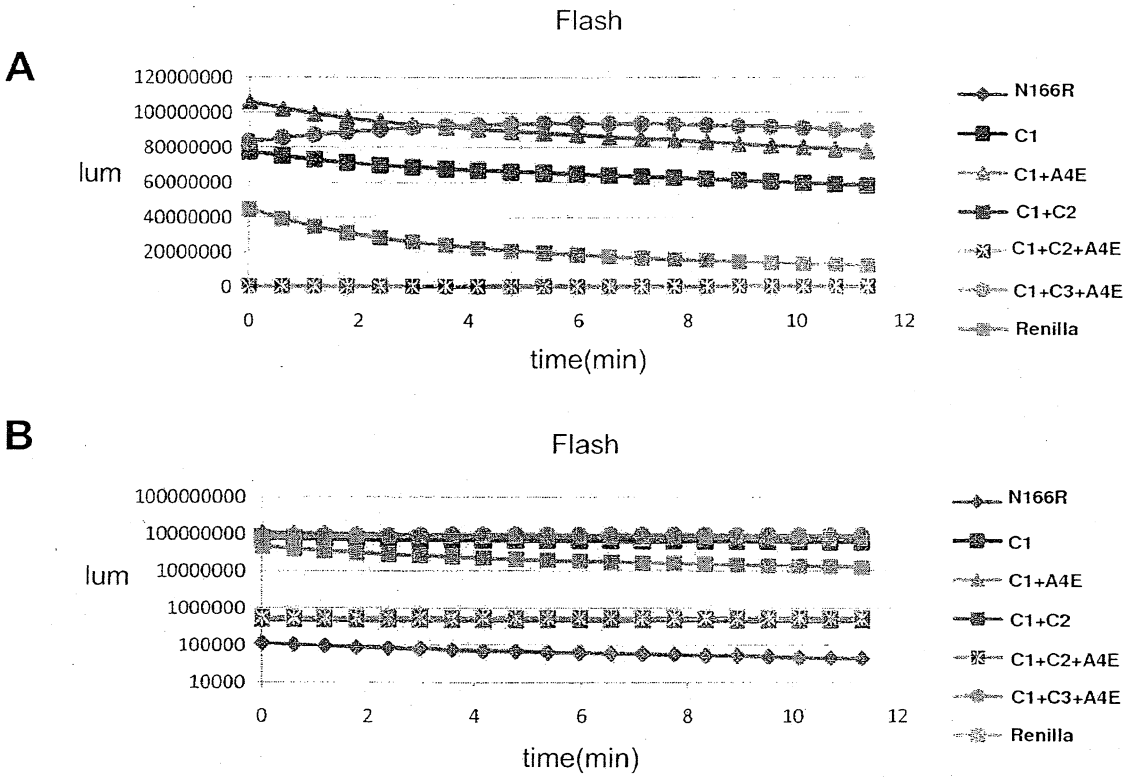
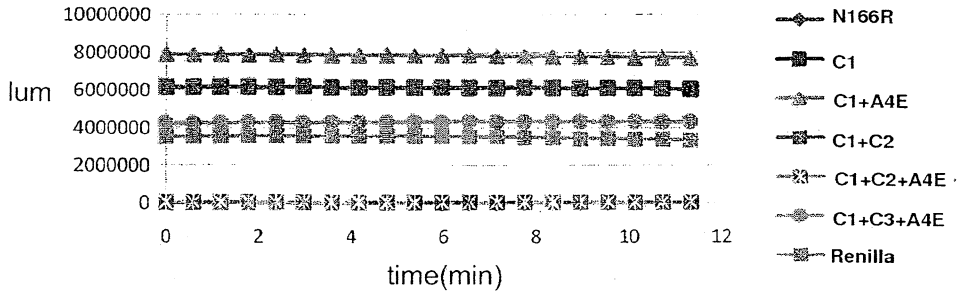

A
Flash
lum
120000000
100000000
80000000
60000000
40000000
20000000
0
0
2
4
6
8
10
12
time(min)
N166R
C1
C1+A4E
C1+C2
C1+C2+A4E
C1+C3+A4E
Renilla
B
Flash
lum
1000000000
100000000
10000000
1000000
100000
10000
0
2
4
6
8
10
12
time(min)
N166R
C1
C1+A4E
C1+C2
C1+C2+A4E
C1+C3+A4E
Renilla

FIG. 4

C



D

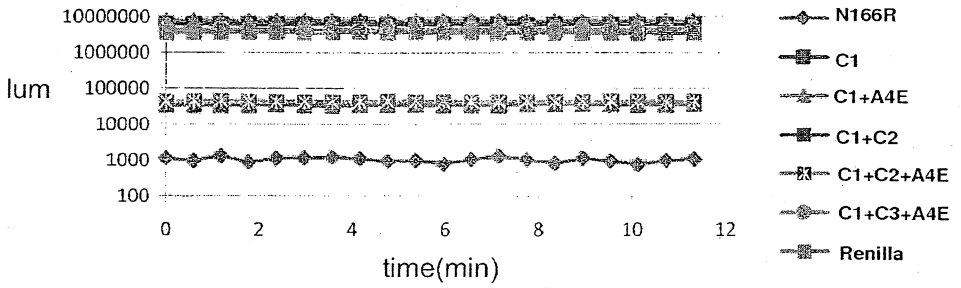


FIG. 4 cont'd

T=0 (injector) 0.5% tergitol assay buffer

Sample	Average	Stdev	CV
WT	116,132	13,032	11.2%
N166R	160,334	18,014	11.2%
T2T	259,374	17,488	6.7%
T2T+N166R	367,468	30,406	8.3%
A4E	193,837	10,272	5.3%
Q11R	172,425	16,389	9.5%
V44I	2,155,303	231,323	10.7%
A54F	1,559,763	121,554	7.8%
A54F+N166R	8,052,686	981,637	12.2%
A54I	15,676,789	2,266,808	14.5%
P115E	406,670	18,160	4.5%
P115E+N166R	489,722	26,228	5.4%
Y138I	1,119,166	128,394	11.5%
Q124K	322,475	30,313	9.4%
Y138C+N166R	590,665	40,138	6.8%
I90V	387,765	29,162	7.5%

FIG. 5A

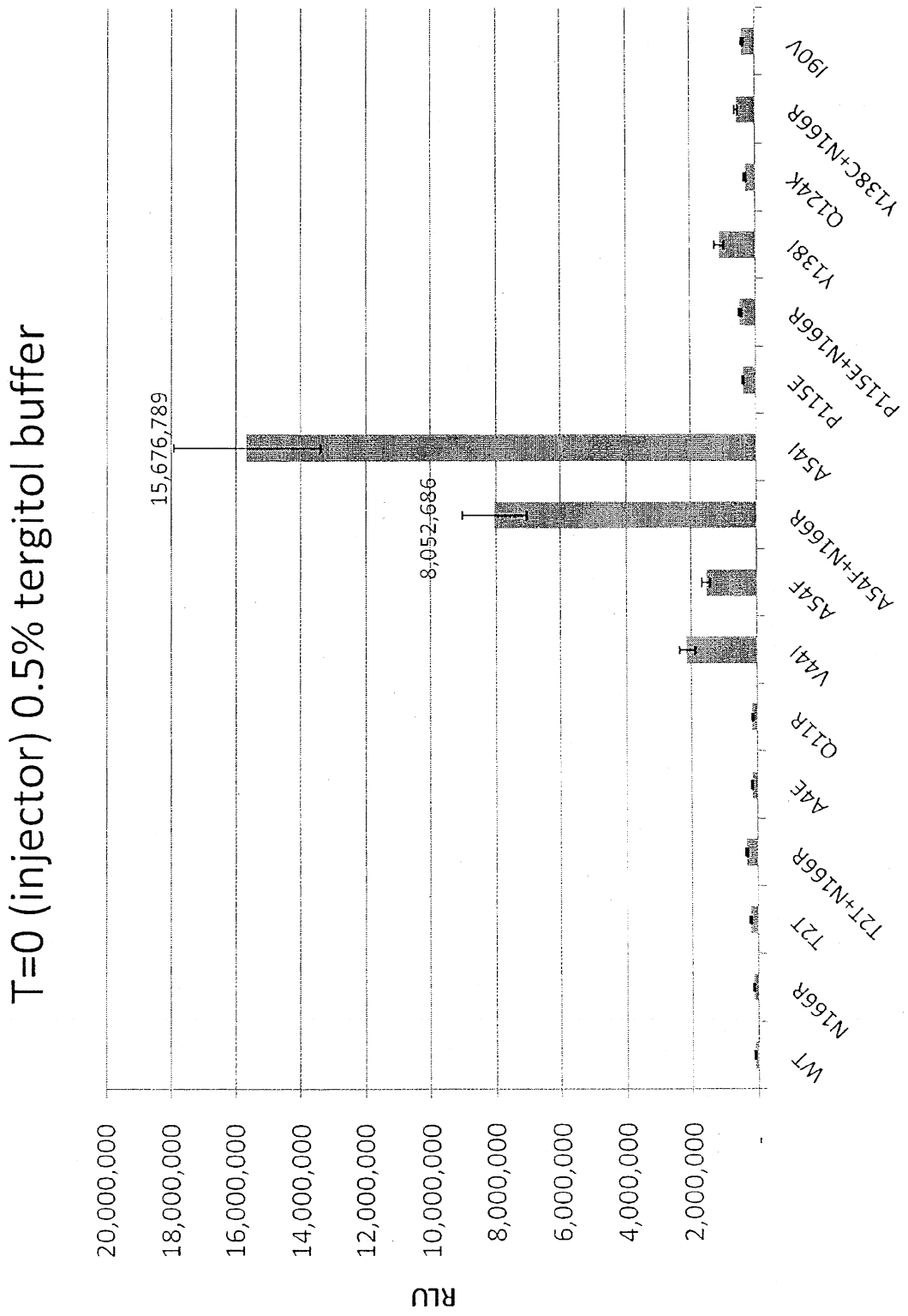

T=0 (injector) 0.5% tergitol buffer
RLU
20,000,000
18,000,000
16,000,000
14,000,000
12,000,000
10,000,000
8,000,000
6,000,000
4,000,000
2,000,000
-
15,676,789
8,052,686
WT
N166R
T21T
T21T+N166R
A4E
Q11R
V4A1
A54F
A54F+N166R
A54I
P115E
P115E+N166R
V138I
Q124K
V138C+N166R
I90V

FIG. 5B

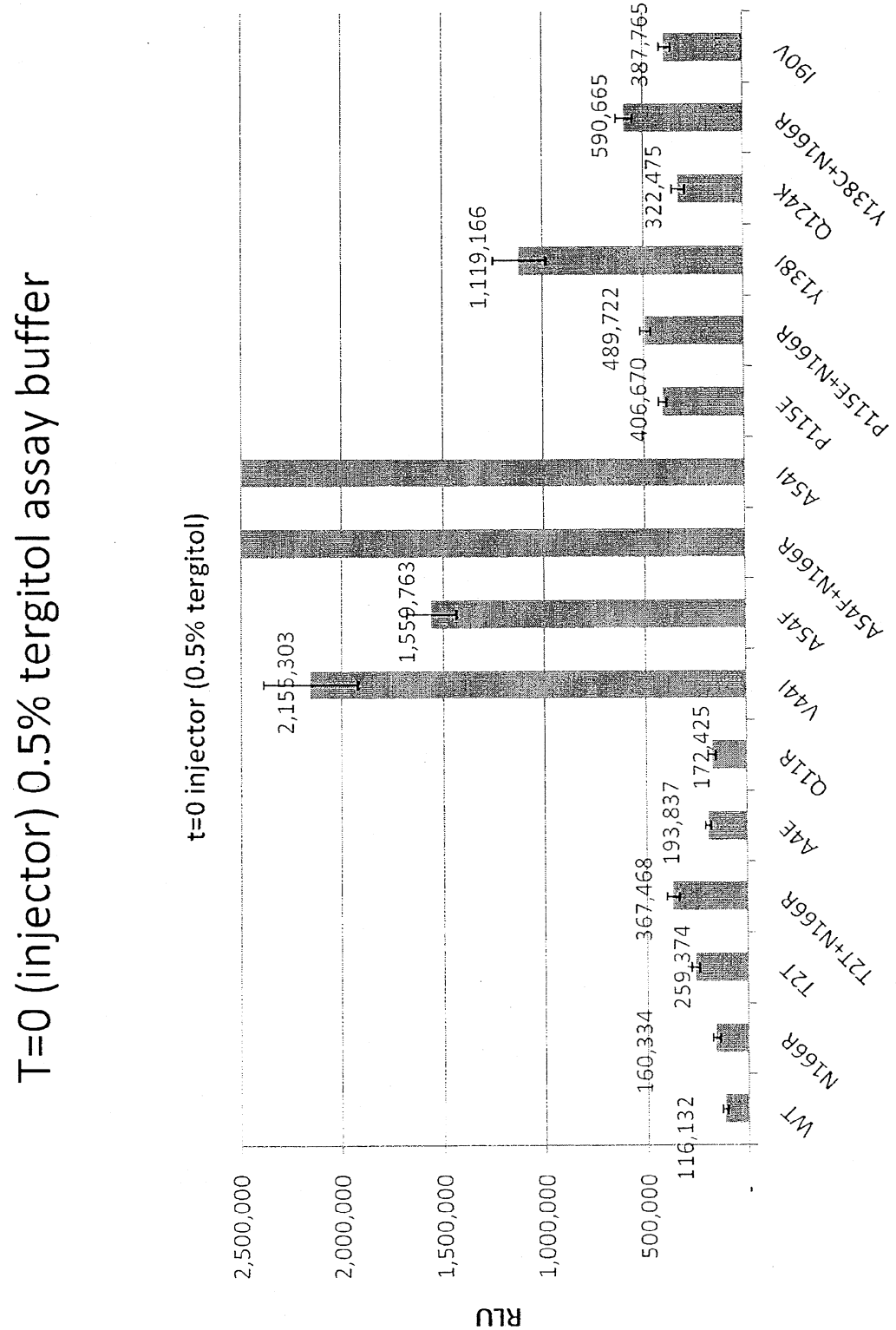

T=0 (injector) 0.5% tergitol assay buffer
t=0 injector (0.5% tergitol)
RLU
2,500,000
2,000,000
1,500,000
1,000,000
500,000
-
2,156,303
1,559,763
1,119,166
590,665
489,722
406,670
387,765
322,475
259,374
193,837
172,425
160,334
116,132
367,468
WT
N166R
T2T
T2T+N166R
A4E
Q11R
V4AI
A54F
A54F+N166R
A54I
P115E
P115E+N166R
V138I
Q124K
V138C+N166R
I90V

FIG. 5C

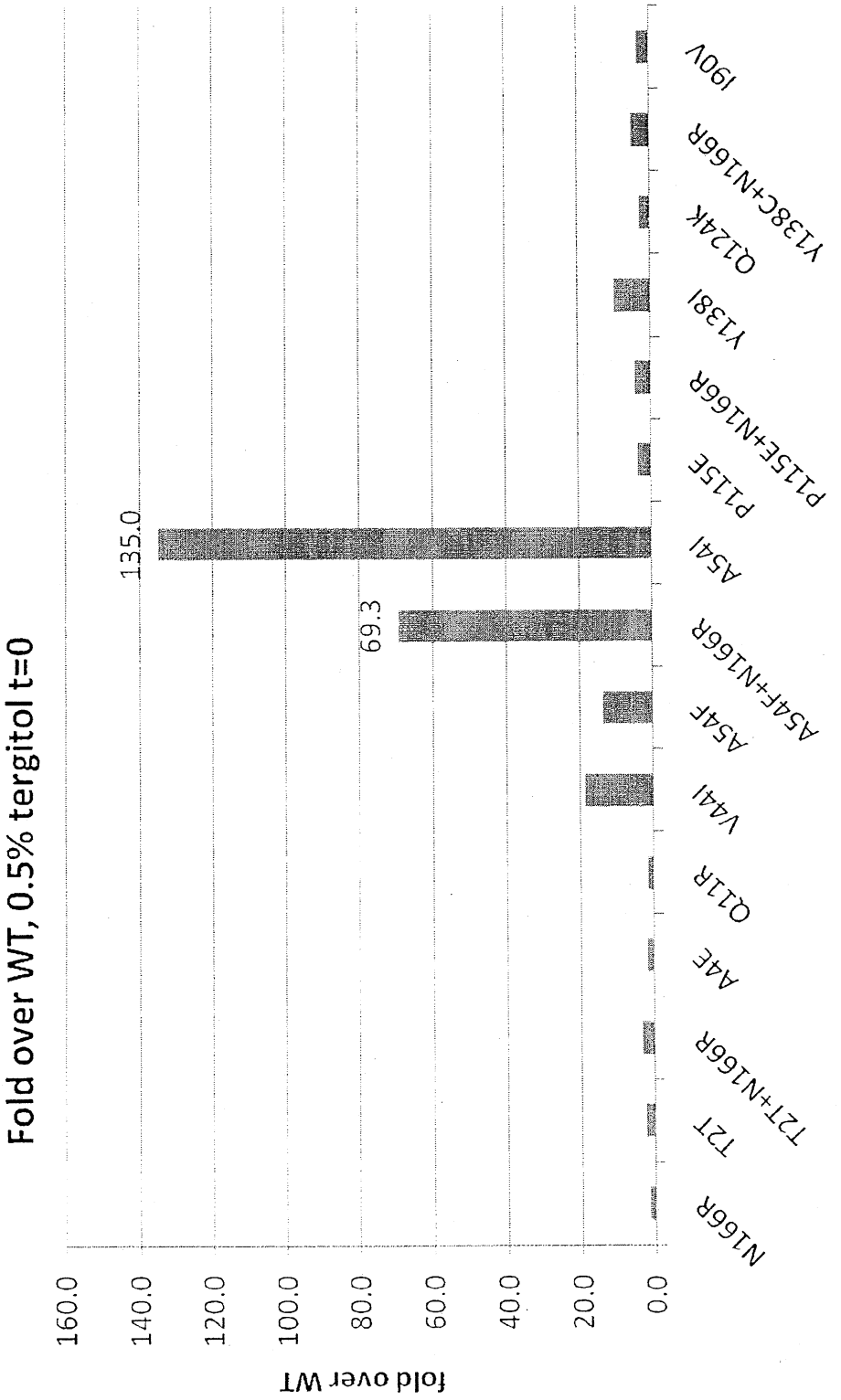

Fold over WT, 0.5% tergitol t=0
fold over WT
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
135.0
69.3
N166R
T2T
T2T+N166R
A4E
Q11R
V4I
A5F
A5F+N166R
A5I
P15E
P15E+N166R
V38I
Q124K
V38C+N166R
I90V

FIG. 6A

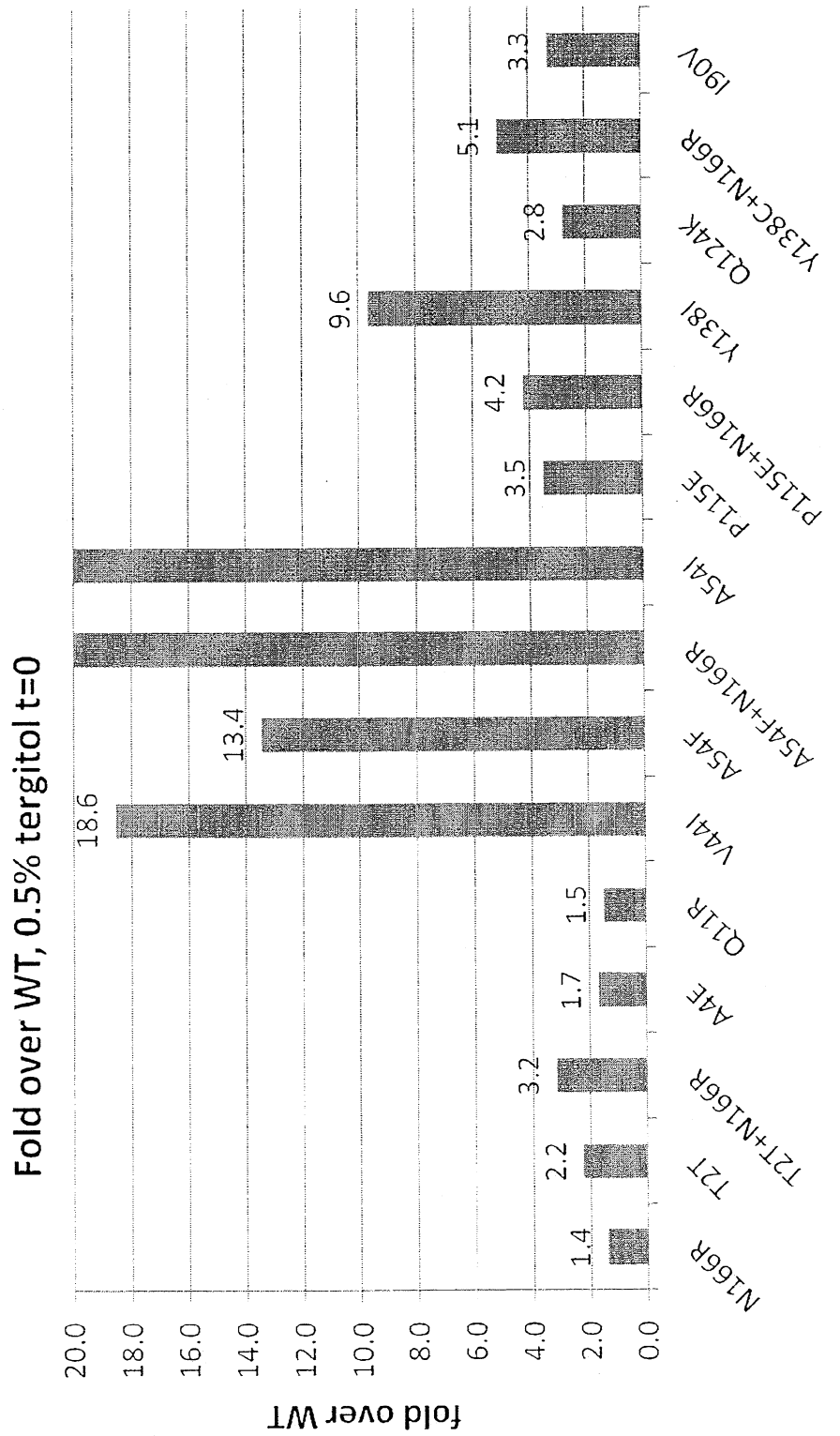

Fold over WT, 0.5% tergitol t=0
fold over WT
20.0
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
N166R
T2T
T2T+N166R
A4E
Q11R
V4AI
A54F
A54F+N166R
A5AI
P115E
P115E+N166R
V13BI
Q124K
V13BC+N166R
I90V
1.4
2.2
3.2
1.7
1.5
18.6
13.4
9.6
4.2
3.5
5.1
2.8
3.3

FIG. 6B

T=0 RLAB with injector

Sample	Average	Stdev	CV
WT	2,266,822	222,850	9.8%
N166R	5,582,331	514,426	9.2%
T2T	3,599,341	244,441	6.8%
T2T+N166R	9,823,129	457,592	4.7%
A4E	2,888,005	231,055	8.0%
Q11R	4,043,391	179,037	4.4%
V44I	12,935,691	546,014	4.2%
A54F	8,572,288	464,442	5.4%
A54F+N166R	79,773,644	3,157,533	4.0%
A54I	32,309,914	2,594,059	8.0%
P115E	7,400,192	416,225	5.6%
P115E+N166R	16,167,230	481,714	3.0%
Y138I	14,573,569	619,909	4.3%
Q124K	5,473,417	517,799	9.5%
Y138C+N166R	15,516,040	1,147,103	7.4%
I90V	4,474,794	297,720	6.7%

FIG. 7A

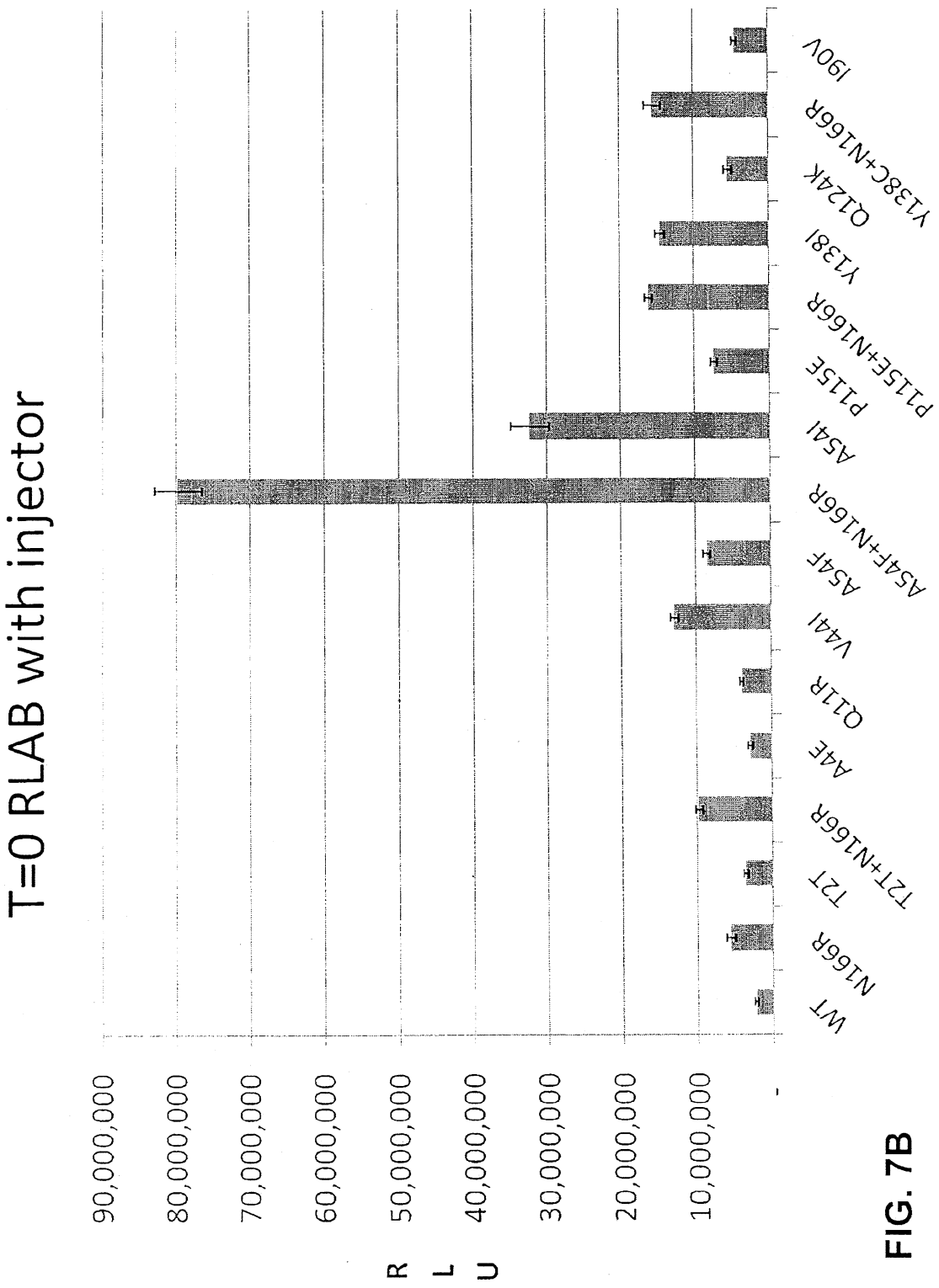

T=0 RLAB with injector
RLU
90,000,000
80,000,000
70,000,000
60,000,000
50,000,000
40,000,000
30,000,000
20,000,000
10,000,000
-
WT
N166R
T2T
T2T+N166R
A4E
Q11R
V4AI
A54F
A54F+N166R
A54I
P115E
P115E+N166R
V138I
Q124K
V138C+N166R
I90V

FIG. 7B

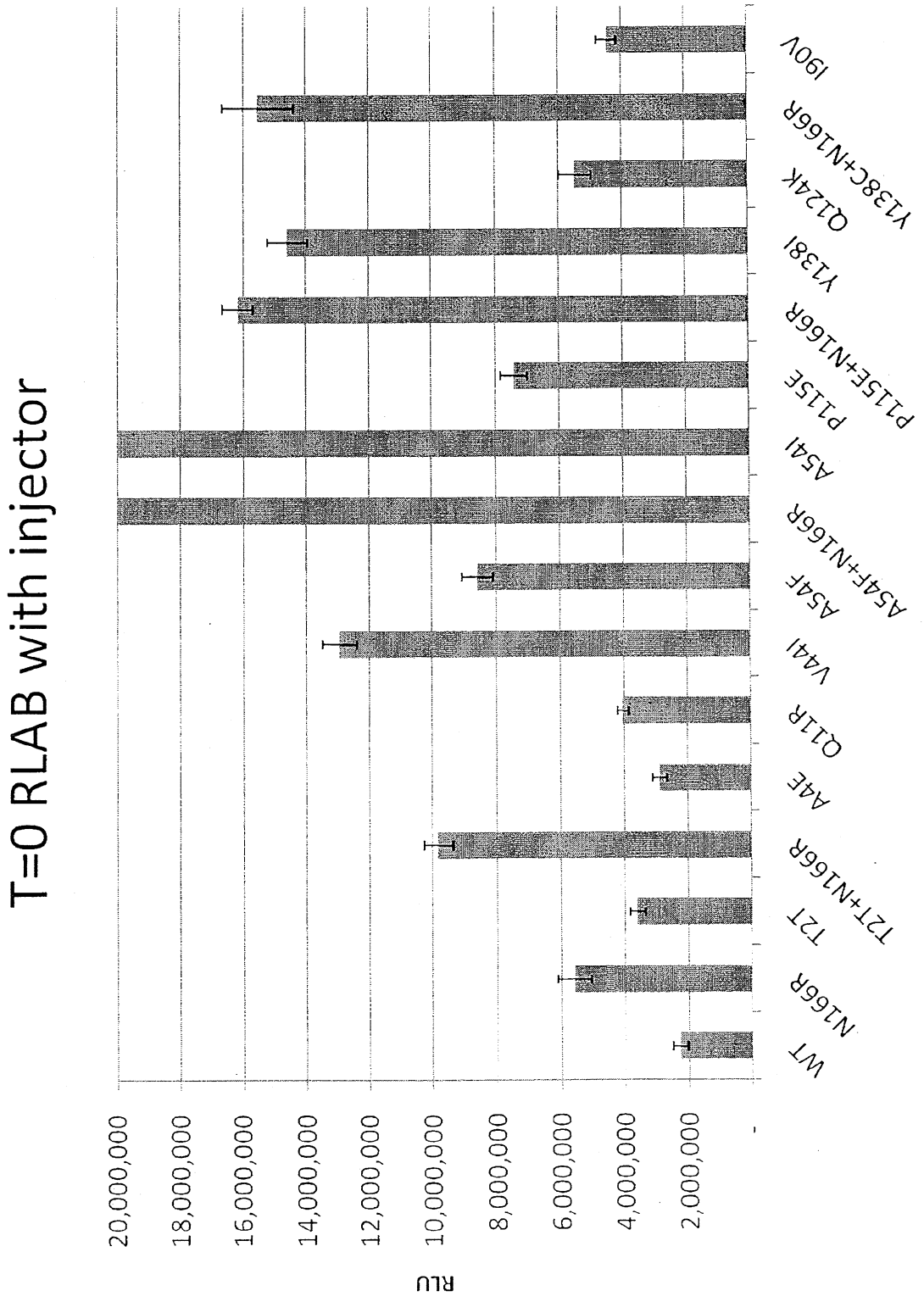

T=0 RLAB with injector
RLU
20,000,000
18,000,000
16,000,000
14,000,000
12,000,000
10,000,000
8,000,000
6,000,000
4,000,000
2,000,000
-
WT
N166R
T2T
T2T+N166R
A4E
Q11R
V4A1
A54F
A54F+N166R
A54I
P115E
P115E+N166R
Y138I
Q124K
Y138C+N166R
I90V

FIG. 7C

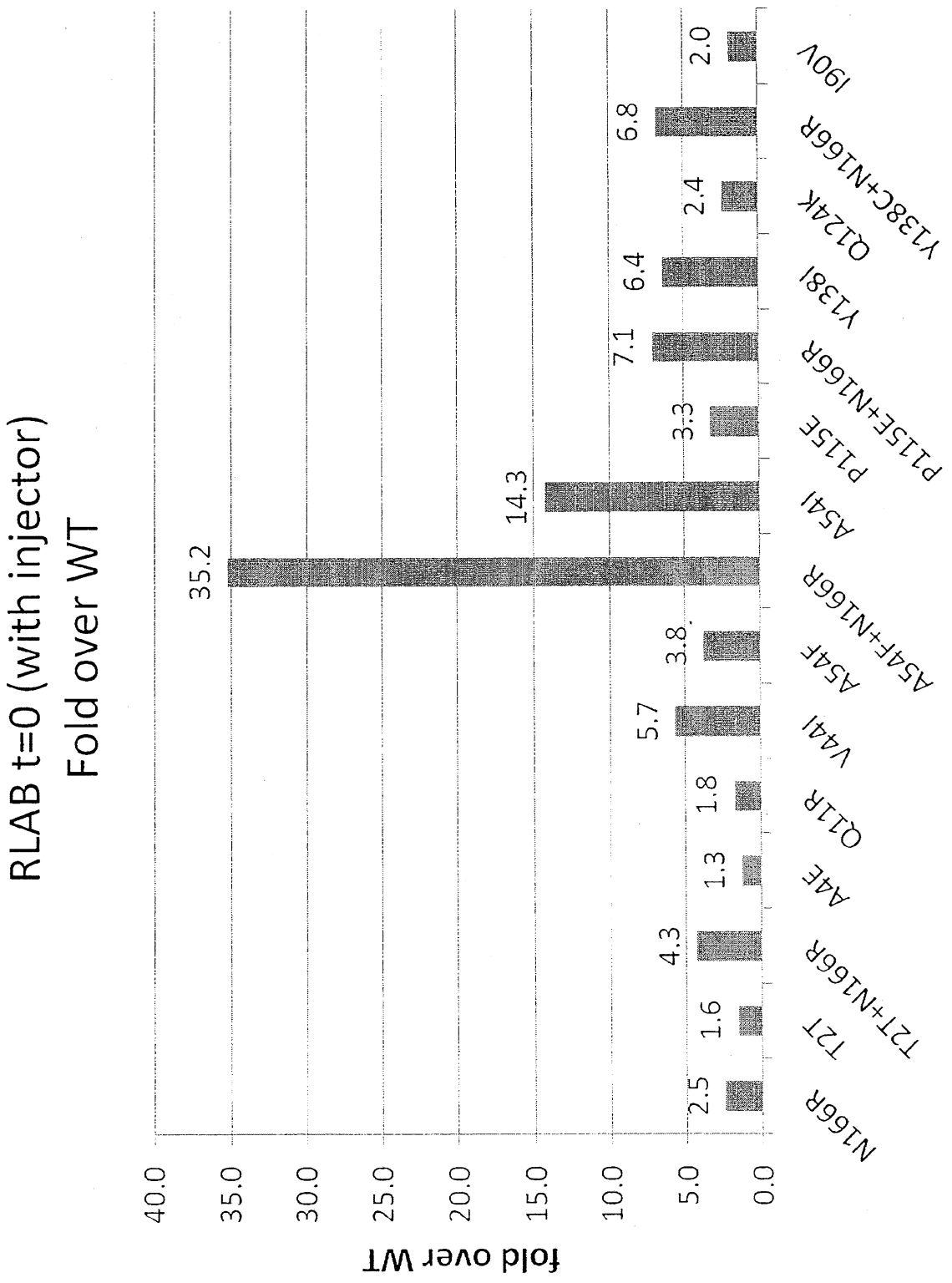

RLAB t=0 (with injector)
Fold over WT
fold over WT
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
35.2
14.3
7.1
6.4
6.8
5.7
3.8
4.3
3.3
2.4
2.5
1.6
1.3
1.8
2.0
N166R
T2T
T2T+N166R
A4E
Q11R
V4AI
A54F
A54F+N166R
A54I
P115E
P115E+N166R
V138I
Q124K
V138C+N166R
I90V

FIG. 8

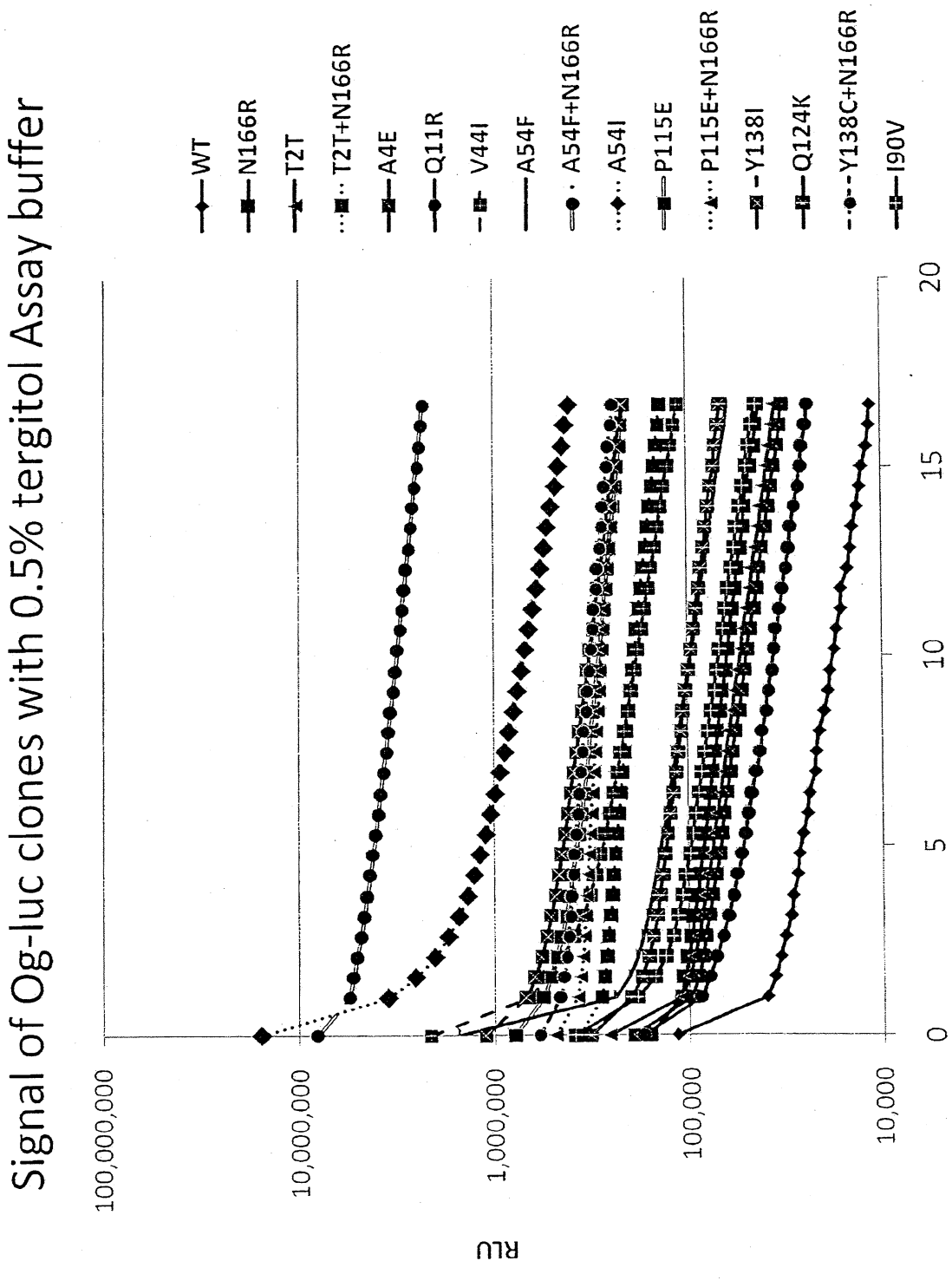

Signal of Og-luc clones with 0.5% tergitol Assay buffer
WT
N166R
T2T
T2T+N166R
A4E
Q11R
V44I
A54F
A54F+N166R
A54I
P115E
P115E+N166R
Y138I
Q124K
Y138C+N166R
I90V
100,000,000
10,000,000
1,000,000
100,000
10,000
RLU
0
5
10
15
20
time(min)

FIG. 9A

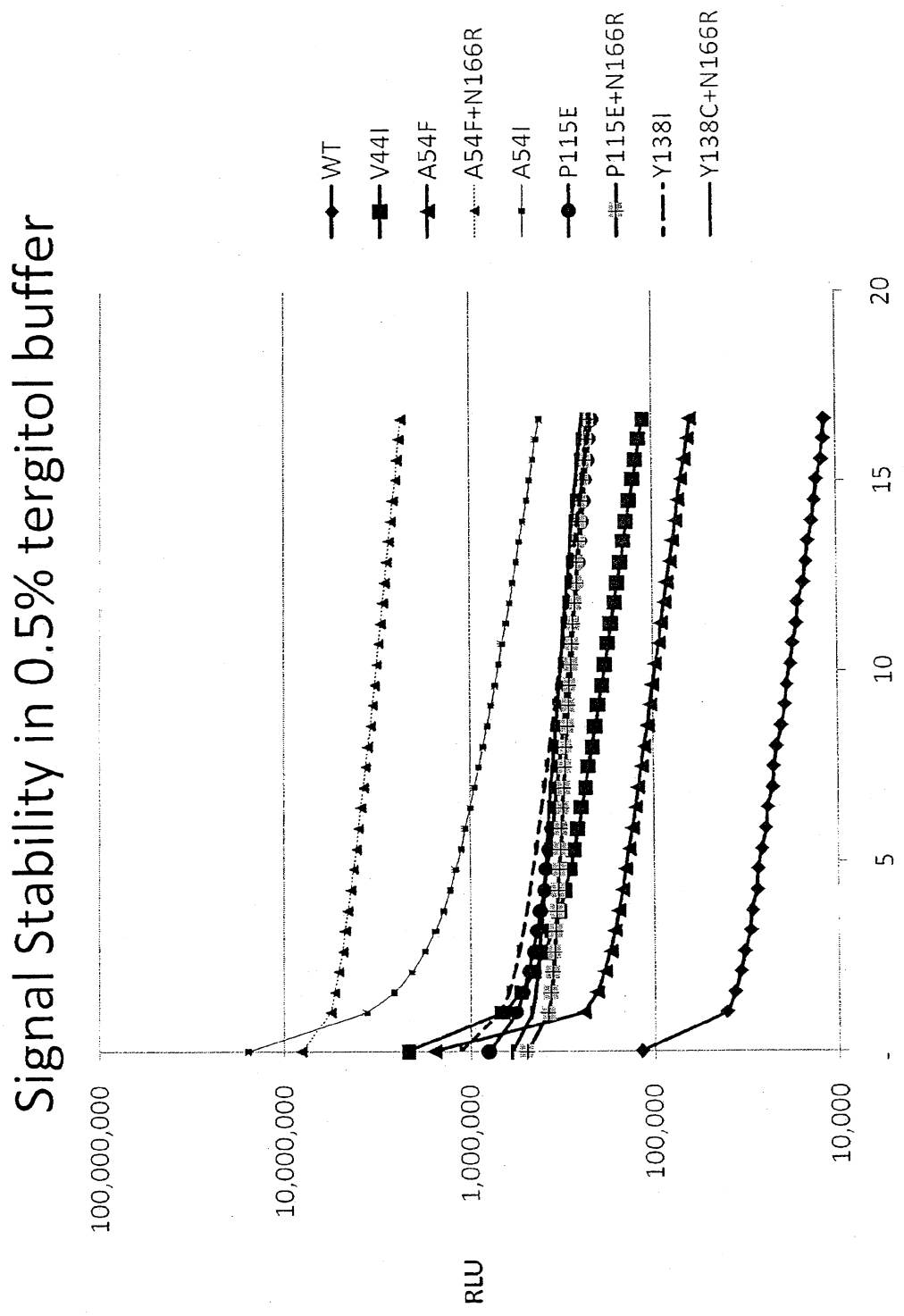

Signal Stability in 0.5% tergitol buffer
WT
V44I
A54F
A54F+N166R
A54I
P115E
P115E+N166R
Y138I
Y138C+N166R
RLU
100,000,000
10,000,000
1,000,000
100,000
10,000
-
5
10
15
20
time(min)

FIG. 9B

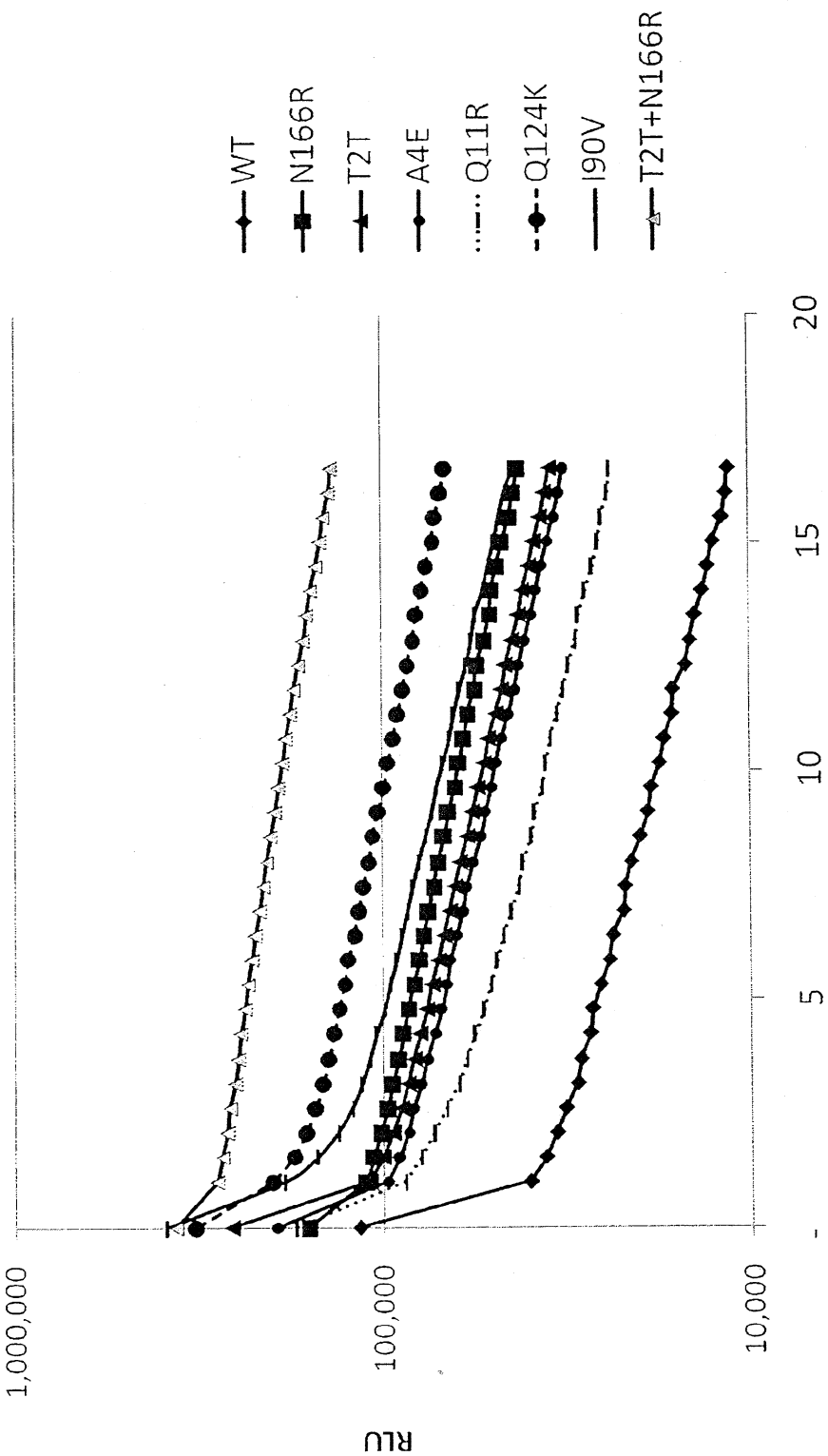

Signal stability in 0.5% tergitol buffer
WT
N166R
T2T
A4E
Q11R
Q124K
I90V
T2T+N166R
1,000,000
100,000
10,000
RLU
-
5
10
15
20
time(min)

FIG. 9C

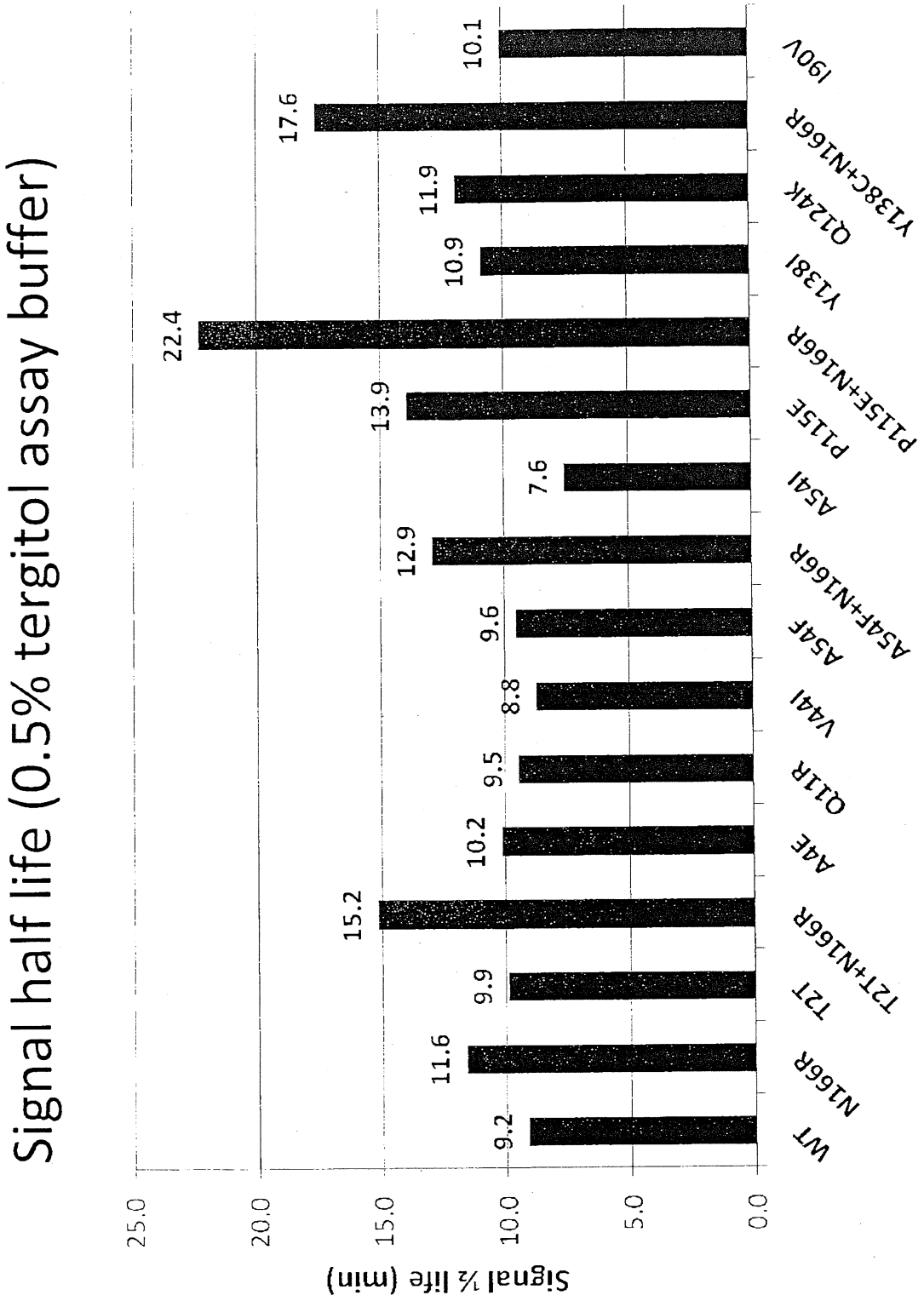

Signal half life (0.5% tergitol assay buffer)
Signal ½ life (min)
25.0
20.0
15.0
10.0
5.0
0.0
WT
N166R
T21
T21+N166R
A4E
Q11R
V44I
A54F
A54F+N166R
A54I
P115E
P115E+N166R
V138I
Q124K
V138C+N166R
I90V
9.2
11.6
9.9
15.2
10.2
9.5
8.8
9.6
12.9
7.6
13.9
22.4
10.9
11.9
17.6
10.1

FIG. 9D

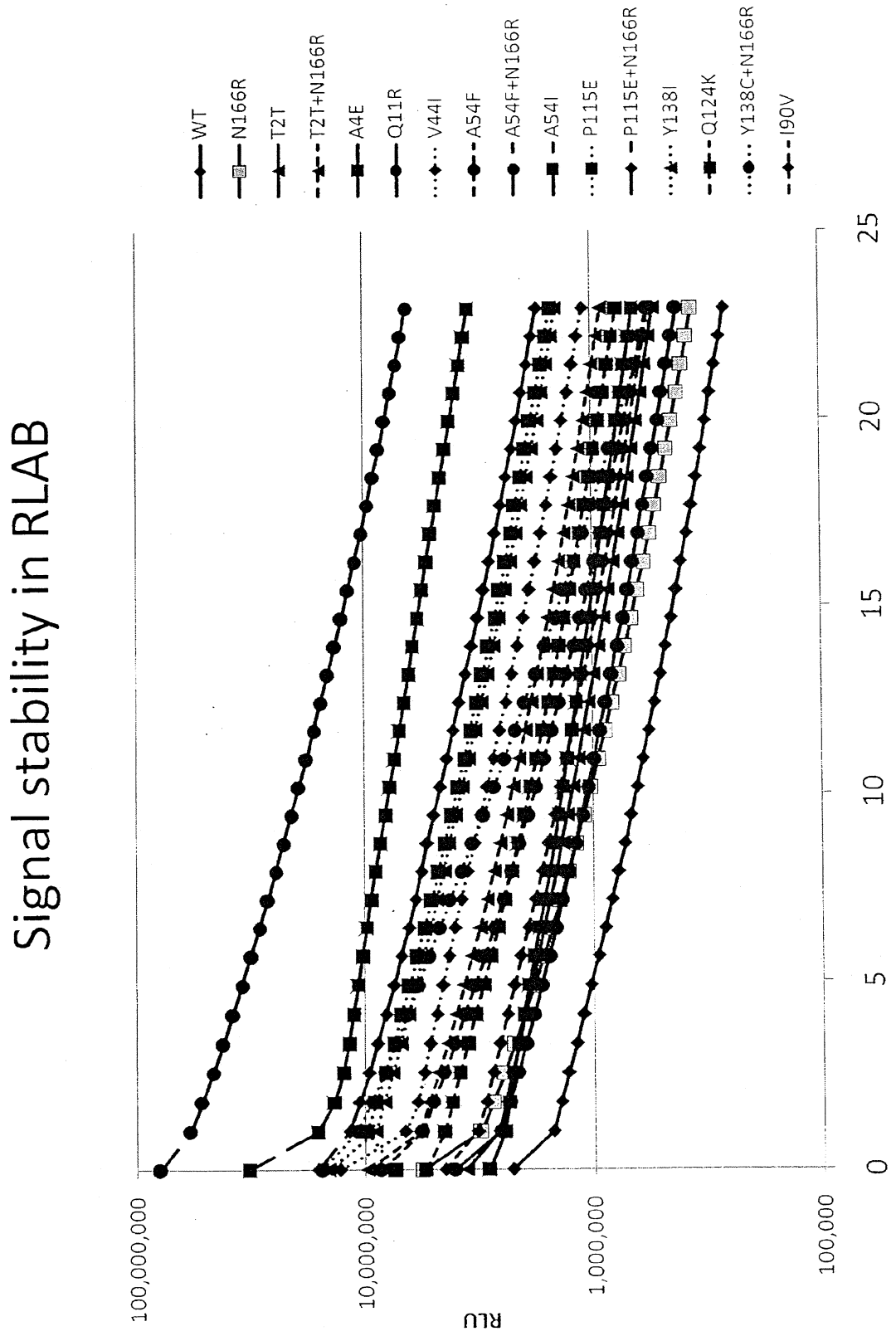

Signal stability in RLAB
WT
N166R
T2T
T2T+N166R
A4E
Q11R
V44I
A54F
A54F+N166R
A54I
P115E
P115E+N166R
Y138I
Q124K
Y138C+N166R
I90V
100,000,000
10,000,000
1,000,000
100,000
RLU
0
5
10
15
20
25
time(min)

FIG. 10A

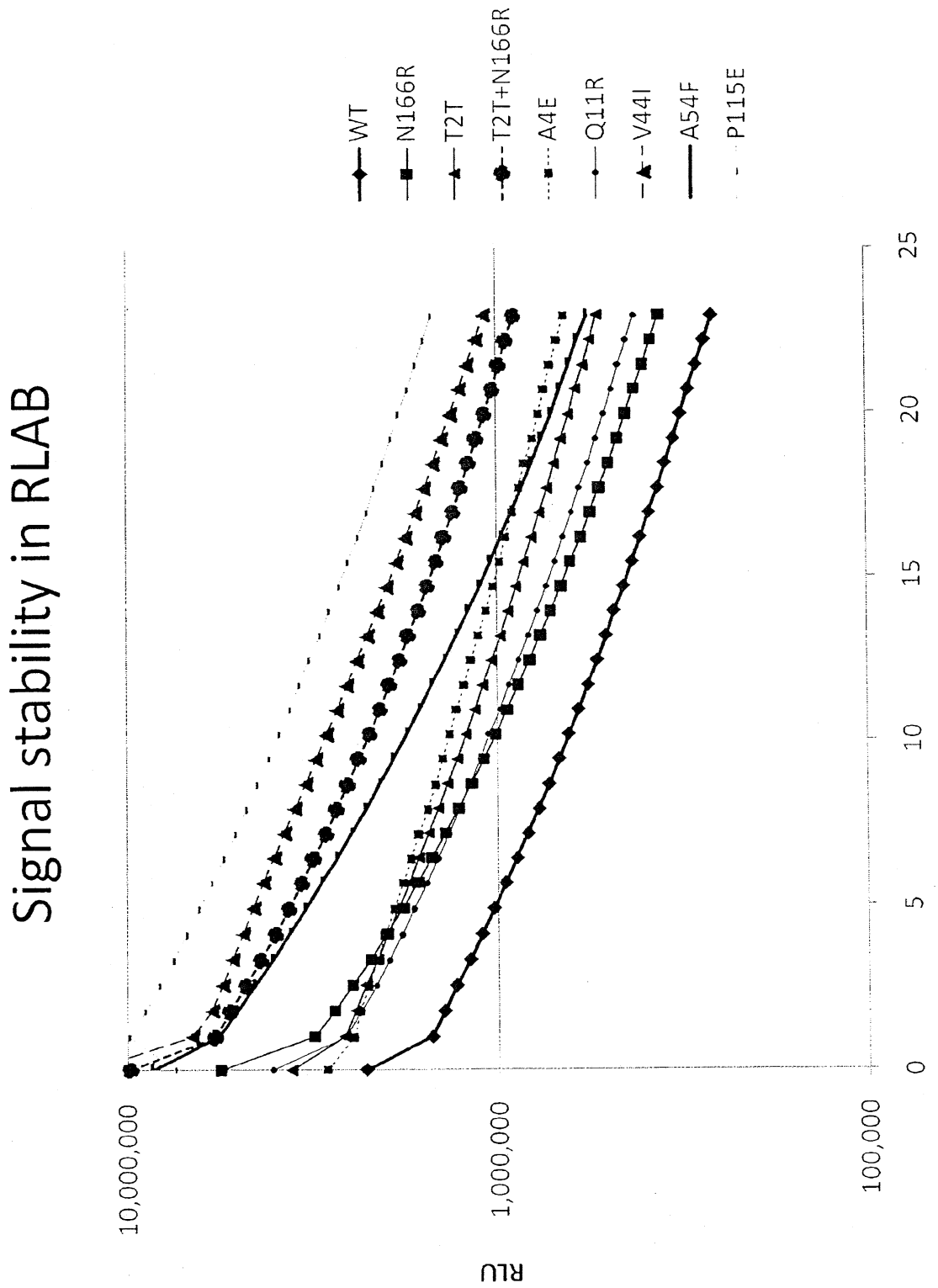

Signal stability in RLAB
WT
N166R
T2T
T2T+N166R
A4E
Q11R
V44I
A54F
P115E
RLU
10,000,000
1,000,000
100,000
0
5
10
15
20
25
time(min)

FIG. 10B

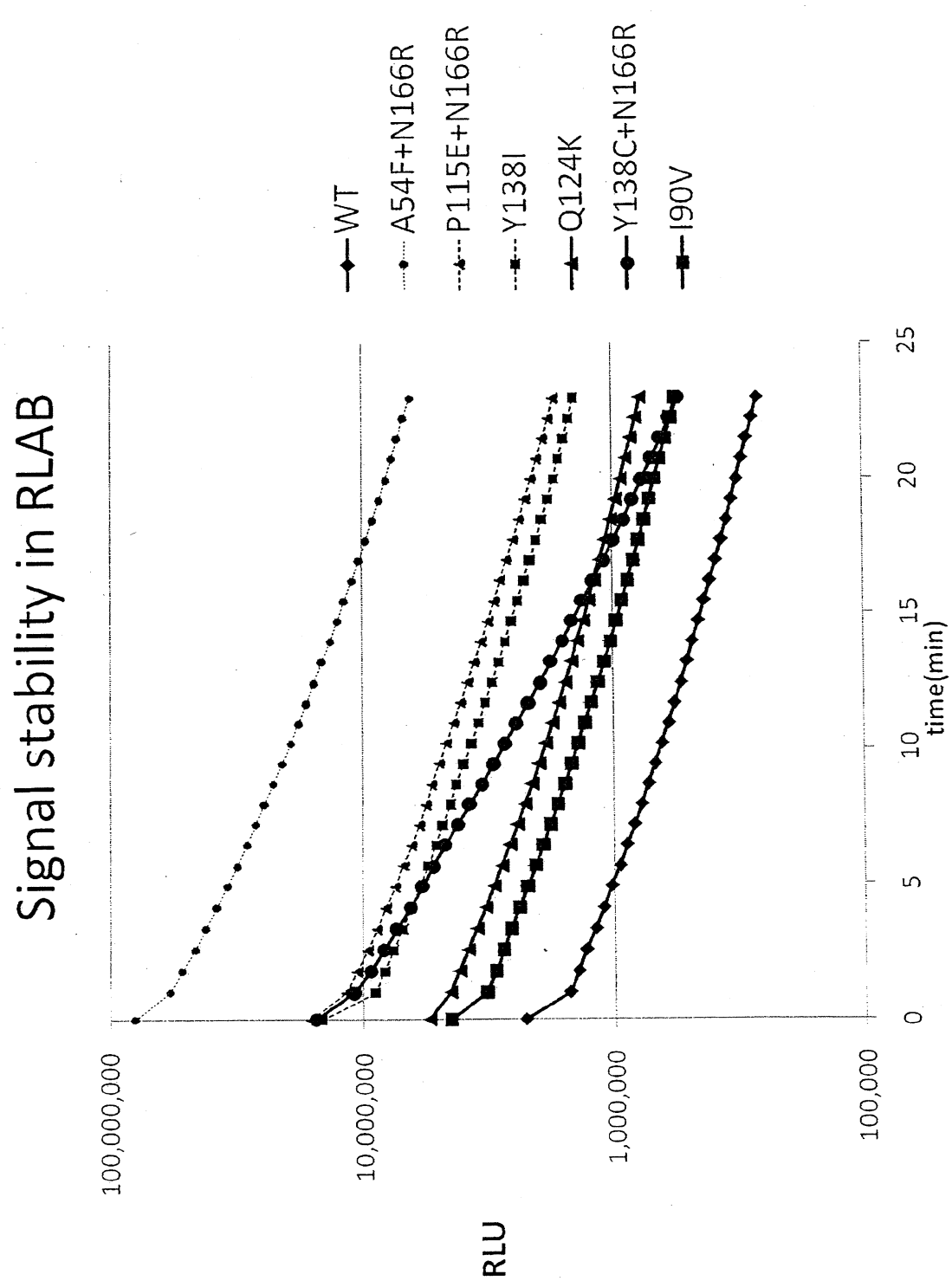

Signal stability in RLAB
WT
A54F+N166R
P115E+N166R
Y138I
Q124K
Y138C+N166R
I90V
RLU
100,000,000
10,000,000
1,000,000
100,000
0
5
10
15
20
25
time(min)

FIG. 10C

Signal ½ life (minutes) RLAB

Sample	Signal 1/2 life (minutes) RLAB
WT	9.03
N166R	8.17
T2T	9.79
T2T+N166R	8.74
A4E	11.48
Q11R	8.69
V44I	8.54
A54F	6.80
A54F+N166R	7.30
A54I	10.88
P115E	8.31
P115E+N166R	8.16
Y138I	8.36
Q124K	8.77
Y138C+N166R	4.97
I90V	8.83

FIG. 11A

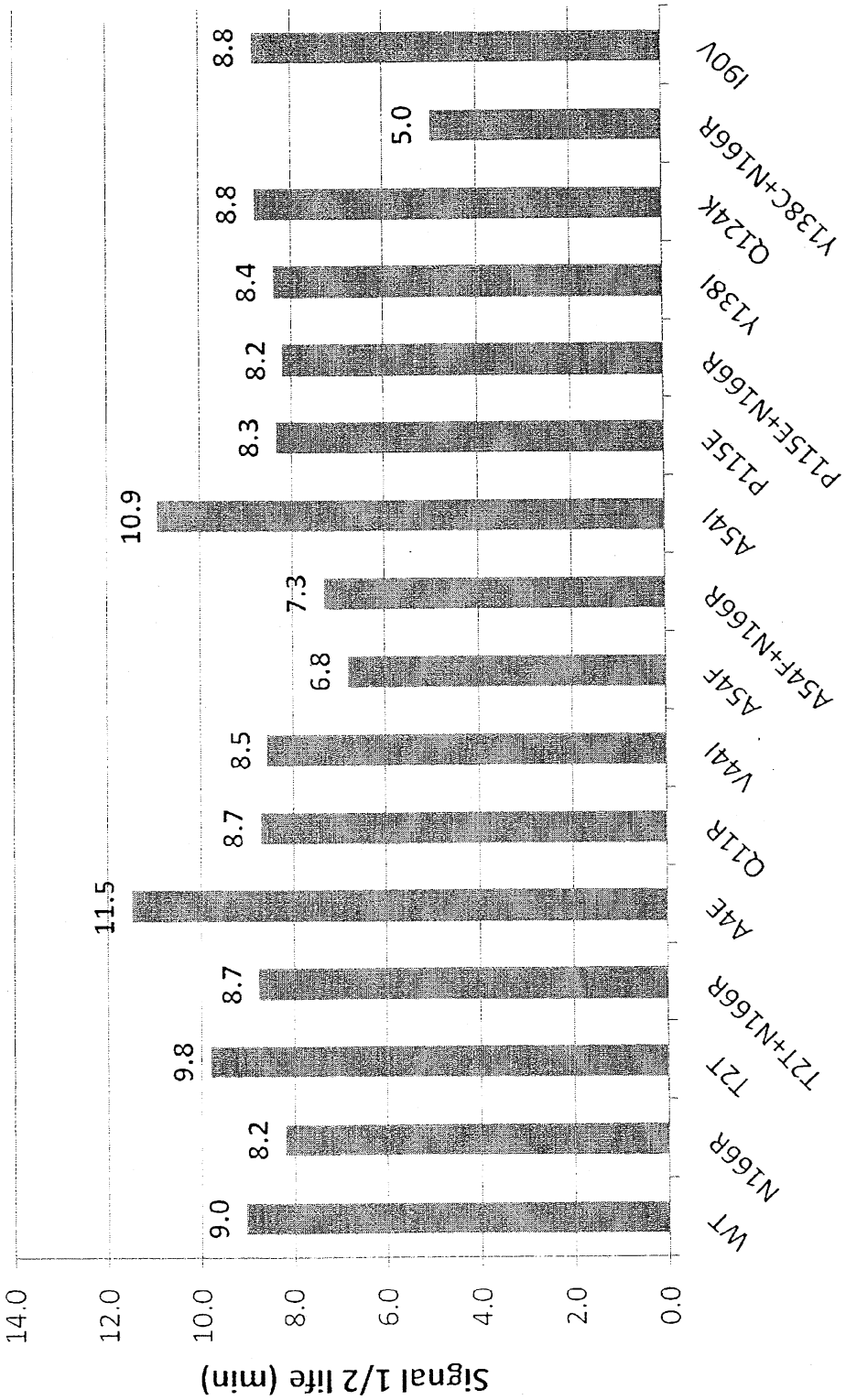

Signal ½ life(minutes) RLAB
Signal 1/2 life (min)
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
9.0
8.2
9.8
8.7
11.5
8.7
8.5
6.8
7.3
10.9
8.3
8.2
8.4
8.8
5.0
8.8
WT
N166R
T2T
T2T+N166R
A4E
Q11R
V4AI
A54F
A54F+N166R
A54I
P115E
P115E+N166R
V138I
Q124K
V138C+N166R
190V

FIG. 11B

Stability at 22°C (1/2 life in minutes)

Sample	stability (1/2 life at 22C)
WT	38.1
N166R	61.3
T2T	43.1
T2T + N166R	68.0
A4E	38.5
Q11R	45.9
V44I	49.5
A54F	56.8
A54F + N166R	177.7
A54I	63.0
P115E	49.9
P115E + N166R	119.5
Y138I	55.9
Q124K	43.9
Y138C + N166R	35.5
I90V	41.3

FIG. 12A

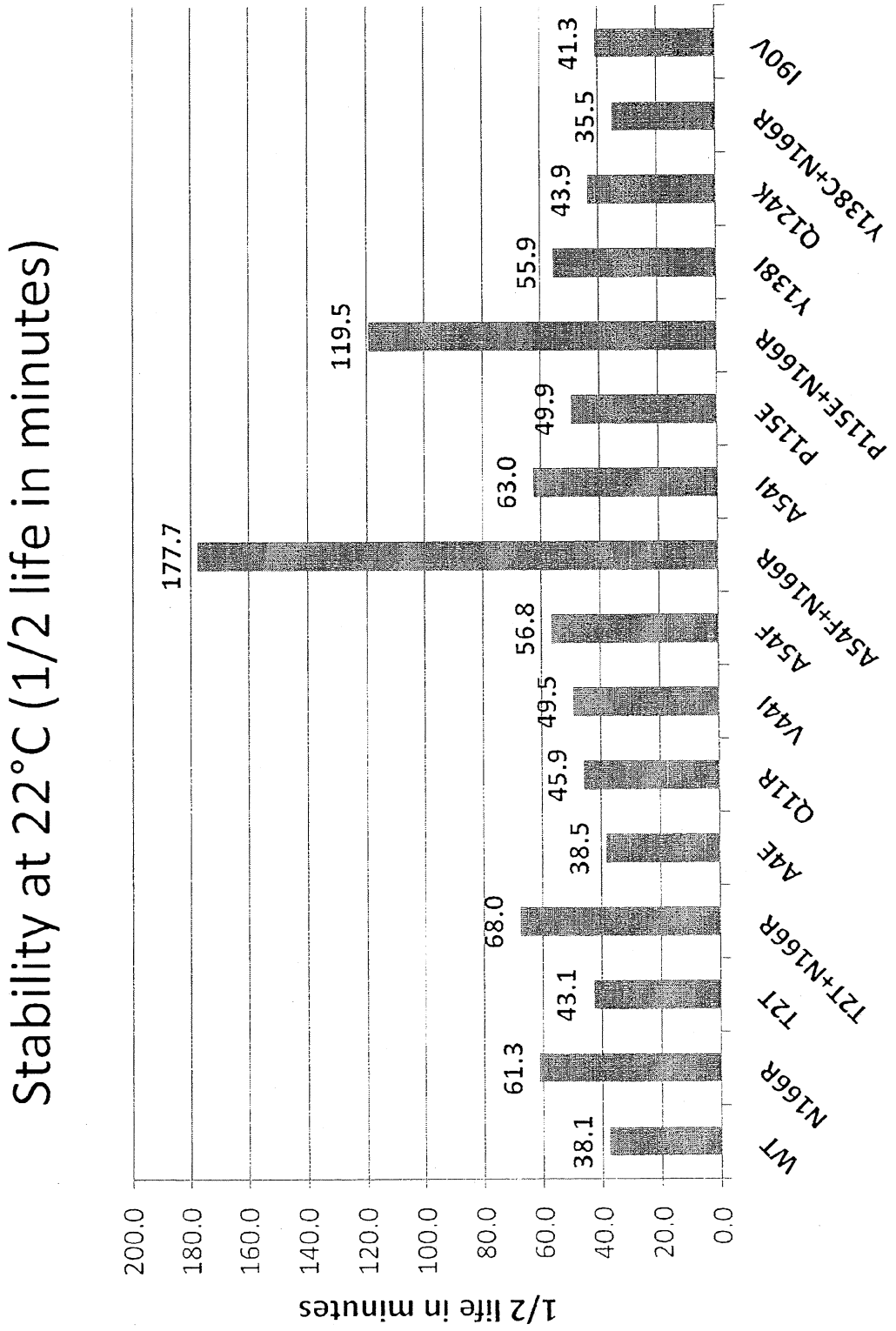

Stability at 22°C (1/2 life in minutes)
1/2 life in minutes
200.0
180.0
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
38.1
61.3
43.1
68.0
38.5
45.9
49.5
56.8
177.7
63.0
49.9
119.5
55.9
43.9
35.5
41.3
WT
N166R
T2T
T2T+N166R
A4E
Q11R
V4AI
A54F
A54F+N166R
A54I
P115E
P115E+N166R
Y138I
Q124K
Y138C+N166R
I90V

FIG. 12B

T=0 0.5% tergitol assay buffer

Sample	Average	% cv
WT	1151904	8.6%
N166R	1666647	12.2%
WT A33K	4384455	7.8%
WT F68Y	4490833	6.5%
N166R A33K	1054387	8.0%
N166R F68Y	5713471	4.7%

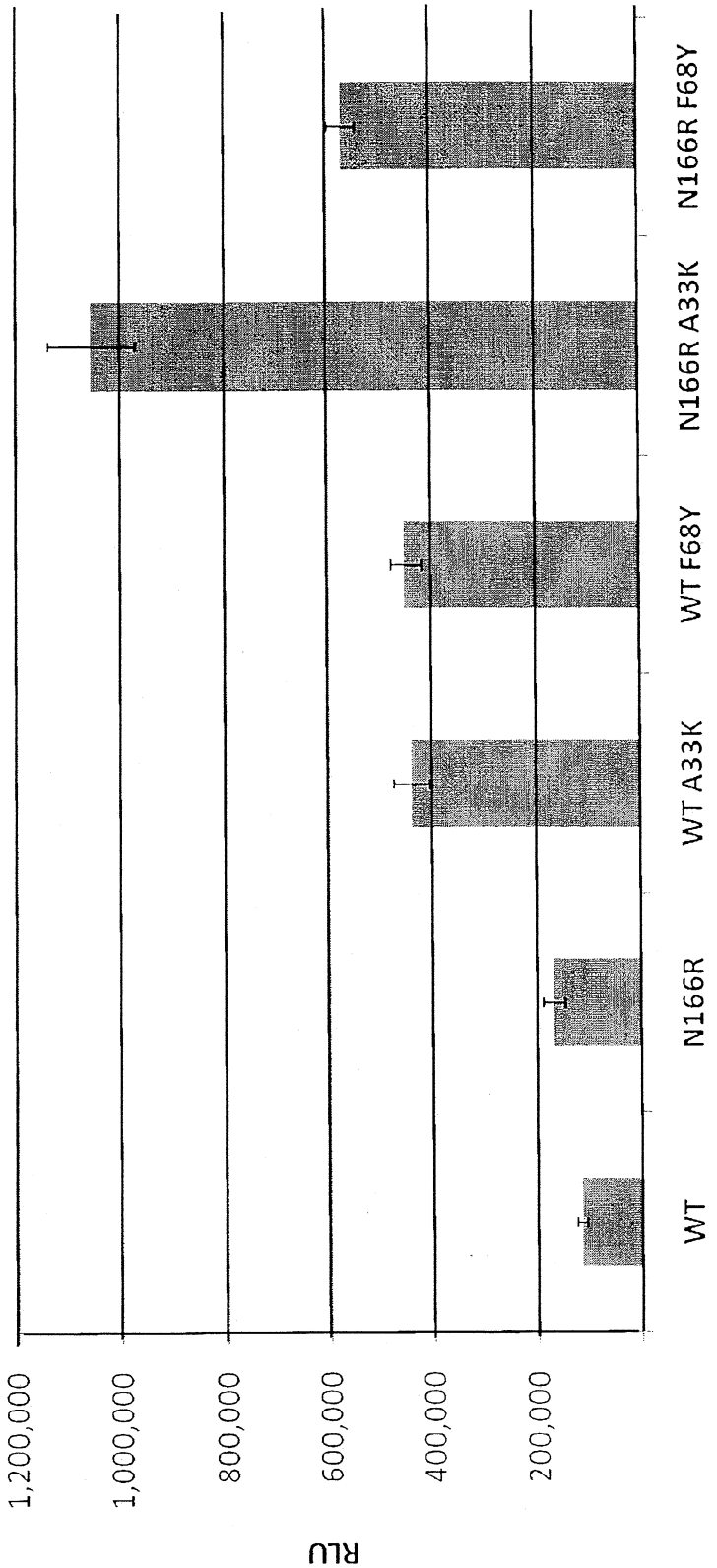


FIG. 13A

RLAB t=0 RLU

Sample	Average	cv
WT	27,306,288	8.9%
N166R	69,445,779	9.2%
WT A33K	44,140,390	14.2%
WT F68Y	45,593,349	10.3%
N166R A33K	138,736,058	8.0%
N166R F68Y	89,324,499	12.9%

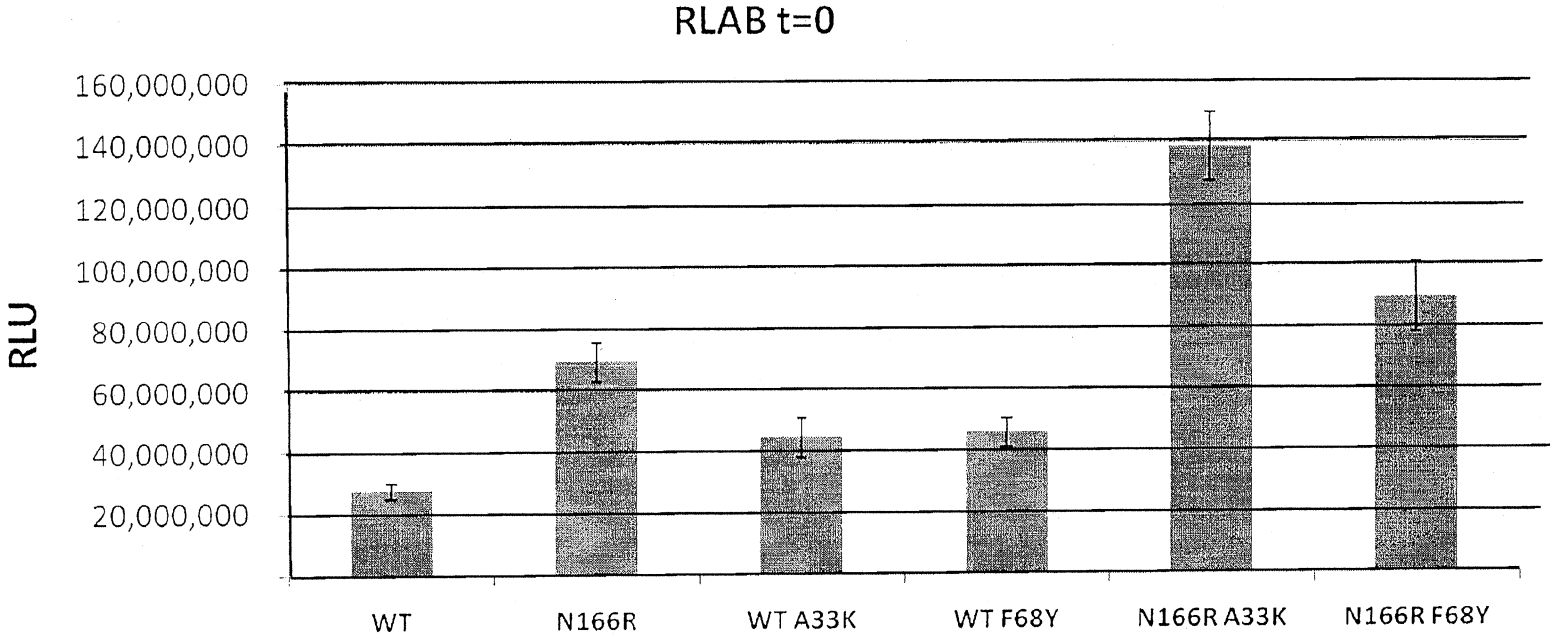


FIG. 13B

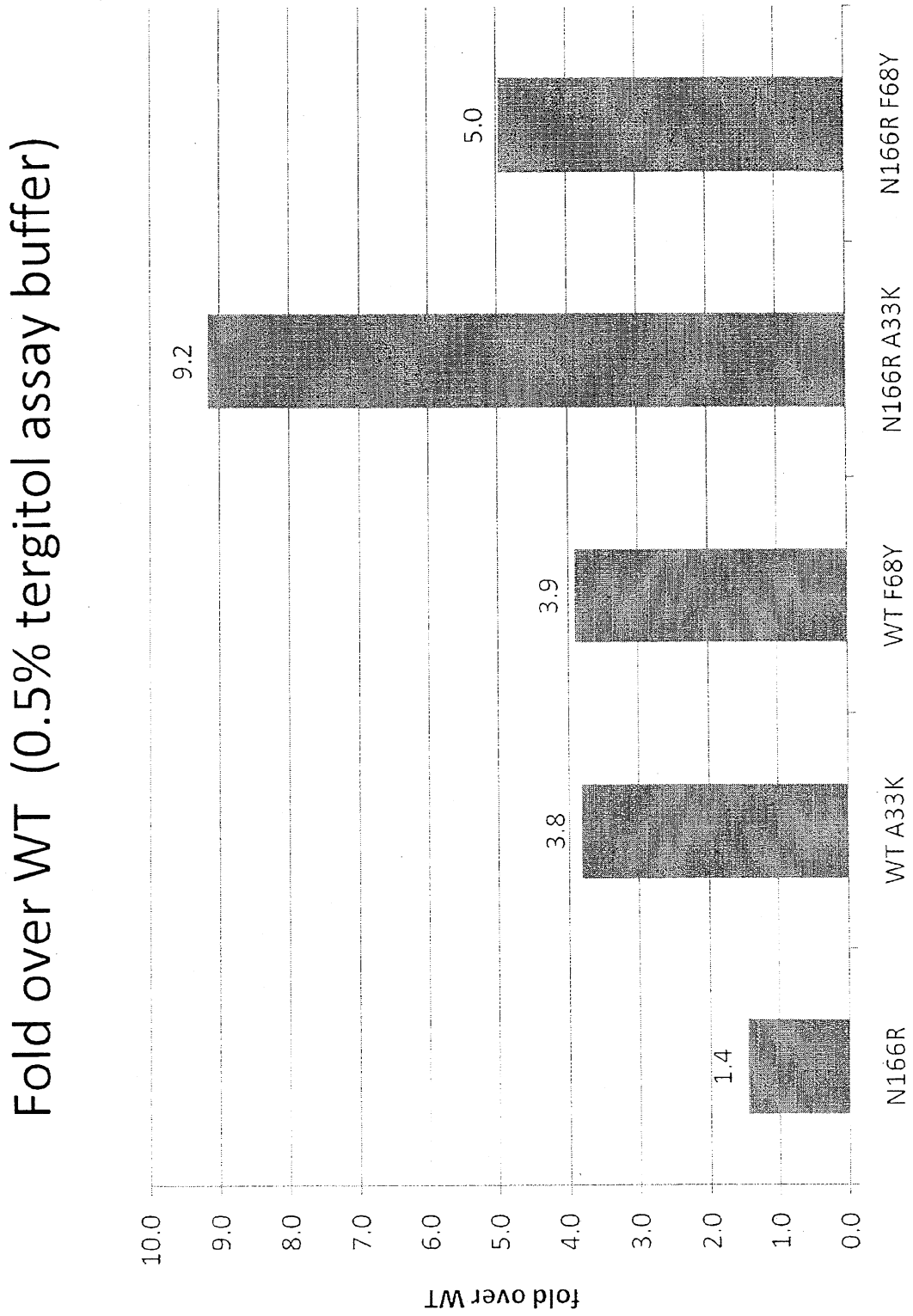

Fold over WT (0.5% tergitol assay buffer)
fold over WT
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
1.4
3.8
3.9
9.2
5.0
N166R
WT A33K
WT F68Y
N166R A33K
N166R F68Y

FIG. 14A

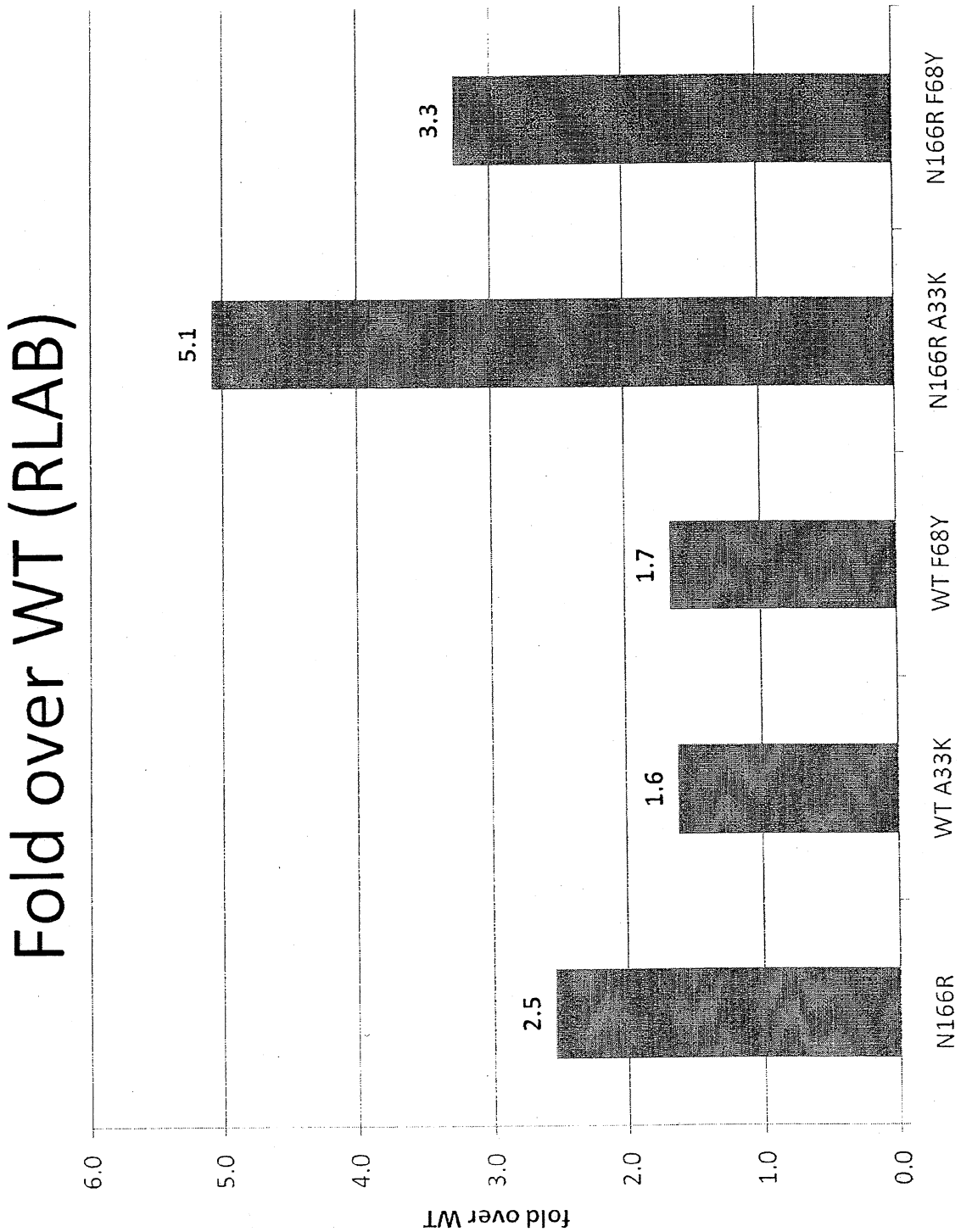

Fold over WT (RLAB)
fold over WT
6.0
5.0
4.0
3.0
2.0
1.0
0.0
2.5
1.6
1.7
5.1
3.3
N166R
WT A33K
WT F68Y
N166R A33K
N166R F68Y

FIG. 14B

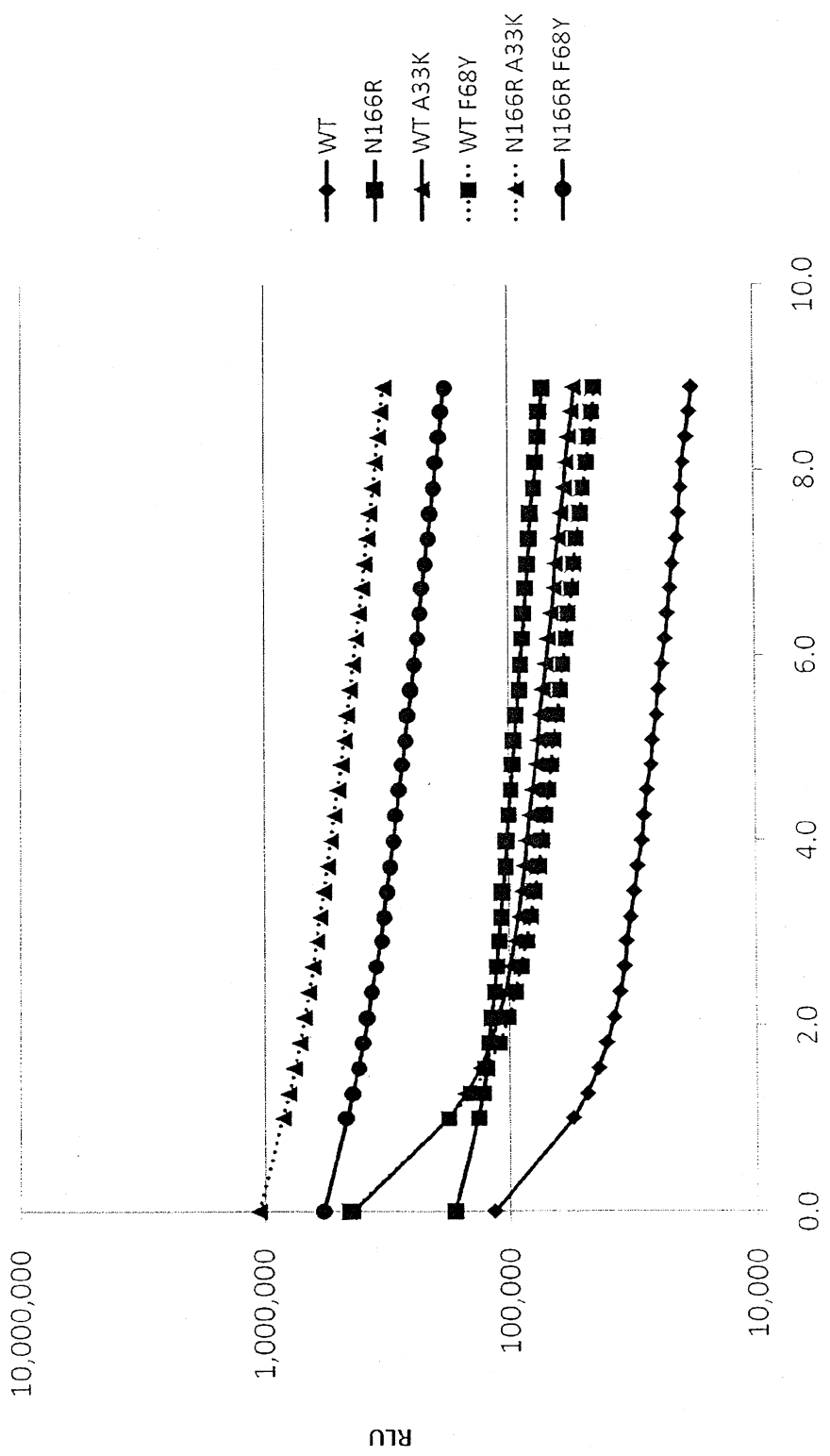

Signal kinetics (0.5% tergitol assay buffer)
WT
N166R
WT A33K
WT F68Y
N166R A33K
N166R F68Y
RLU
10,000,000
1,000,000
100,000
10,000
0.0
2.0
4.0
6.0
8.0
10.0
time(min)

FIG. 15A

Signal stability 0.5% tergitol assay buffer

Sample	Signal 1/2 life (0.5% tertitol assay buffer)
WT	6.5
N166R	9.6
WTA33K	7.4
WTF68Y	6.3
N166R A33K	6.3
N166R F68Y	6.2

FIG. 15B

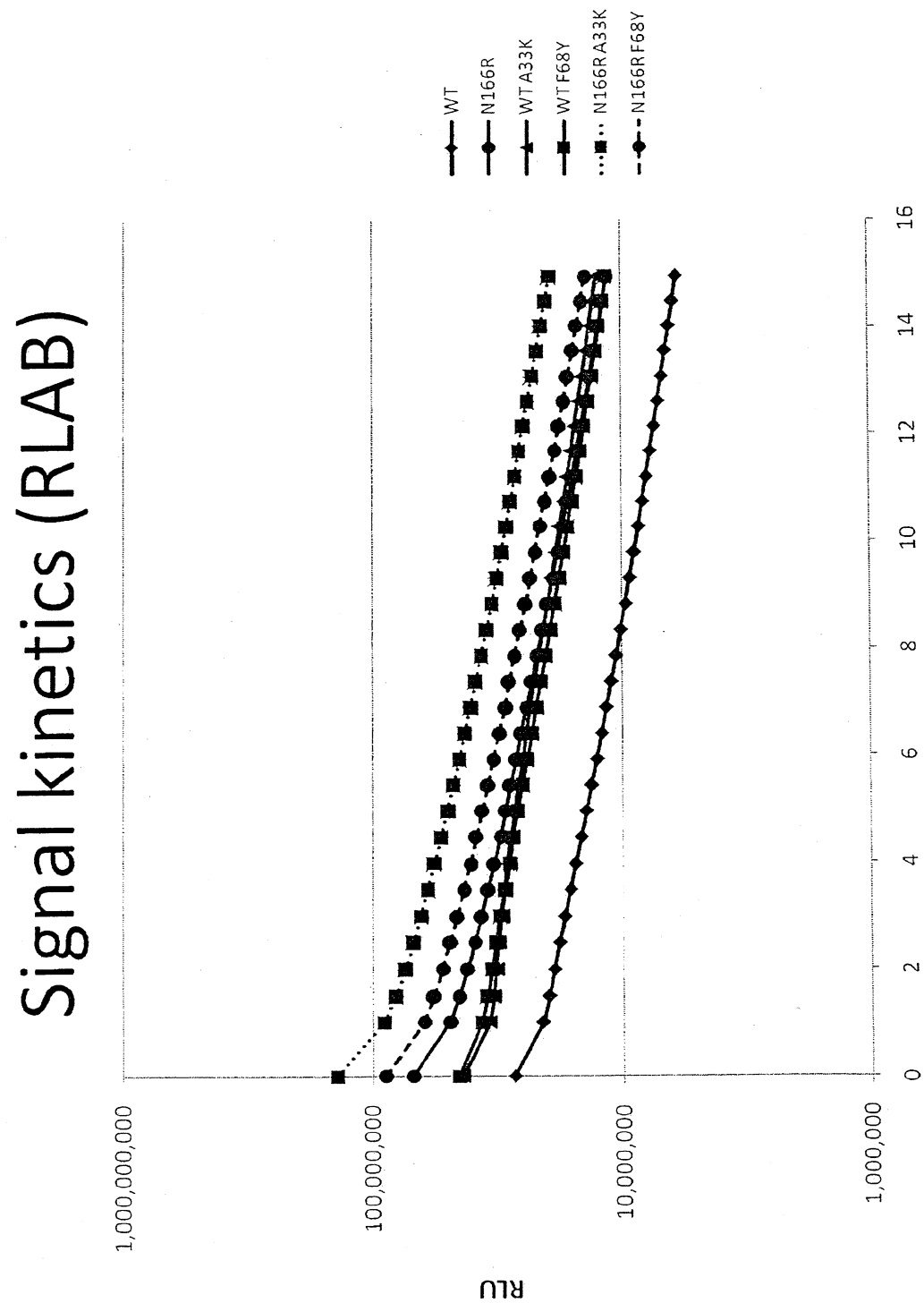

Signal kinetics (RLAB)
WT
N166R
WT A33K
WT F68Y
N166R A33K
N166R F68Y
RLU
1,000,000,000
100,000,000
10,000,000
1,000,000
0
2
4
6
8
10
12
14
16
time(min)

FIG. 16A

Signal stability RLAB

Sample	signal 1/2 life minutes
WT	7.64
N166R	6.73
A33K	9.22
F68Y	8.13
N166R/A33K	6.58
N166R/F68Y	6.60

FIG. 16B

Stability ½ life at 22°C

Sample	1/2 life (min) 22C
WT	54.6
N166R	67.3
A33K	58.2
F68Y	57.3
N166R, A33K	72.2
N166R, F68Y	77.9

FIG. 17

0.5% tergitol assay buffer

time(min)	N166R	C1+A4E	C1+A4E+F54T	C1+C2+A4E	C1+C3+A4E	C1+C3+A4E+F54T	Renilla
0.0	1,110	7,910,000	3,583,750	43,371	4,217,500	3,322,500	3,542,500
0.6	970	7,876,250	3,587,500	43,902	4,217,500	3,326,250	3,543,750
1.2	1,277	7,888,750	3,576,250	45,133	4,243,750	3,351,250	3,557,500
1.8	872	7,868,750	3,566,250	43,553	4,257,500	3,338,750	3,563,750
2.4	1,110	7,887,500	3,575,000	42,476	4,260,000	3,366,250	3,571,250
3.0	1,137	7,880,000	3,576,250	43,036	4,266,250	3,397,500	3,562,500
3.6	1,193	7,860,000	3,577,500	43,637	4,276,250	3,386,250	3,586,250
4.2	1,095	7,855,000	3,556,250	42,896	4,293,750	3,406,250	3,568,750
4.8	928	7,862,500	3,556,250	44,755	4,290,000	3,415,000	3,565,000
5.4	998	7,852,500	3,538,750	44,112	4,315,000	3,422,500	3,568,750
6.0	774	7,843,750	3,542,500	44,196	4,311,250	3,433,750	3,551,250
6.6	1,026	7,841,250	3,520,000	42,938	4,332,500	3,431,250	3,525,000
7.2	1,277	7,786,250	3,516,250	43,217	4,311,250	3,445,000	3,512,500
7.8	1,012	7,783,750	3,511,250	43,986	4,318,750	3,430,000	3,486,250
8.4	816	7,775,000	3,491,250	43,483	4,317,500	3,440,000	3,467,500
9.0	1,110	7,771,250	3,471,250	42,854	4,317,500	3,450,000	3,416,250
9.6	928	7,750,000	3,460,000	44,713	4,308,750	3,465,000	3,422,500
10.1	732	7,705,000	3,457,500	44,168	4,320,000	3,462,500	3,386,250
10.7	970	7,706,250	3,451,250	43,245	4,296,250	3,433,750	3,363,750
11.3	1,068	7,687,500	3,430,000	43,902	4,315,000	3,450,000	3,333,750

FIG. 18A

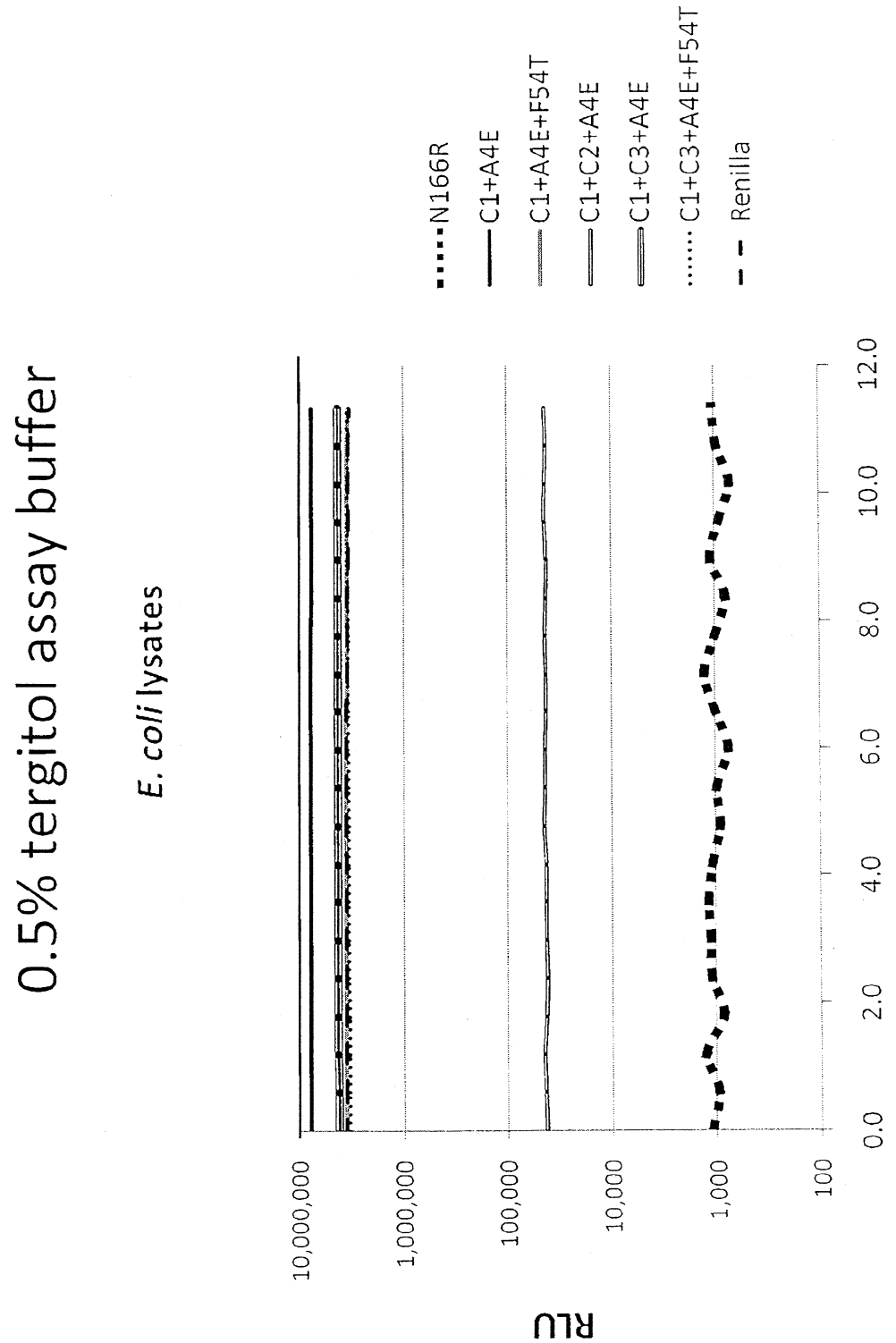

0.5% tergitol assay buffer
E. coli lysates
RLU
10,000,000
1,000,000
100,000
10,000
1,000
100
0.0
2.0
4.0
6.0
8.0
10.0
12.0
time(min)
N166R
C1+A4E
C1+A4E+F54T
C1+C2+A4E
C1+C3+A4E
C1+C3+A4E+F54T
Renilla

FIG. 18B

RLAB with injector *E. coli* lysates

time(min)	C1+A4E	C1+A4E+F54T	C1+C3+A4E	C1+C3+A4E+F54T	renilla	WT
0	6,288,773	10,015,723	3,998,483	19,218,247	5,721,221	10,170
1	3,880,142	1,479,839	3,501,544	6,033,827	2,111,378	3,490
2	2,515,425	242,450	2,948,188	1,422,742	827,548	1,753
3	1,739,960	99,503	2,378,597	526,400	437,314	1,326
4	1,269,359	66,080	1,926,802	273,345	277,539	966
5	957,041	51,848	1,587,709	172,383	195,944	843
6	734,186	41,485	1,320,817	120,617	147,764	729
7	573,084	34,985	1,106,277	89,734	116,523	609
8	453,838	29,690	944,146	69,148	95,257	469
9	363,570	25,892	803,433	55,316	78,655	513
10	296,648	22,868	696,959	45,239	66,302	423
11	244,349	19,838	606,222	38,464	55,581	296
12	203,980	17,906	533,878	33,612	48,518	289
13	171,296	16,269	469,833	29,523	40,784	253
14	145,640	14,579	420,908	26,163	36,657	236

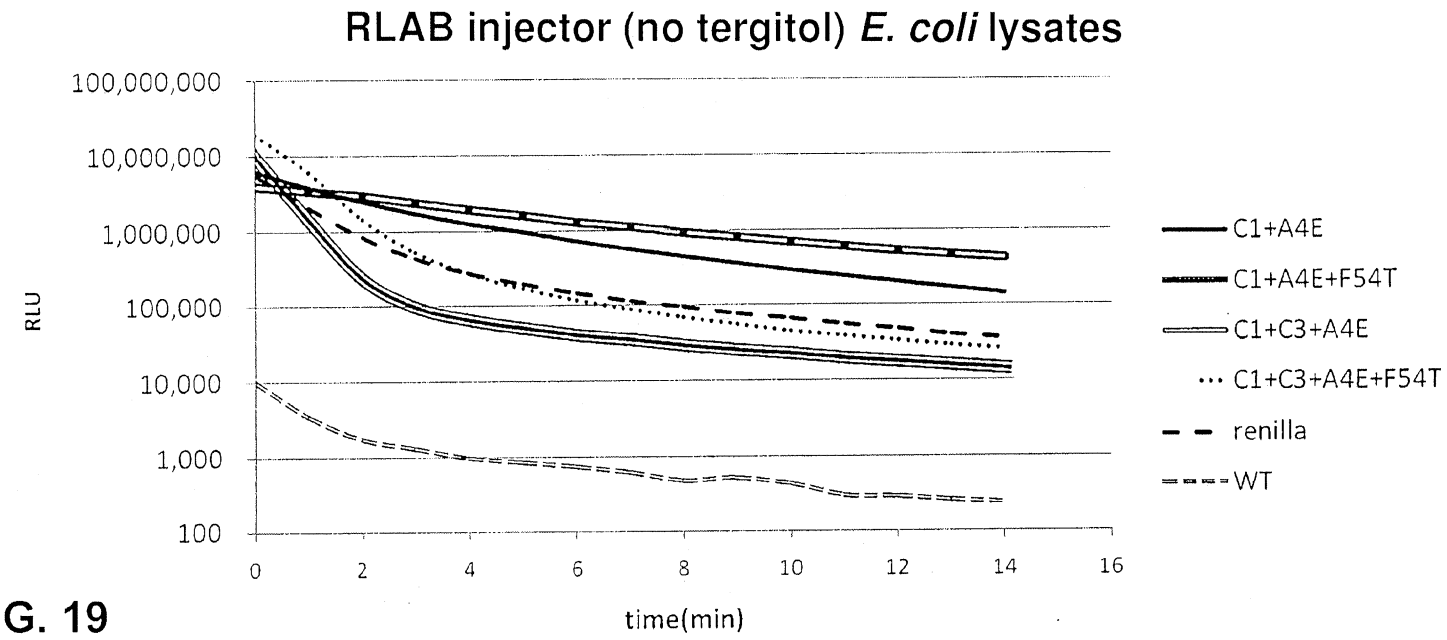


FIG. 19

Og luc composites 0.5% tergitol

time(min)	C1+C2+A4E	C1+A4	hRL	Og-Luc	T2T	A54F
0	3,283,757	705,604,545	82,499,609	40,946	129,209	3,196,722
1	3,272,774	655,089,672	81,682,809	43,269	144,141	3,053,210
2	3,263,848	607,514,297	80,390,118	39,618	141,818	3,051,850
3	3,323,641	561,553,077	78,958,229	45,923	164,050	2,972,811
4	3,251,173	520,696,293	77,087,348	29,000	165,045	2,863,212
5	3,259,501	481,737,466	75,545,993	54,218	178,318	2,773,522
6	3,332,268	443,689,477	73,595,608	38,955	168,031	2,774,186
7	3,290,426	409,149,917	71,668,815	37,296	184,622	2,630,011
8	3,389,076	375,439,634	69,682,029	44,264	180,641	2,614,748
9	3,320,986	346,313,863	67,570,182	42,936	192,586	2,575,859

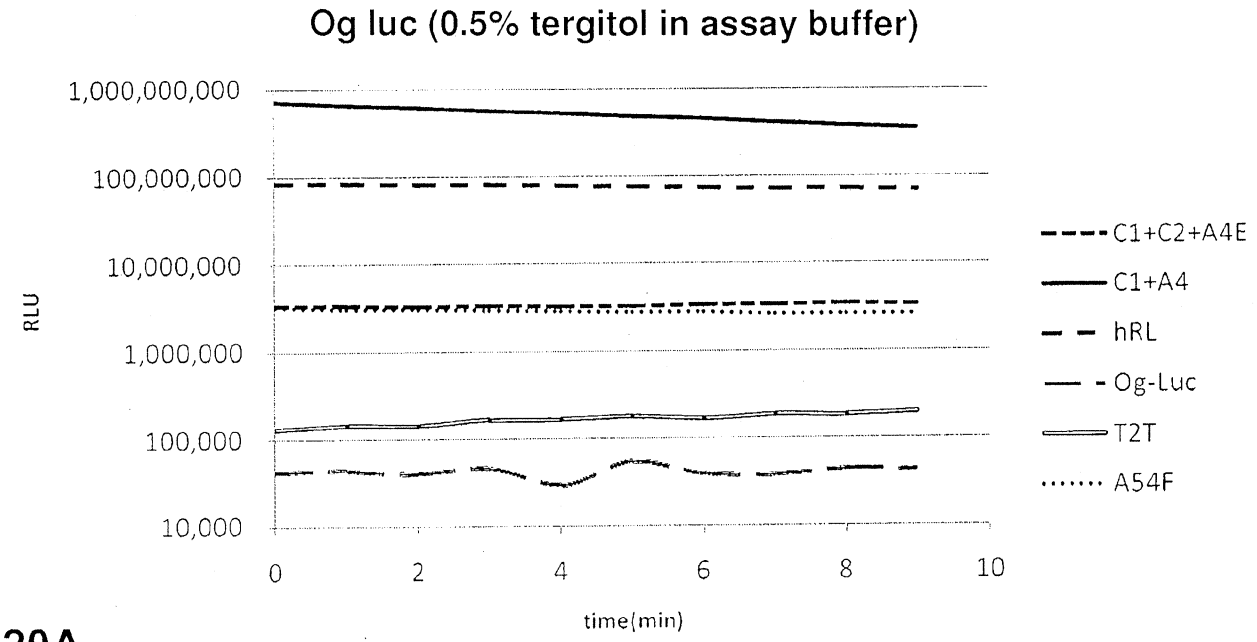


FIG. 20A

E. Coli lysates RLAB injector

Time(min)	WT	Ren	C1+A4E	C1+C2+A4E	C1+C3+A4E
0	1,516	5,224,047	7,334,767	41,049	3,886,812
1	1,146	1,400,228	4,282,362	35,602	3,162,835
2	690	628,021	2,940,985	31,649	2,451,582
3	493	362,496	2,127,271	29,607	1,842,268
4	393	243,436	1,606,017	27,491	1,412,564
5	266	176,676	1,248,100	26,635	1,117,276
6	253	137,402	998,258	25,333	906,594
7	203	111,480	820,954	24,741	751,344
8	200	90,889	681,497	23,637	634,050
9	156	78,227	573,912	23,222	542,510
10	156	66,966	487,929	22,152	469,571
11	110	58,172	422,367	21,129	413,198
12	103	51,130	368,901	21,209	363,911
13	83	44,578	321,104	21,032	327,210

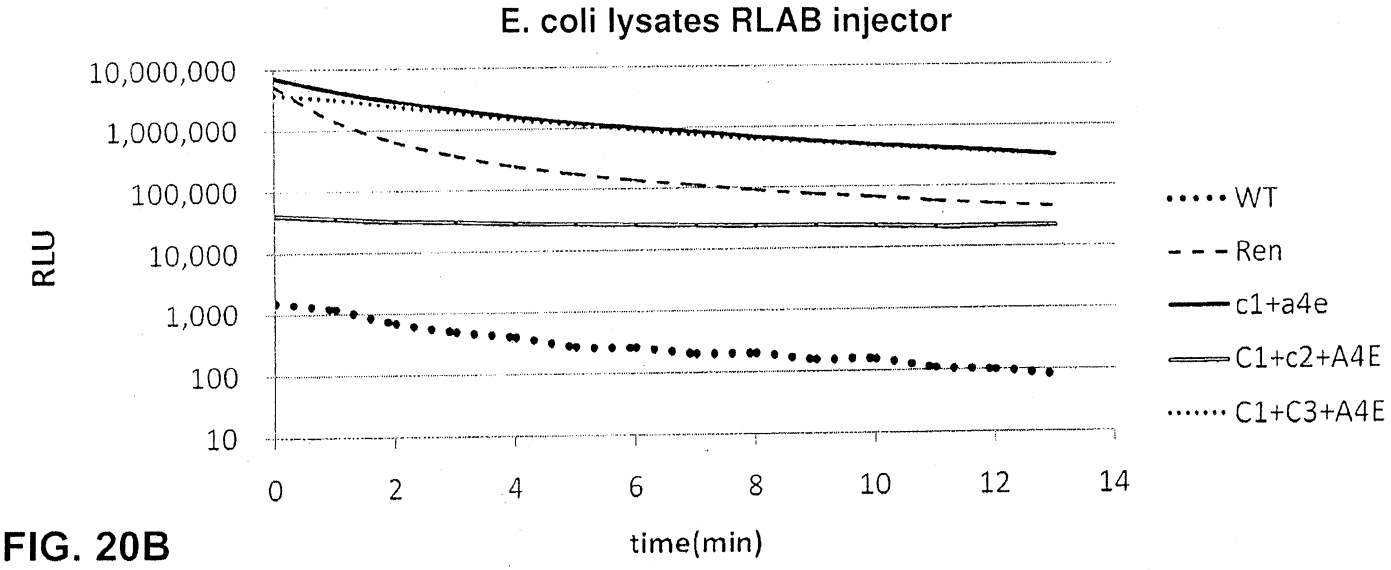


FIG. 20B

Results in *E. coli* (tergitol in assay buffer to stabilize signal)

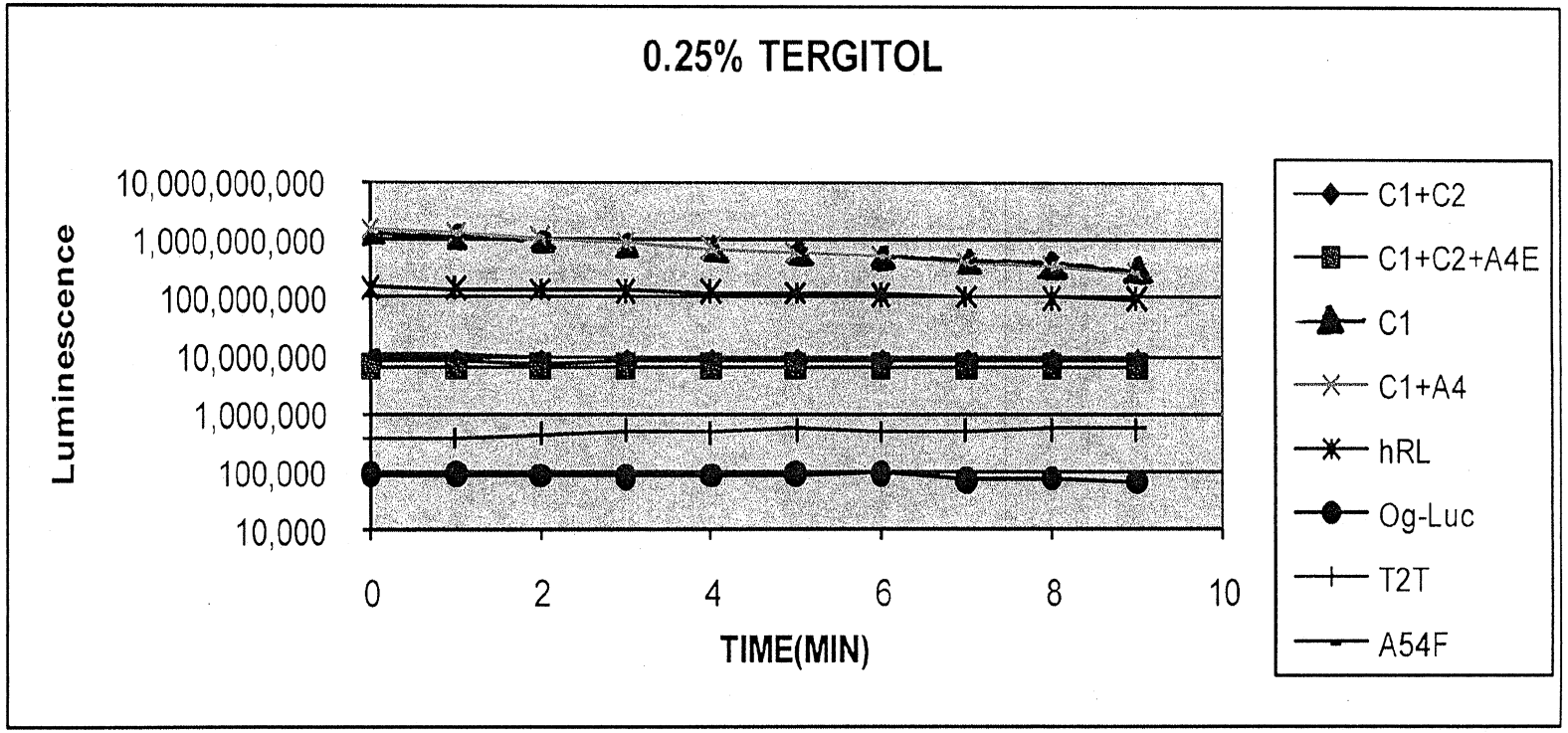


FIG. 21

Og-Luc composites in 293 cells

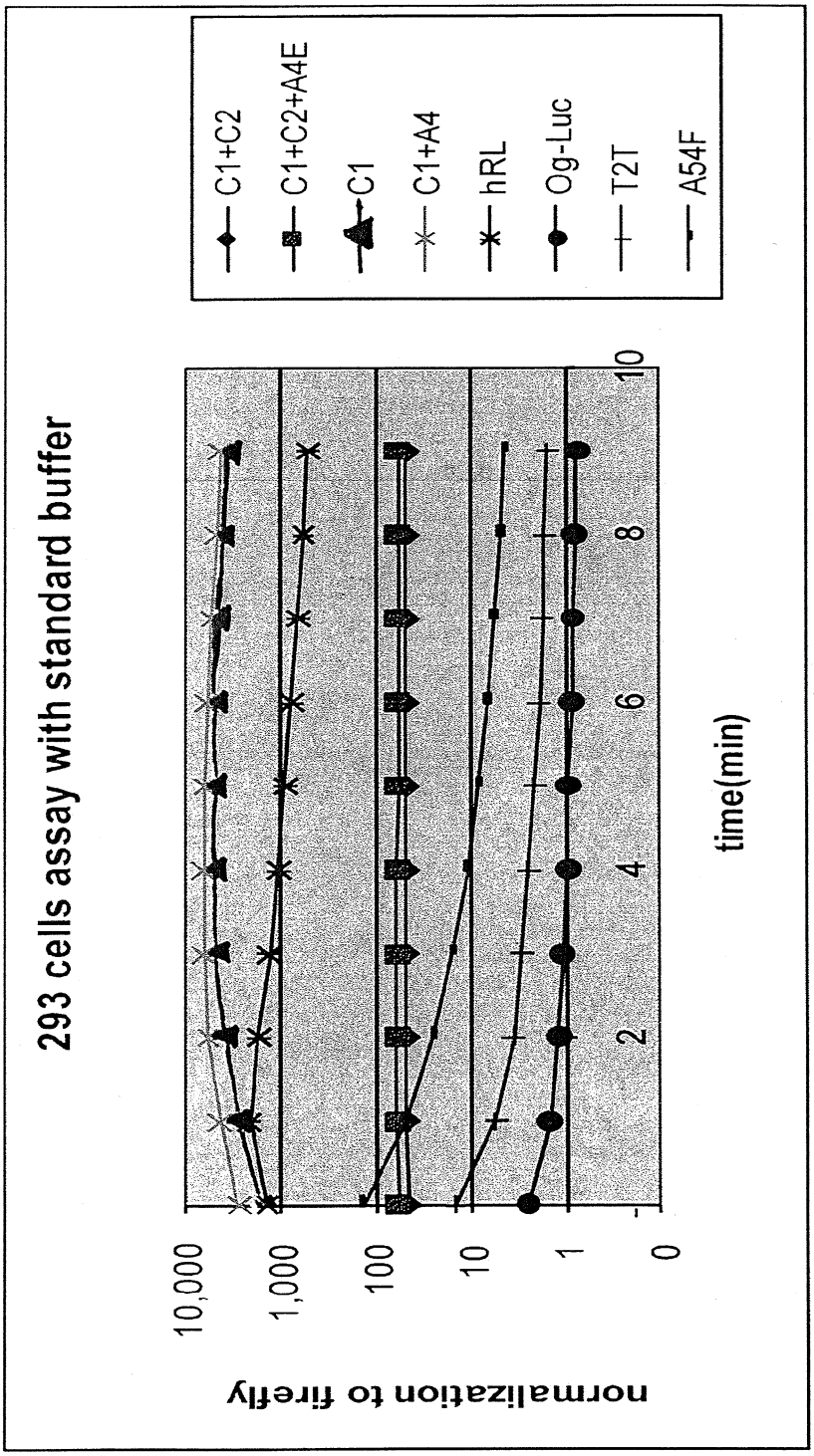


FIG. 22

293 cells normalized to firefly

0.25% tergitol normalized to firefly
293 cells

time(min)	C1+C2+A4E	C1+A4	hRL	Og-Luc (wt)
0	2.71	741.28	344.96	0.07
1	2.78	731.25	325.17	0.07
2	2.65	722.31	306.02	0.08
3	2.90	715.06	288.69	0.05
4	2.73	706.89	269.87	0.07
5	2.92	697.22	254.60	0.05
6	2.69	693.01	239.46	0.08
7	2.61	687.04	228.25	0.07
8	2.75	676.30	215.62	0.04
9	2.76	671.85	204.51	0.04

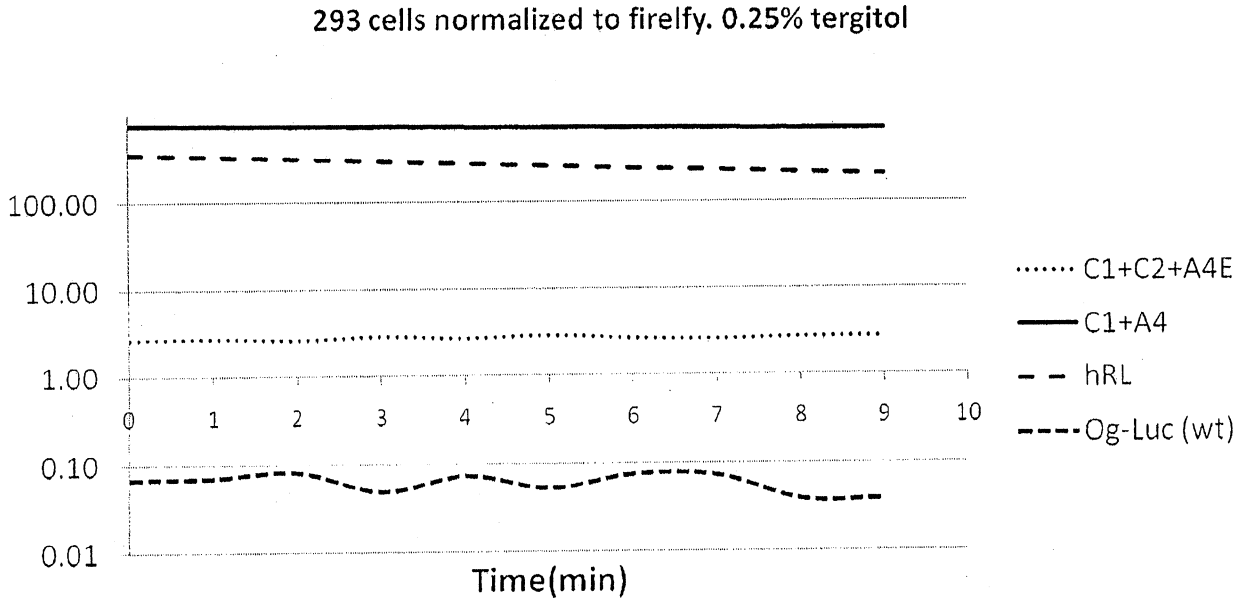


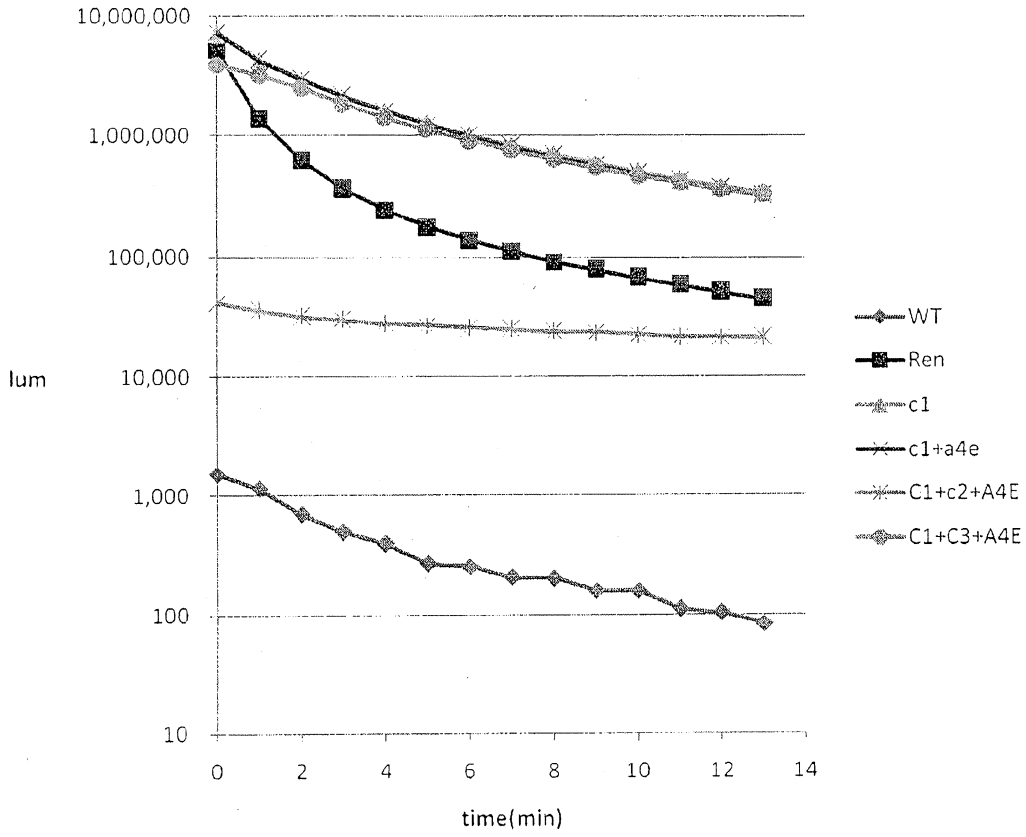
FIG. 23

Stability of og-luc clones

Temp	Clone	1/2 life
RT (22°C)	WT	1 hour
RT (22°C)	og-luc C1	9.4 hours
RT (22°C)	Renilla	~24 hours
30°C	N166R	30 minutes
30°C	og-luc C1+A4E	No decay in 6 hours
30°C	Renilla	7.9 hours
37°C	N166R	2 min
37°C	og-luc C1+A4E	no loss 3 hours
42°C	og-luc C1+A4E	6.4 hours
54°C	og-luc C1	7.1 min
54°C	og-luc C1+A4E	8 (7.1, 7.7) min
54°C	og-luc C1 +C2+A4E	128 min
54°C	og-luc C1 +C3+A4E	23.7 min

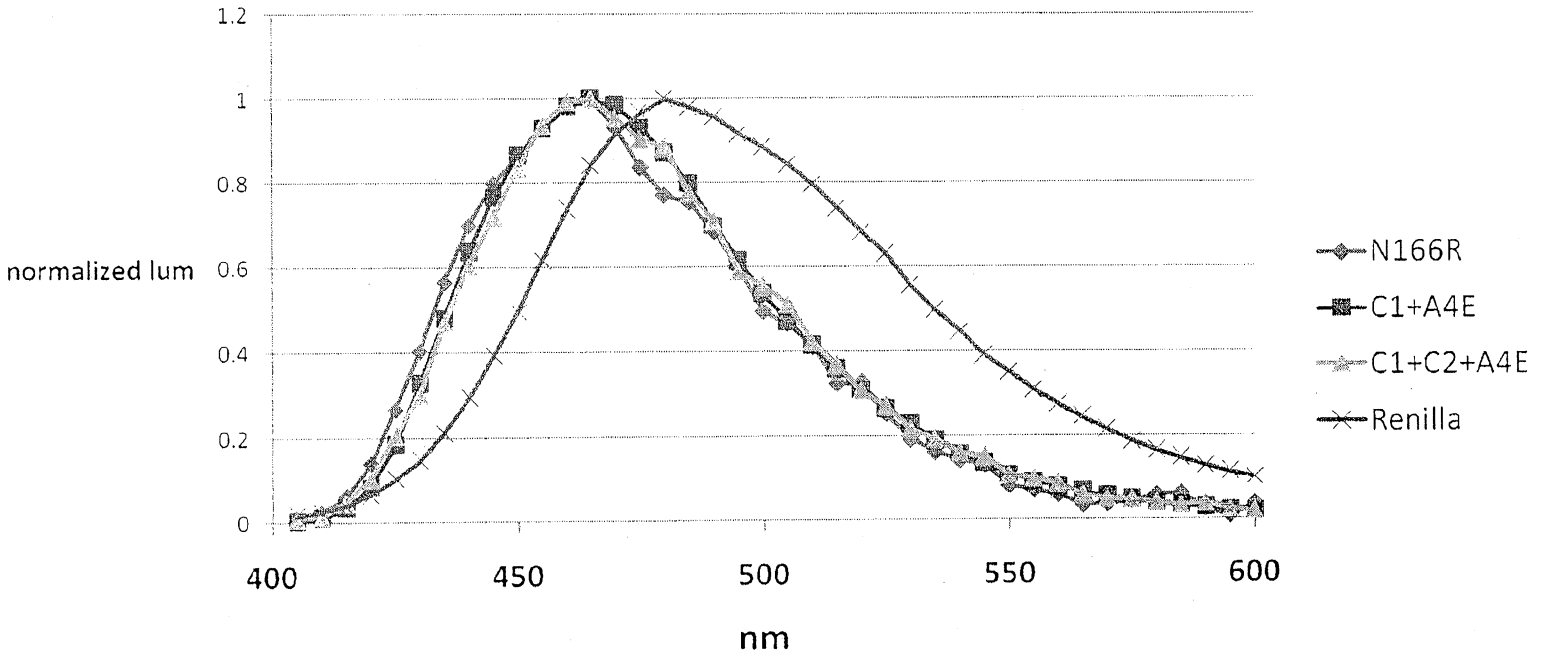
FIG. 24

Signal ½ life or signal stability



Sample	1/2 life (min)
WT	1.8
Ren	0.8
c1	1.7
c1+a4e	1.7
C1+c2+A4E	12.6
C1+C3+A4E	3.3

FIG. 25



Sample	Spectral peak
N166R	465
C1+A4E	465
C1+C2+A4E	465
Renilla	480

FIG. 26

Mutations: C1+A4E random mutagenesis

C1A4E random mutagenesis	AA change	Fold over C1A4E
04g7	L72Q	3
10f2	I90T	1.9
16c5	Q20R	4.1
25a1	C164S	3.1
28b2	V79I	2.9
29h7	F54I	10.3
35d11	K89E	2.2

FIG. 27A

Mutations: C1+A4E random mutagenesis

Amino Acid change	Fold over C1+A4E
I90V	1.5
F77W	2

FIG. 27B

Changes at position 92

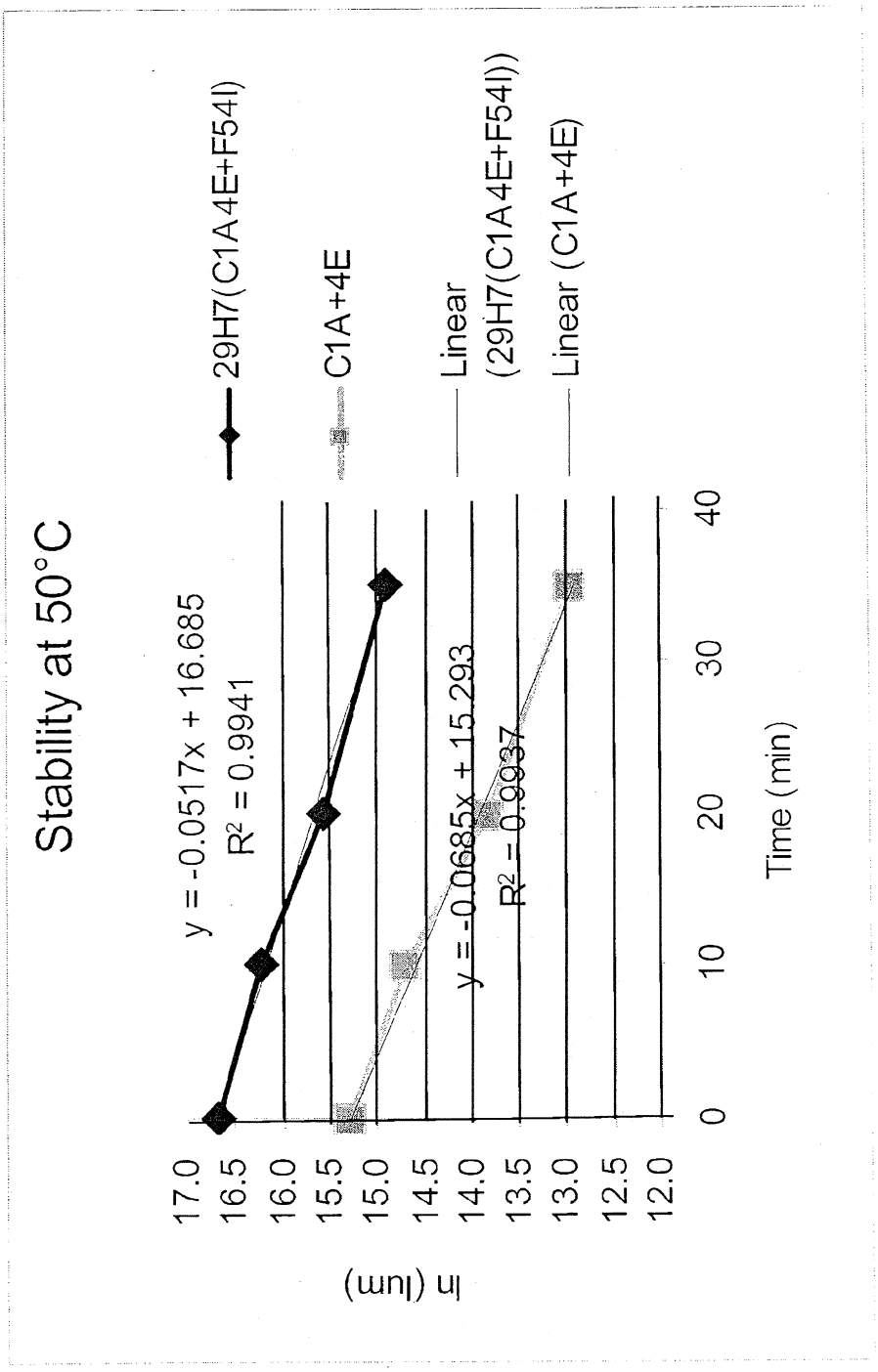
Amino Acid change	Fold over C1A4E (20uM coelenterazine)
L92G	2.2
L92Q	2
L92S	2.9
L92A	2.5

FIG. 28

Combinations that improve light output

Sample ID (Combination experiment)	Fold over C1A4E	Amino Acid change from C1A4E
17A6	19.3	F54I, F68S, I90V
14A12	17.7	F54I, M75K
1G4	17.5	F54I, M75K, I90V
3C6	17.5	F54I, F68S, M75K
14B7	17.5	F54I, I90V

FIG. 29



Sample	½ life 50°C (min)
C1A4E	10.1
29H7(C1A4E+F54I)	13.3

FIG. 30

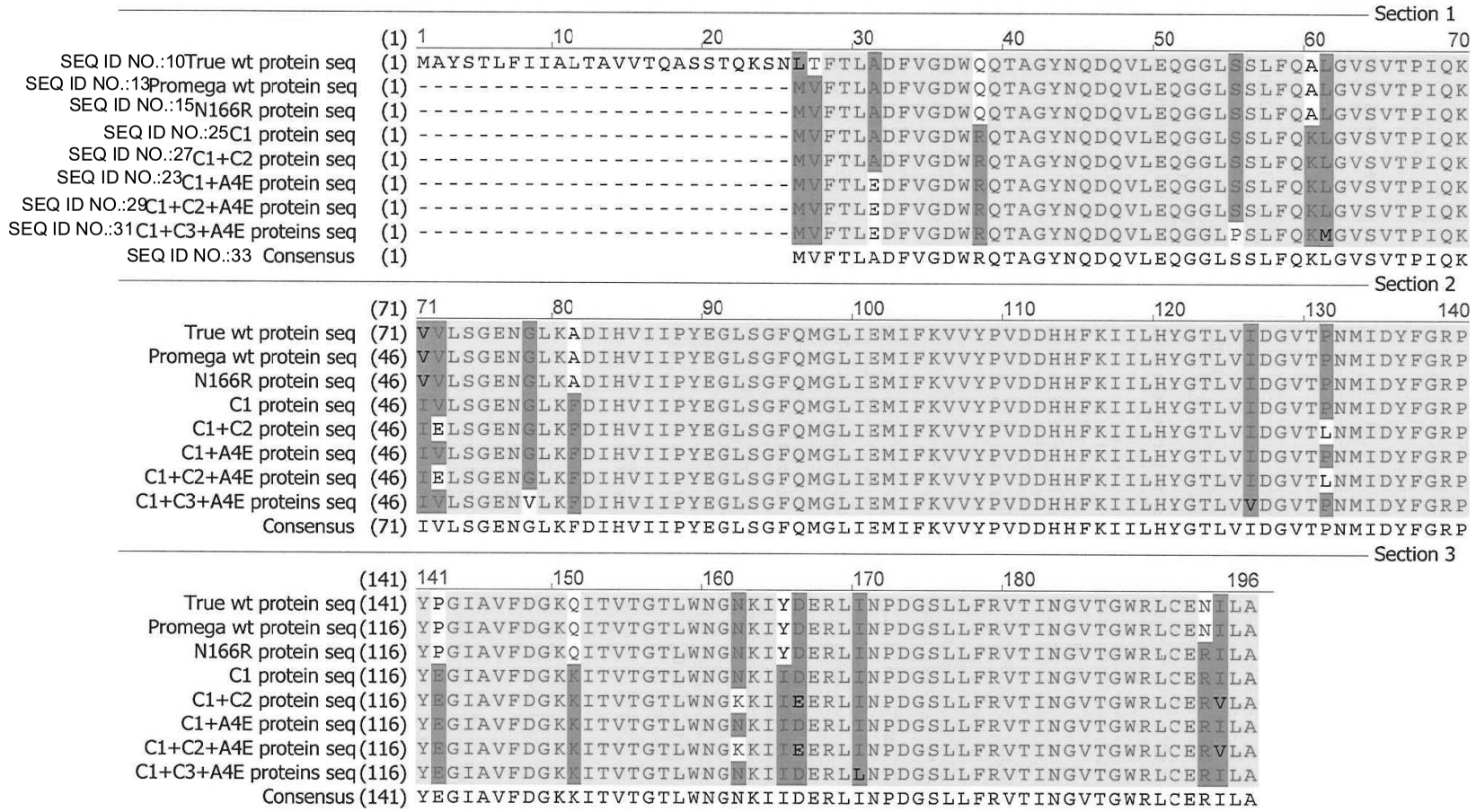

Section 1
(1) 1 10 20 30 40 50 60 70
SEQ ID NO.:10 True wt protein seq (1) MAYSTLFIIALTAVVTQASSTQKSNLTFTLADFVGDWQQTAGYNQDQVLEQGGLSSLFQALGVSVTPIQK
SEQ ID NO.:13 Promega wt protein seq (1) ----------MVFTLADFVGDWQQTAGYNQDQVLEQGGLSSLFQALGVSVTPIQK
SEQ ID NO.:15 N166R protein seq (1) ----------MVFTLADFVGDWQQTAGYNQDQVLEQGGLSSLFQALGVSVTPIQK
SEQ ID NO.:25 C1 protein seq (1) ----------MVFTLADFVGDWRQTAGYNQDQVLEQGGLSSLFQKLGVSVTPIQK
SEQ ID NO.:27 C1+C2 protein seq (1) ----------MVFTLADFVGDWRQTAGYNQDQVLEQGGLSSLFQKLGVSVTPIQK
SEQ ID NO.:23 C1+A4E protein seq (1) ----------MVFTLEDFVGDWRQTAGYNQDQVLEQGGLSSLFQKLGVSVTPIQK
SEQ ID NO.:29 C1+C2+A4E protein seq (1) ----------MVFTLEDFVGDWRQTAGYNQDQVLEQGGLSSLFQKLGVSVTPIQK
SEQ ID NO.:31 C1+C3+A4E proteins seq (1) ----------MVFTLEDFVGDWRQTAGYNQDQVLEQGGLPSLFQKMGVSVTPIQK
SEQ ID NO.:33 Consensus (1) MVFTLADFVGDWRQTAGYNQDQVLEQGGLSSLFQKLGVSVTPIQK
Section 2
(71) 71 80 90 100 110 120 130 140
True wt protein seq (71) VVLSGENGLKADIHVIIPYEGLSGFQMGLIEMIFKVVYPVDDHHFKIILHYGTLVIDGVTPNMIDYFGRP
Promega wt protein seq (46) VVLSGENGLKADIHVIIPYEGLSGFQMGLIEMIFKVVYPVDDHHFKIILHYGTLVIDGVTPNMIDYFGRP
N166R protein seq (46) VVLSGENGLKADIHVIIPYEGLSGFQMGLIEMIFKVVYPVDDHHFKIILHYGTLVIDGVTPNMIDYFGRP
C1 protein seq (46) IVLSGENGLKFDIHVIIPYEGLSGFQMGLIEMIFKVVYPVDDHHFKIILHYGTLVIDGVTPNMIDYFGRP
C1+C2 protein seq (46) IELSGENGLKFDIHVIIPYEGLSGFQMGLIEMIFKVVYPVDDHHFKIILHYGTLVIDGVTLNMIDYFGRP
C1+A4E protein seq (46) IVLSGENGLKFDIHVIIPYEGLSGFQMGLIEMIFKVVYPVDDHHFKIILHYGTLVIDGVTPNMIDYFGRP
C1+C2+A4E protein seq (46) IELSGENGLKFDIHVIIPYEGLSGFQMGLIEMIFKVVYPVDDHHFKIILHYGTLVIDGVTLNMIDYFGRP
C1+C3+A4E proteins seq (46) IVLSGENVLKFDIHVIIPYEGLSGFQMGLIEMIFKVVYPVDDHHFKIILHYGTLVVDGVTPNMIDYFGRP
Consensus (71) IVLSGENGLKFDIHVIIPYEGLSGFQMGLIEMIFKVVYPVDDHHFKIILHYGTLVIDGVTPNMIDYFGRP
Section 3
(141) 141 150 160 170 180 196
True wt protein seq (141) YPGIAVFDGKQITVTGTLWNGNKIYDERLINPDGSLLFRVTINGVTGWRLCENILA
Promega wt protein seq (116) YPGIAVFDGKQITVTGTLWNGNKIYDERLINPDGSLLFRVTINGVTGWRLCENILA
N166R protein seq (116) YPGIAVFDGKQITVTGTLWNGNKIYDERLINPDGSLLFRVTINGVTGWRLCERILA
C1 protein seq (116) YPGIAVFDGKKITVTGTLWNGNKIIDERLINPDGSLLFRVTINGVTGWRLCERILA
C1+C2 protein seq (116) YPGIAVFDGKKITVTGTLWNGKKIIEERLINPDGSLLFRVTINGVTGWRLCERVLA
C1+A4E protein seq (116) YPGIAVFDGKKITVTGTLWNGNKIIDERLINPDGSLLFRVTINGVTGWRLCERILA
C1+C2+A4E protein seq (116) YPGIAVFDGKKITVTGTLWNGKKIIEERLINPDGSLLFRVTINGVTGWRLCERVLA
C1+C3+A4E proteins seq (116) YPGIAVFDGKKITVTGTLWNGNKIIDERLLNPDGSLLFRVTINGVTGWRLCERILA
Consensus (141) YEGIAVFDGKKITVTGTLWNGNKIIDERLINPDGSLLFRVTINGVTGWRLCERILA

FIG. 31

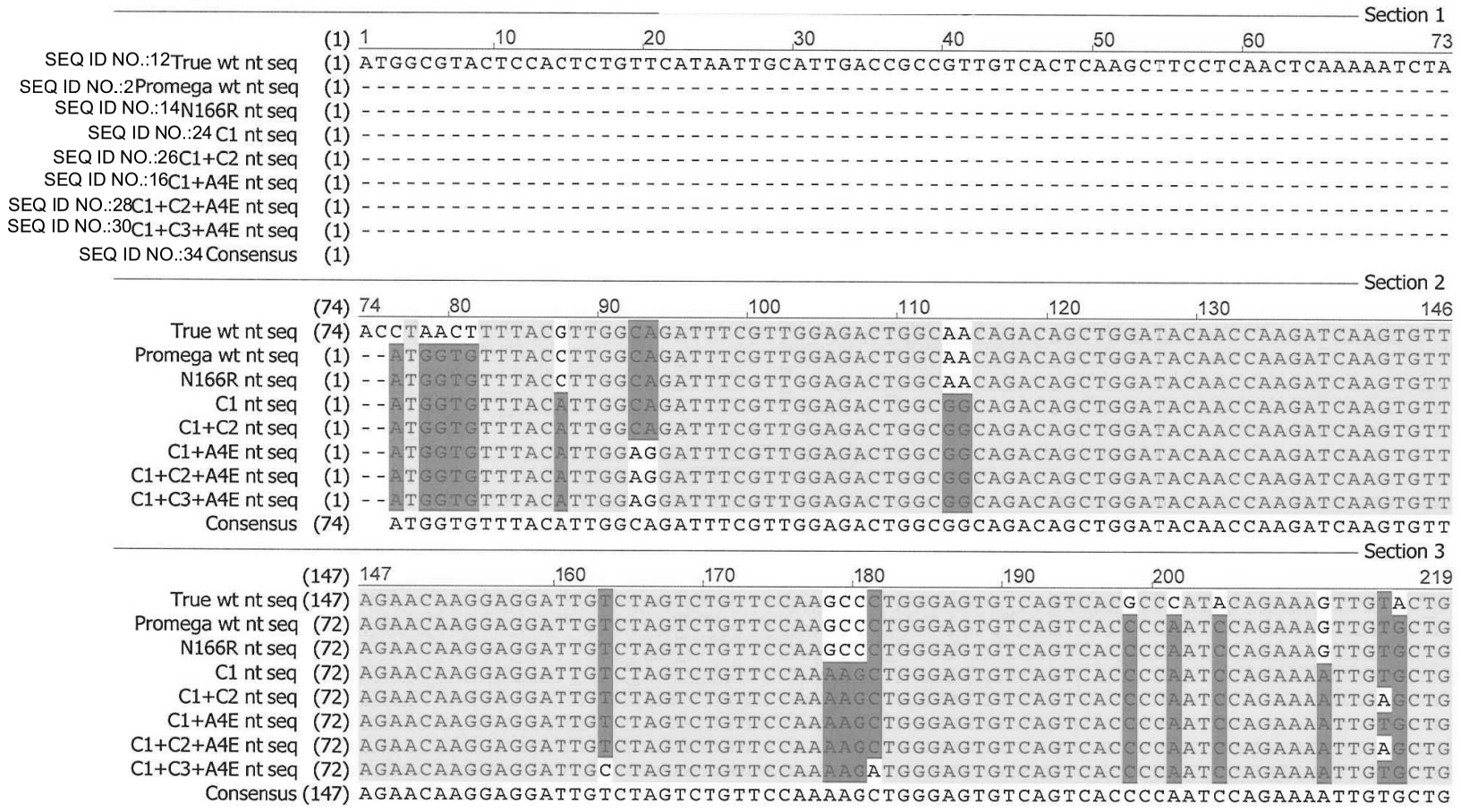

Section 1
(1) 1 10 20 30 40 50 60 73
SEQ ID NO.:12 True wt nt seq (1) ATGGCGTACTCCACTCTGTTCATAATTGCATTGACCGCCGTTGTCACTCAAGCTTCCTCAACTCAAAAATCTA
SEQ ID NO.:2 Promega wt nt seq (1)
SEQ ID NO.:14 N166R nt seq (1)
SEQ ID NO.:24 C1 nt seq (1)
SEQ ID NO.:26 C1+C2 nt seq (1)
SEQ ID NO.:16 C1+A4E nt seq (1)
SEQ ID NO.:28 C1+C2+A4E nt seq (1)
SEQ ID NO.:30 C1+C3+A4E nt seq (1)
SEQ ID NO.:34 Consensus (1)
Section 2
(74) 74 80 90 100 110 120 130 146
True wt nt seq (74) ACCTAACTTTTACGTTGGCAGATTTCGTTGGAGACTGGCAACAGACAGCTGGATACAACCAAGATCAAGTGTT
Promega wt nt seq (1) --ATGGTGTTTACCTTGGCAGATTTCGTTGGAGACTGGCAACAGACAGCTGGATACAACCAAGATCAAGTGTT
N166R nt seq (1) --ATGGTGTTTACCTTGGCAGATTTCGTTGGAGACTGGCAACAGACAGCTGGATACAACCAAGATCAAGTGTT
C1 nt seq (1) --ATGGTGTTTACATTGGCAGATTTCGTTGGAGACTGGCGGCAGACAGCTGGATACAACCAAGATCAAGTGTT
C1+C2 nt seq (1) --ATGGTGTTTACATTGGCAGATTTCGTTGGAGACTGGCGGCAGACAGCTGGATACAACCAAGATCAAGTGTT
C1+A4E nt seq (1) --ATGGTGTTTACATTGGAGGATTTCGTTGGAGACTGGCGGCAGACAGCTGGATACAACCAAGATCAAGTGTT
C1+C2+A4E nt seq (1) --ATGGTGTTTACATTGGAGGATTTCGTTGGAGACTGGCGGCAGACAGCTGGATACAACCAAGATCAAGTGTT
C1+C3+A4E nt seq (1) --ATGGTGTTTACATTGGAGGATTTCGTTGGAGACTGGCGGCAGACAGCTGGATACAACCAAGATCAAGTGTT
Consensus (74) ATGGTGTTTACATTGGCAGATTTCGTTGGAGACTGGCGGCAGACAGCTGGATACAACCAAGATCAAGTGTT
Section 3
(147) 147 160 170 180 190 200 219
True wt nt seq (147) AGAACAAGGAGGATTGTCTAGTCTGTTCCAAGCCCTGGGAGTGTCAGTCACGCCCATACAGAAAGTTGTACTG
Promega wt nt seq (72) AGAACAAGGAGGATTGTCTAGTCTGTTCCAAGCCCTGGGAGTGTCAGTCACCCCAATCCAGAAAGTTGTGCTG
N166R nt seq (72) AGAACAAGGAGGATTGTCTAGTCTGTTCCAAGCCCTGGGAGTGTCAGTCACCCCAATCCAGAAAGTTGTGCTG
C1 nt seq (72) AGAACAAGGAGGATTGTCTAGTCTGTTCCAAAAGCTGGGAGTGTCAGTCACCCCAATCCAGAAAATTGTGCTG
C1+C2 nt seq (72) AGAACAAGGAGGATTGTCTAGTCTGTTCCAAAAGCTGGGAGTGTCAGTCACCCCAATCCAGAAAATTGAGCTG
C1+A4E nt seq (72) AGAACAAGGAGGATTGTCTAGTCTGTTCCAAAAGCTGGGAGTGTCAGTCACCCCAATCCAGAAAATTGTGCTG
C1+C2+A4E nt seq (72) AGAACAAGGAGGATTGTCTAGTCTGTTCCAAAAGCTGGGAGTGTCAGTCACCCCAATCCAGAAAATTGAGCTG
C1+C3+A4E nt seq (72) AGAACAAGGAGGATTGCCTAGTCTGTTCCAAAAGATGGGAGTGTCAGTCACCCCAATCCAGAAAATTGTGCTG
Consensus (147) AGAACAAGGAGGATTGTCTAGTCTGTTCCAAAAGCTGGGAGTGTCAGTCACCCCAATCCAGAAAATTGTGCTG

FIG. 32

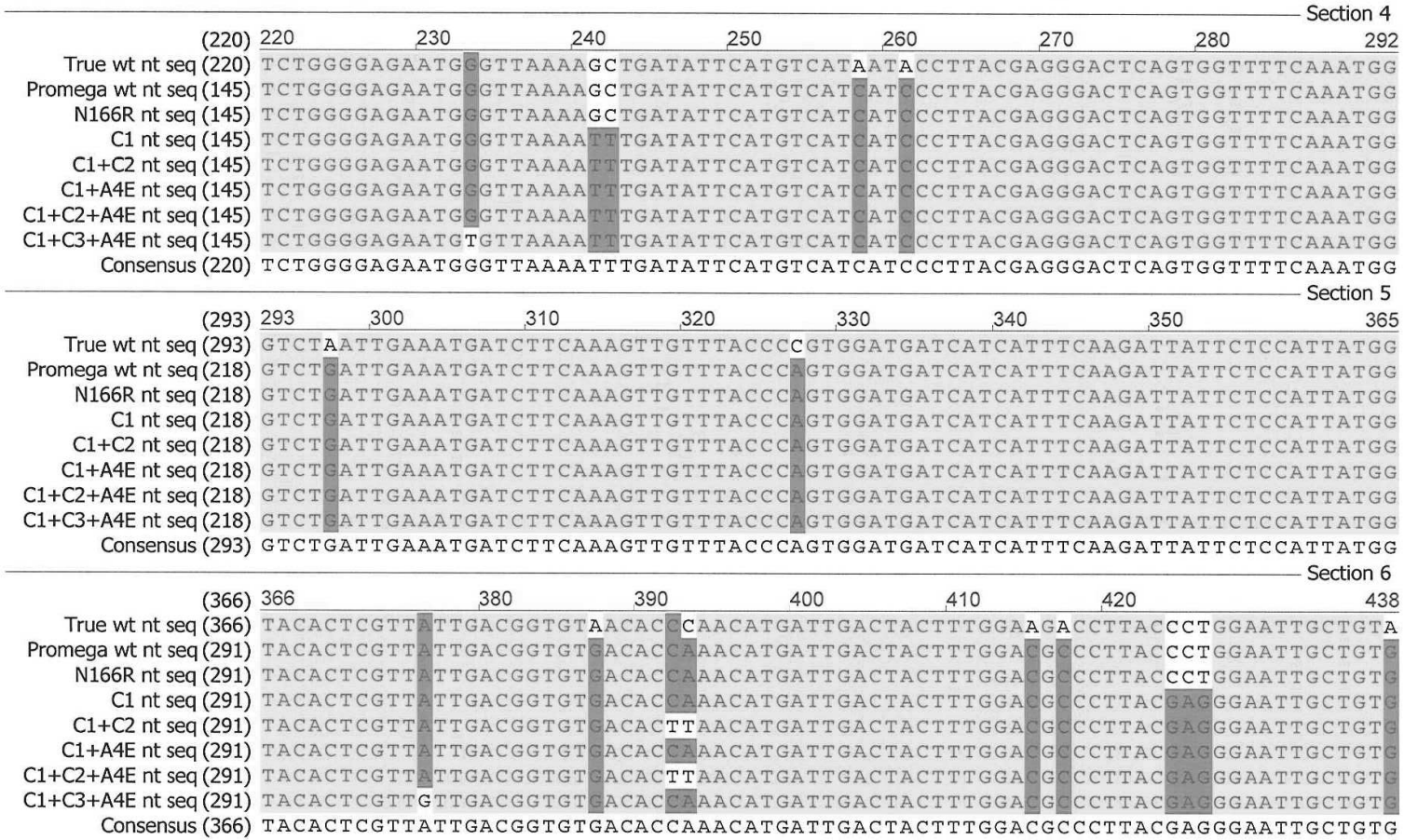

Section 4
(220) 220 230 240 250 260 270 280 292
True wt nt seq (220) TCTGGGGAGAATGGGTTAAAAGCTGATATTCATGTCATAATACCTTACGAGGGACTCAGTGGTTTTCAAATGG
Promega wt nt seq (145) TCTGGGGAGAATGGGTTAAAAGCTGATATTCATGTCATCATCCCTTACGAGGGACTCAGTGGTTTTCAAATGG
N166R nt seq (145) TCTGGGGAGAATGGGTTAAAAGCTGATATTCATGTCATCATCCCTTACGAGGGACTCAGTGGTTTTCAAATGG
C1 nt seq (145) TCTGGGGAGAATGGGTTAAAATTTGATATTCATGTCATCATCCCTTACGAGGGACTCAGTGGTTTTCAAATGG
C1+C2 nt seq (145) TCTGGGGAGAATGGGTTAAAATTTGATATTCATGTCATCATCCCTTACGAGGGACTCAGTGGTTTTCAAATGG
C1+A4E nt seq (145) TCTGGGGAGAATGGGTTAAAATTTGATATTCATGTCATCATCCCTTACGAGGGACTCAGTGGTTTTCAAATGG
C1+C2+A4E nt seq (145) TCTGGGGAGAATGGGTTAAAATTTGATATTCATGTCATCATCCCTTACGAGGGACTCAGTGGTTTTCAAATGG
C1+C3+A4E nt seq (145) TCTGGGGAGAATGTGTTAAAATTTGATATTCATGTCATCATCCCTTACGAGGGACTCAGTGGTTTTCAAATGG
Consensus (220) TCTGGGGAGAATGGGTTAAAATTTGATATTCATGTCATCATCCCTTACGAGGGACTCAGTGGTTTTCAAATGG
Section 5
(293) 293 300 310 320 330 340 350 365
True wt nt seq (293) GTCTAATTGAAATGATCTTCAAAGTTGTTTACCCCGTGGATGATCATCATTTCAAGATTATTCTCCATTATGG
Promega wt nt seq (218) GTCTGATTGAAATGATCTTCAAAGTTGTTTACCCAGTGGATGATCATCATTTCAAGATTATTCTCCATTATGG
N166R nt seq (218) GTCTGATTGAAATGATCTTCAAAGTTGTTTACCCAGTGGATGATCATCATTTCAAGATTATTCTCCATTATGG
C1 nt seq (218) GTCTGATTGAAATGATCTTCAAAGTTGTTTACCCAGTGGATGATCATCATTTCAAGATTATTCTCCATTATGG
C1+C2 nt seq (218) GTCTGATTGAAATGATCTTCAAAGTTGTTTACCCAGTGGATGATCATCATTTCAAGATTATTCTCCATTATGG
C1+A4E nt seq (218) GTCTGATTGAAATGATCTTCAAAGTTGTTTACCCAGTGGATGATCATCATTTCAAGATTATTCTCCATTATGG
C1+C2+A4E nt seq (218) GTCTGATTGAAATGATCTTCAAAGTTGTTTACCCAGTGGATGATCATCATTTCAAGATTATTCTCCATTATGG
C1+C3+A4E nt seq (218) GTCTGATTGAAATGATCTTCAAAGTTGTTTACCCAGTGGATGATCATCATTTCAAGATTATTCTCCATTATGG
Consensus (293) GTCTGATTGAAATGATCTTCAAAGTTGTTTACCCAGTGGATGATCATCATTTCAAGATTATTCTCCATTATGG
Section 6
(366) 366 380 390 400 410 420 438
True wt nt seq (366) TACACTCGTTATTGACGGTGTAACACCCAACATGATTGACTACTTTGGAAGACCTTACCCTGGAATTGCTGTA
Promega wt nt seq (291) TACACTCGTTATTGACGGTGTGACACCAAACATGATTGACTACTTTGGACGCCCTTACCCTGGAATTGCTGTG
N166R nt seq (291) TACACTCGTTATTGACGGTGTGACACCAAACATGATTGACTACTTTGGACGCCCTTACCCTGGAATTGCTGTG
C1 nt seq (291) TACACTCGTTATTGACGGTGTGACACCAAACATGATTGACTACTTTGGACGCCCTTACGAGGGAATTGCTGTG
C1+C2 nt seq (291) TACACTCGTTATTGACGGTGTGACACTTAACATGATTGACTACTTTGGACGCCCTTACGAGGGAATTGCTGTG
C1+A4E nt seq (291) TACACTCGTTATTGACGGTGTGACACCAAACATGATTGACTACTTTGGACGCCCTTACGAGGGAATTGCTGTG
C1+C2+A4E nt seq (291) TACACTCGTTATTGACGGTGTGACACTTAACATGATTGACTACTTTGGACGCCCTTACGAGGGAATTGCTGTG
C1+C3+A4E nt seq (291) TACACTCGTTGTTGACGGTGTGACACCAAACATGATTGACTACTTTGGACGCCCTTACGAGGGAATTGCTGTG
Consensus (366) TACACTCGTTATTGACGGTGTGACACCAAACATGATTGACTACTTTGGACGCCCTTACGAGGGAATTGCTGTG

FIG. 32 CONT.

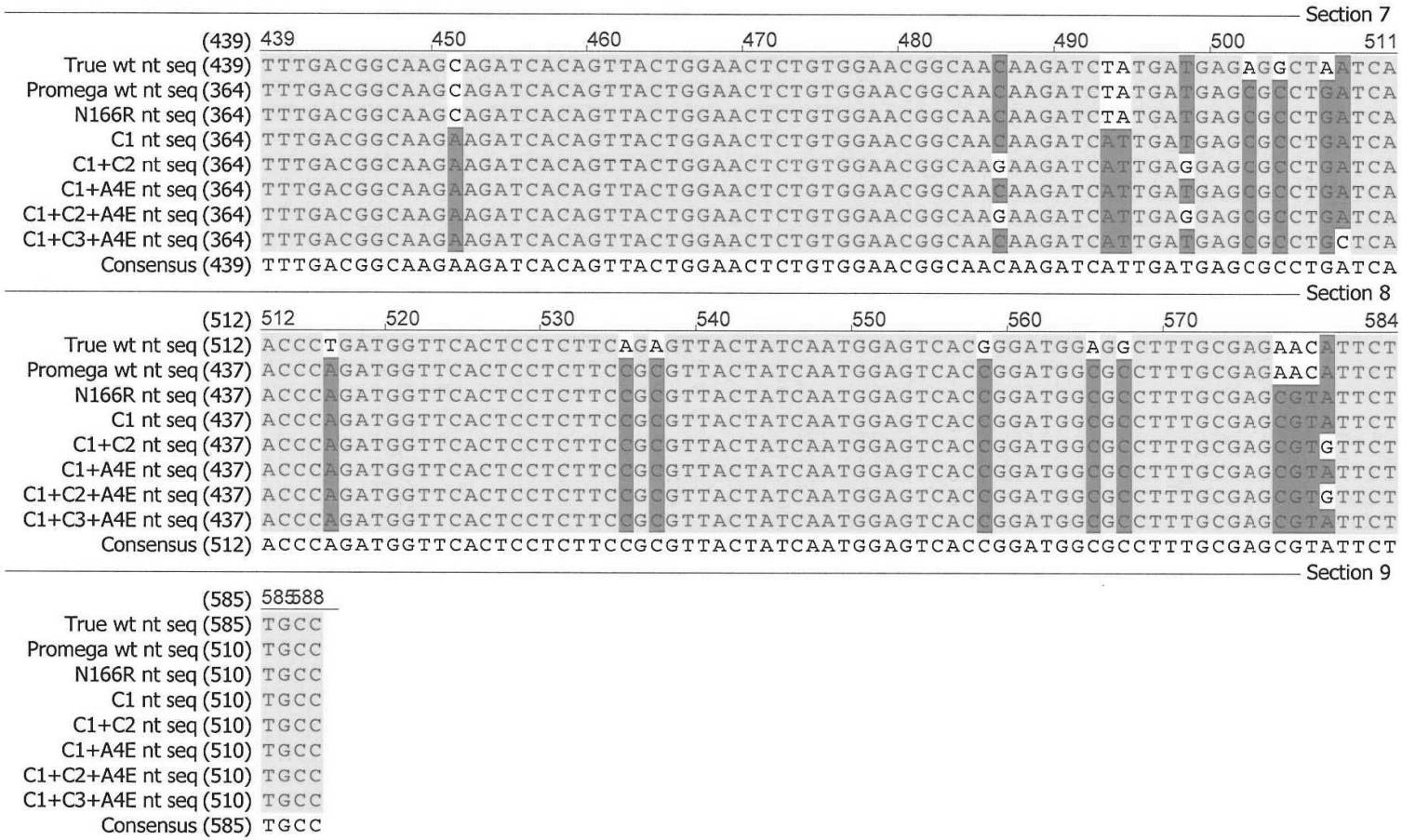

Section 7
(439) 439 450 460 470 480 490 500 511
True wt nt seq (439) TTTGACGGCAAGCAGATCACAGTTACTGGAACTCTGTGGAACGGCAACAAGATCTATGATGAGAGGCTAATCA
Promega wt nt seq (364) TTTGACGGCAAGCAGATCACAGTTACTGGAACTCTGTGGAACGGCAACAAGATCTATGATGAGCGCCTGATCA
N166R nt seq (364) TTTGACGGCAAGCAGATCACAGTTACTGGAACTCTGTGGAACGGCAACAAGATCTATGATGAGCGCCTGATCA
C1 nt seq (364) TTTGACGGCAAGAAGATCACAGTTACTGGAACTCTGTGGAACGGCAACAAGATCATTGATGAGCGCCTGATCA
C1+C2 nt seq (364) TTTGACGGCAAGAAGATCACAGTTACTGGAACTCTGTGGAACGGCAAGAAGATCATTGAGGAGCGCCTGATCA
C1+A4E nt seq (364) TTTGACGGCAAGAAGATCACAGTTACTGGAACTCTGTGGAACGGCAACAAGATCATTGATGAGCGCCTGATCA
C1+C2+A4E nt seq (364) TTTGACGGCAAGAAGATCACAGTTACTGGAACTCTGTGGAACGGCAAGAAGATCATTGAGGAGCGCCTGATCA
C1+C3+A4E nt seq (364) TTTGACGGCAAGAAGATCACAGTTACTGGAACTCTGTGGAACGGCAACAAGATCATTGATGAGCGCCTGCTCA
Consensus (439) TTTGACGGCAAGAAGATCACAGTTACTGGAACTCTGTGGAACGGCAACAAGATCATTGATGAGCGCCTGATCA
Section 8
(512) 512 520 530 540 550 560 570 584
True wt nt seq (512) ACCCTGATGGTTCACTCCTCTTCAGAGTTACTATCAATGGAGTCACGGGATGGAGGCTTTGCGAGAACATTCT
Promega wt nt seq (437) ACCCAGATGGTTCACTCCTCTTCCGCGTTACTATCAATGGAGTCACCGGATGGCGCCTTTGCGAGAACATTCT
N166R nt seq (437) ACCCAGATGGTTCACTCCTCTTCCGCGTTACTATCAATGGAGTCACCGGATGGCGCCTTTGCGAGCGTATTCT
C1 nt seq (437) ACCCAGATGGTTCACTCCTCTTCCGCGTTACTATCAATGGAGTCACCGGATGGCGCCTTTGCGAGCGTATTCT
C1+C2 nt seq (437) ACCCAGATGGTTCACTCCTCTTCCGCGTTACTATCAATGGAGTCACCGGATGGCGCCTTTGCGAGCGTGTTCT
C1+A4E nt seq (437) ACCCAGATGGTTCACTCCTCTTCCGCGTTACTATCAATGGAGTCACCGGATGGCGCCTTTGCGAGCGTATTCT
C1+C2+A4E nt seq (437) ACCCAGATGGTTCACTCCTCTTCCGCGTTACTATCAATGGAGTCACCGGATGGCGCCTTTGCGAGCGTGTTCT
C1+C3+A4E nt seq (437) ACCCAGATGGTTCACTCCTCTTCCGCGTTACTATCAATGGAGTCACCGGATGGCGCCTTTGCGAGCGTATTCT
Consensus (512) ACCCAGATGGTTCACTCCTCTTCCGCGTTACTATCAATGGAGTCACCGGATGGCGCCTTTGCGAGCGTATTCT
Section 9
(585) 585588
True wt nt seq (585) TGCC
Promega wt nt seq (510) TGCC
N166R nt seq (510) TGCC
C1 nt seq (510) TGCC
C1+C2 nt seq (510) TGCC
C1+A4E nt seq (510) TGCC
C1+C2+A4E nt seq (510) TGCC
C1+C3+A4E nt seq (510) TGCC
Consensus (585) TGCC

FIG. 32 CONT.

Mutation	Fold over N166R
T2T	0.6
T2T+N166R	1.8
A4E	0.5
Q11R	0.7
V44I	2.3
A54F	1.5
A54F+N166R	14.3
A54I	5.8
P115E	1.3
P115E+N166R	2.9
Y138I	2.6
Q124K	1.0
Y138C+N166R	2.8
I90V	0.8
A33K	0.6
F68Y	0.7
N166R+ A33K	2.0
N166R +F68Y	1.3

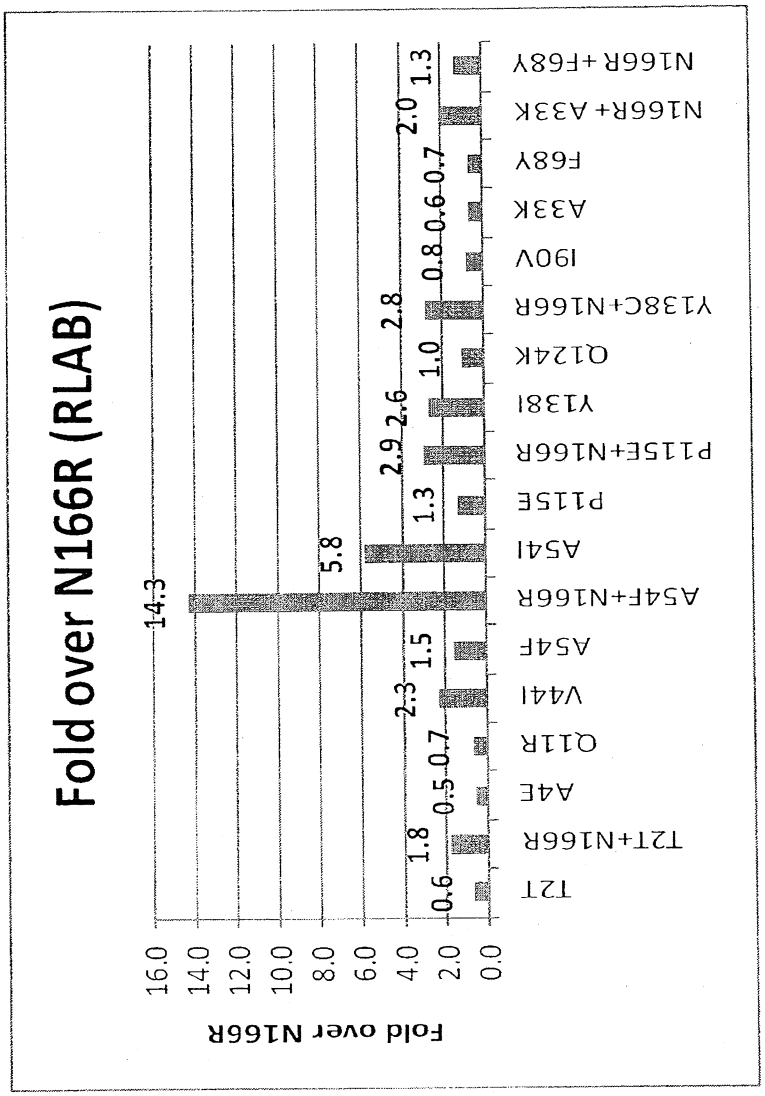


FIG. 33A

Mutation	Fold over N166R
T2T	1.6
T2T+N166R	2.3
A4E	1.2
Q11R	1.1
V44I	13.4
A54F	9.7
A54F+N166R	50.2
A54I	97.8
P115E	2.5
P115E+N166R	3.1
Y138I	7.0
Q124K	2.0
Y138C+N166R	3.7
I90V	2.4
A33K	2.6
F68Y	2.7
N166R+ A33K	6.3
N166R +F68Y	3.4

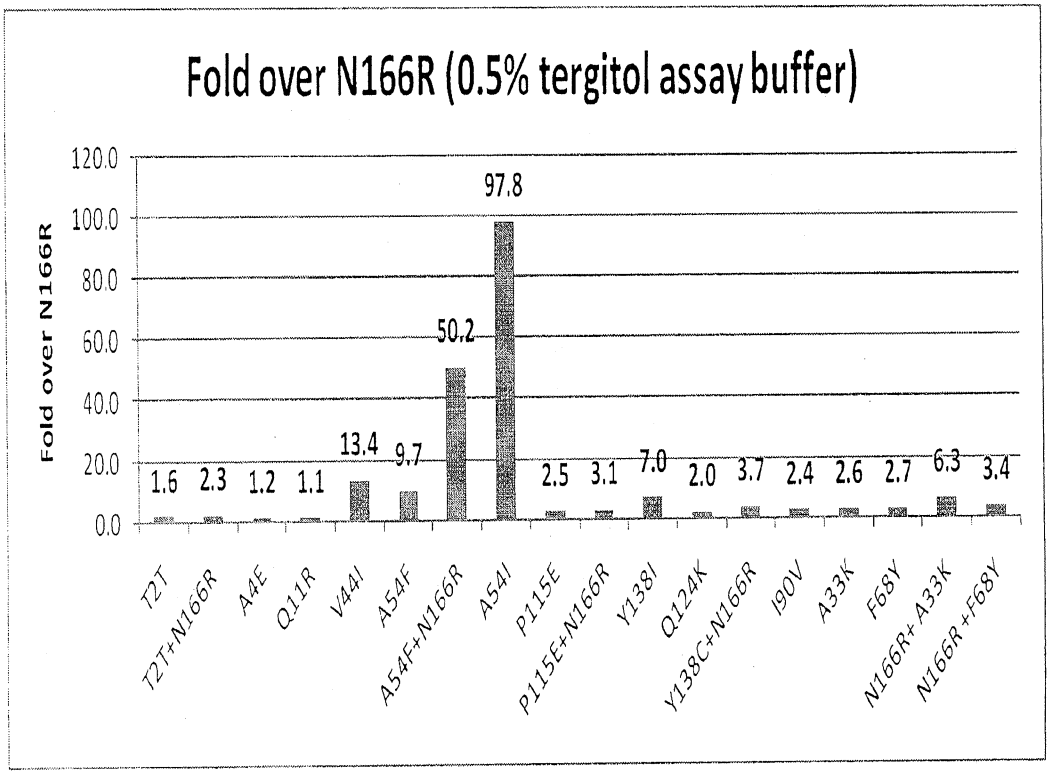


FIG. 33B

Sample	Signal 1/2 life (minutes) RLAB normalized by N166R
T2T	1.2
T2T+N166R	1.1
A4E	1.4
Q11R	1.1
V44I	1.0
A54F	0.8
A54F+N166R	0.9
A54I	1.3
P115E	1.0
P115E+N166R	1.0
Y138I	1.0
Q124K	1.1
Y138C+N166R	0.6
I90V	1.1
A33K	1.4
F68Y	1.2
N166R/A33K	1.0
N166R/F68Y	1.0

Sample	Signal stability 0.5% tergitol buffer normalized by N166R
T2T	0.9
T2T+N166R	1.3
A4E	0.9
Q11R	0.8
V44I	0.8
A54F	0.8
A54F+N166R	1.1
A54I	0.7
P115E	1.2
P115E+N166R	1.9
Y138I	0.9
Q124K	1.0
Y138C+N166R	1.5
I90V	0.9
A33K	0.8
F68Y	0.9
N166R/A33K	1.0
N166R/F68Y	1.0

FIG. 33C

Stability at 22°C normalized by N166R

Sample	stability (1/2 life at 22°C) normalized to N166R
T2T	0.7
T2T+N166R	1.1
A4E	0.6
Q11R	0.7
V44I	0.8
A54F	0.9
A54F+N166R	2.9
A54I	1.0
P115E	0.8
P115E+N166R	1.9
Y138I	0.9
Q124K	0.7
Y138C+N166R	0.6
I90V	0.7

FIG. 33D

Summary

Sample	Signal 1/2 life(mintes) 0.5% tergitol buffer normalized by N166R	Signal 1/2 life (minutes) RLAB normalized by N166R	Fold over N166R 0.5% tergitol assay buffer	Fold over N166R (RLAB)	stability (1/2 life at 22°C normalized to N166R
T2T	0.9	1.2	1.6	0.6	0.7
T2T+N166R	1.3	1.1	2.3	1.8	1.1
A4E	0.9	1.4	1.2	0.5	0.6
Q11R	0.8	1.1	1.1	0.7	0.7
V44I	0.8	1.0	13.4	2.3	0.8
A54F	0.8	0.8	9.7	1.5	0.9
A54F+N166R	1.1	0.9	50.2	14.3	2.9
A54I	0.7	1.3	97.8	5.8	1.0
P115E	1.2	1.0	2.5	1.3	0.8
P115E+N166R	1.9	1.0	3.1	2.9	1.9
Y138I	0.9	1.0	7.0	2.6	0.9
Q124K	1.0	1.1	2.0	1.0	0.7
Y138C+N166R	1.5	0.6	3.7	2.8	0.6
I90V	0.9	1.1	2.4	0.8	0.7
A33K	0.8	1.4	2.6	0.6	0.9
F68Y	0.9	1.2	2.7	0.7	0.9
N166R+ A33K	1.0	1.0	6.3	2.0	1.1
N166R +F68Y	1.0	1.0	3.4	1.3	1.2

FIG. 33E

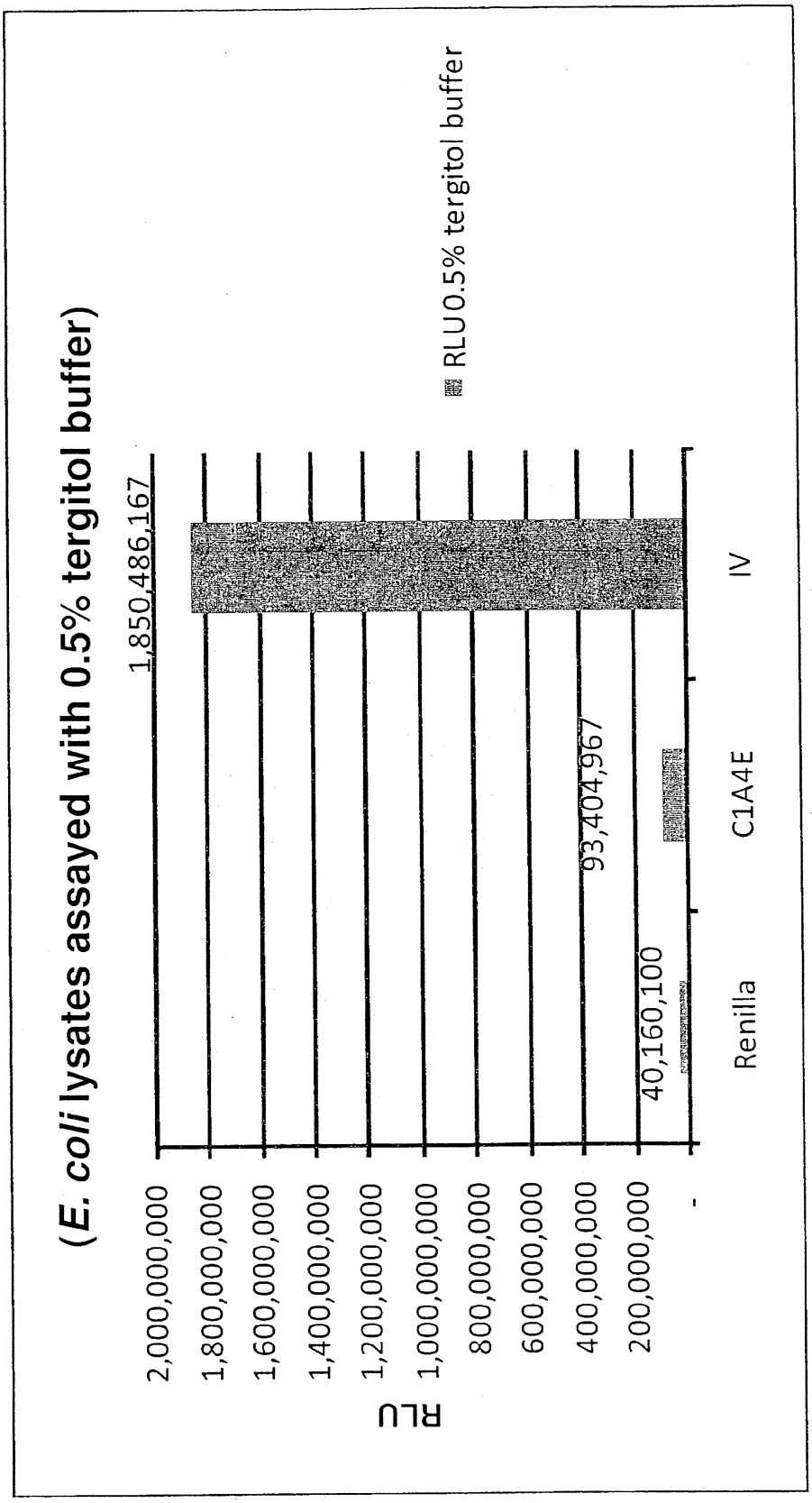
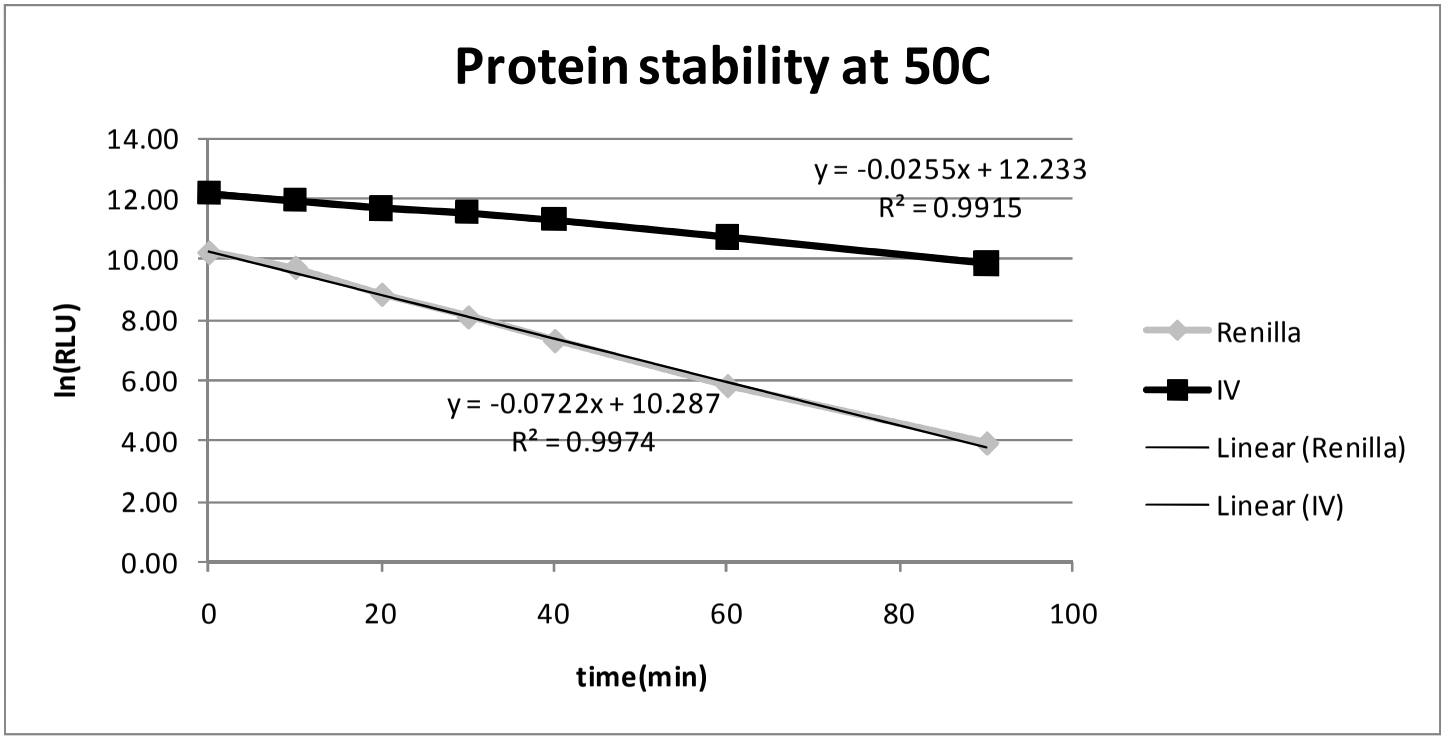

(E. coli lysates assayed with 0.5% tergitol buffer)
RLU
2,000,000,000
1,800,000,000
1,600,000,000
1,400,000,000
1,200,000,000
1,000,000,000
800,000,000
600,000,000
400,000,000
200,000,000
-
1,850,486,167
93,404,967
40,160,100
RLU0.5% tergitol buffer
Renilla
C1A4E
IV

FIG. 34A



Sample	1/2 life (min)
Renilla	9.6
IV	27.2

FIG. 34B