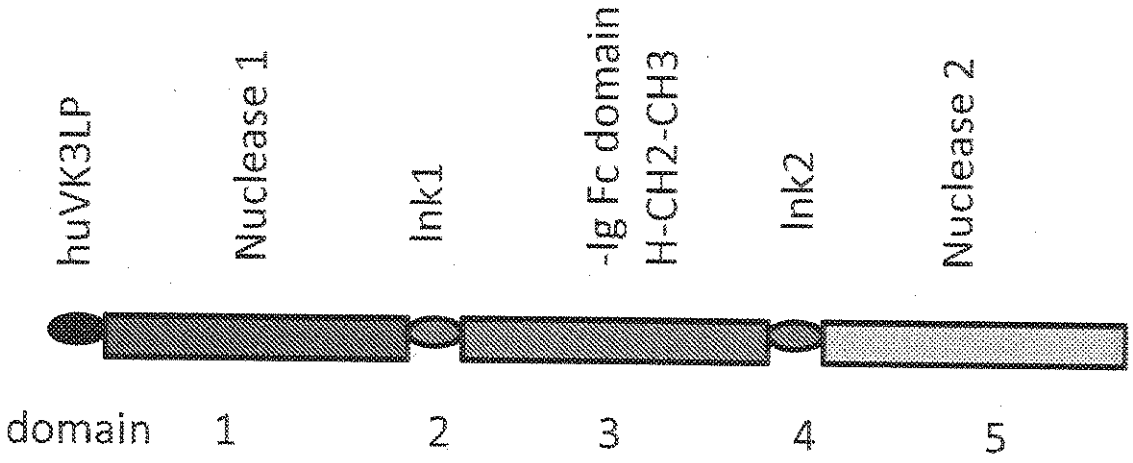
Schematic Showing General Approach to Engineering Hybrid Nuclease Fc Fusion Proteins



Nuclease 1 or 2	lnk 1 or 2	IgGFc
hRNase-WT	(gly4ser)3	SCCH-hIgG1WT
hRNase-G88D	(gly4ser)4	SCCH-hIgG1WT
hDNase1 WT	(gly4ser)5	hIgG1-P238S
hDNAse1-A114F	NLG	hIgG1-N297S
hDNase1-G105R;A114F	none	hIgG1-P331S
hDNase1-G105R	(gly4ser)n	mIgG2a-WT
hTREX1-72AA		mIgG2c-WT
hDNase1L3		mIgG2A-C WT or MT
hTREX1-lnk-hTREX1		

Figure 1

RSLV-124 Shows Bi-Phasic PK response in mice

In Vivo half-life of RSLV-124 in mice (single 150 ug i.v. injection)

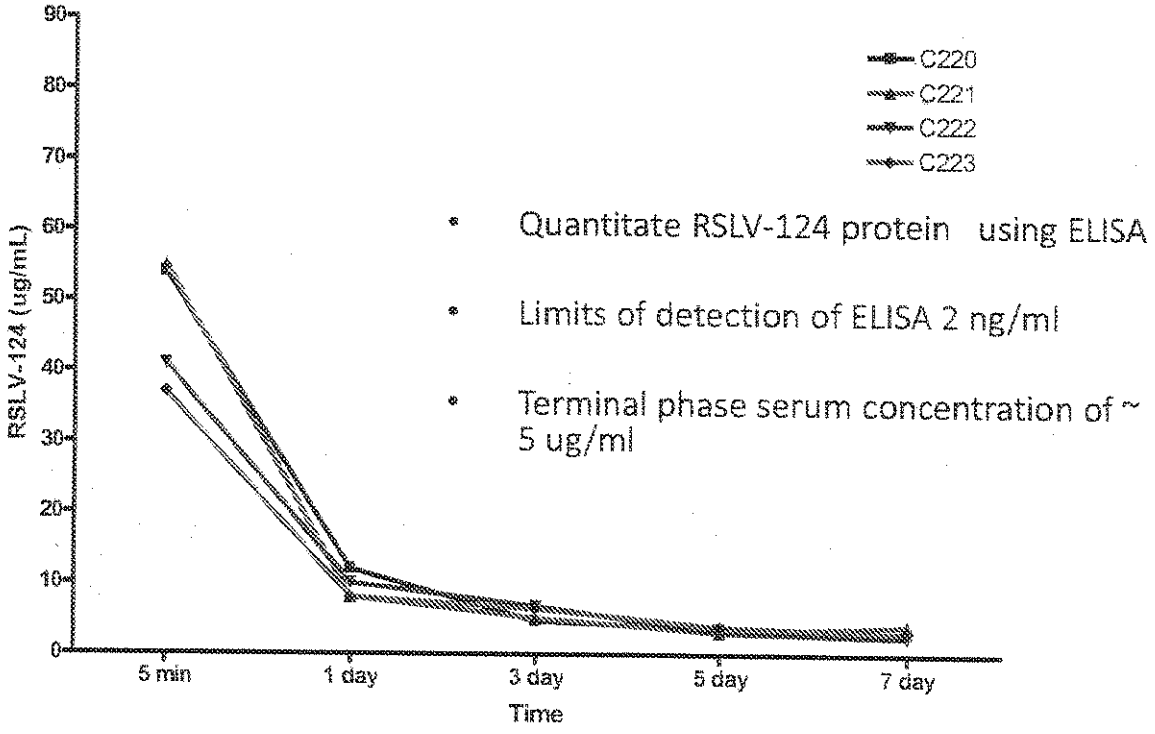


Figure 2

RSLV-124 Retains Full Enzyme Activity In Vivo

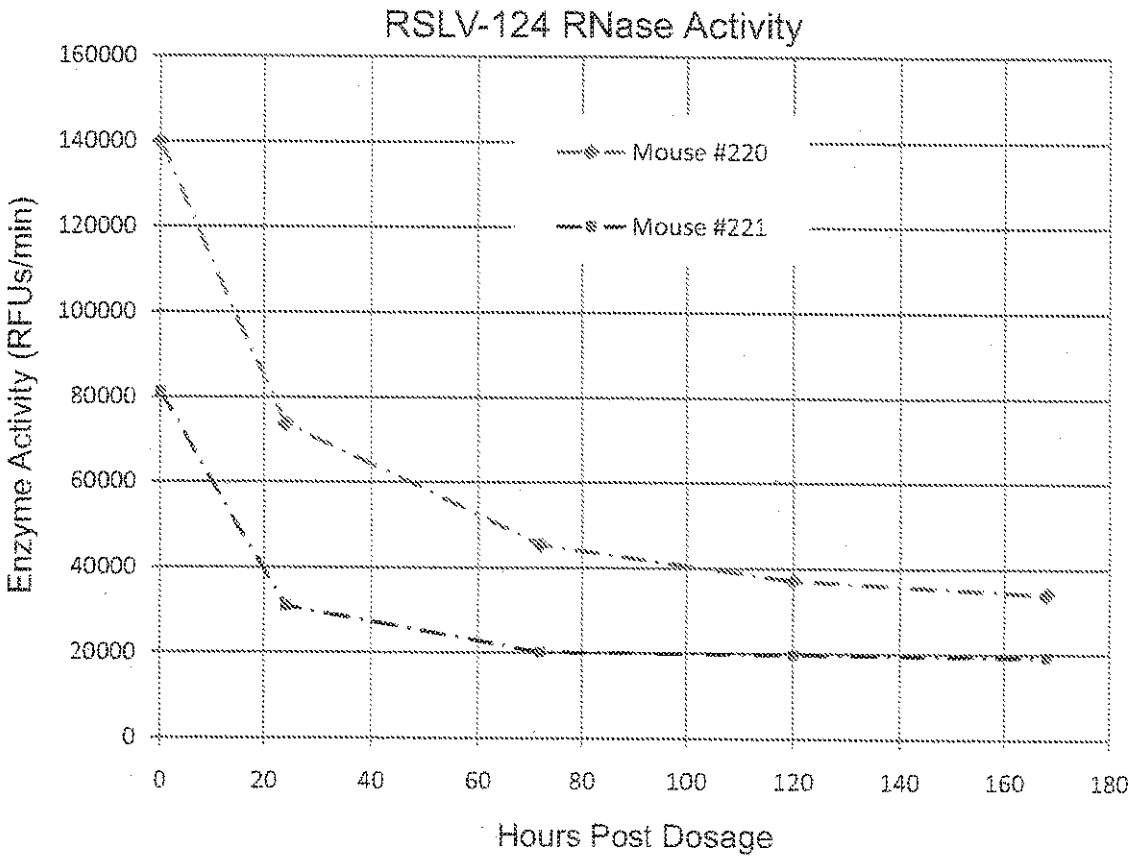
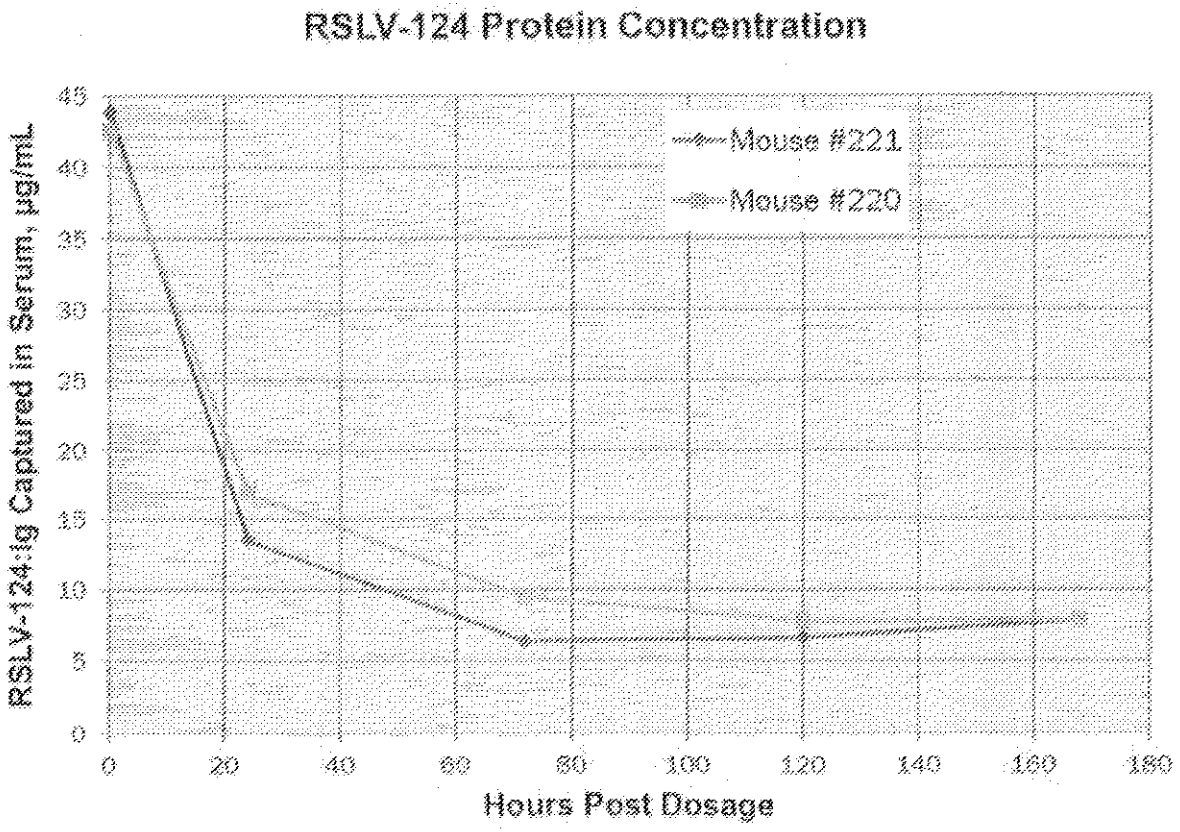


Figure 3



- Following administration to mice, RSLV-124 in serum was captured via plate-bound anti-human Fc.
- The associated RNase activity was assessed.
- Based on comparison to RNase, the quantity of RSLV-124 was estimated (ug/ml).

Derived protein concentrations agreed well with those determined by a direct ELISA. As such, RSLV-124 appears to maintain full activity in vivo.

Figure 4

SRED Analysis of RNase Enzymatic Activity
In the RNase Tg. Mice

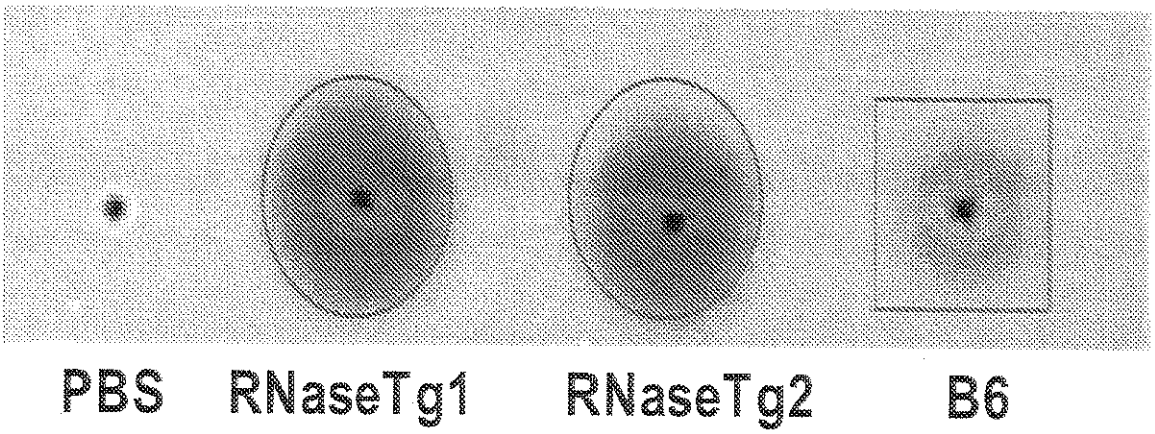


Figure 5

ELISA Quantitation of Serum RNase Levels
In the RNase Tg. Mice

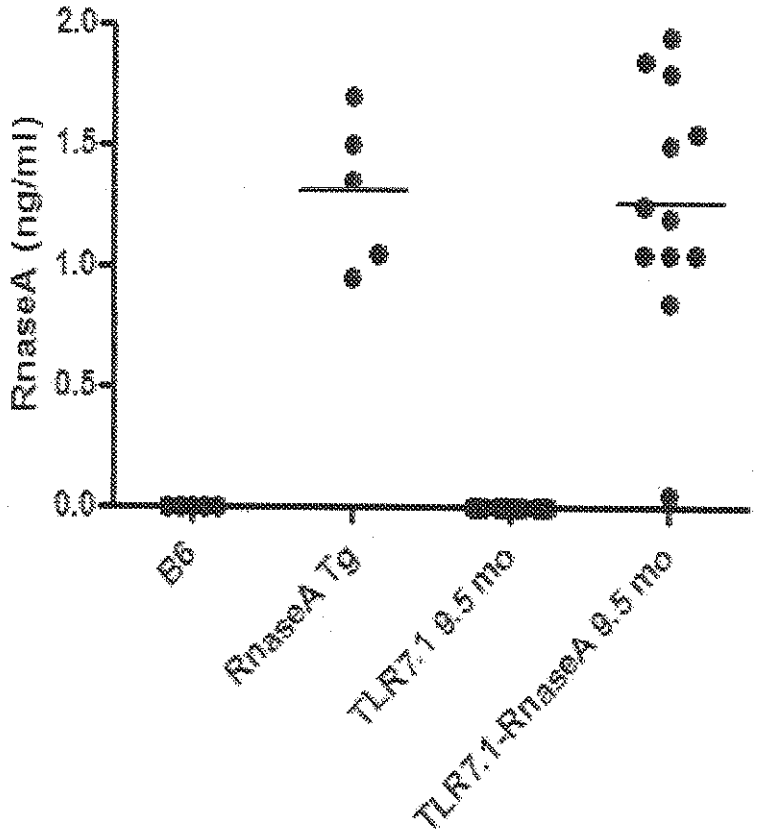


Figure 6

RNase Expression Reduces Mortality in the TLR7 Mice

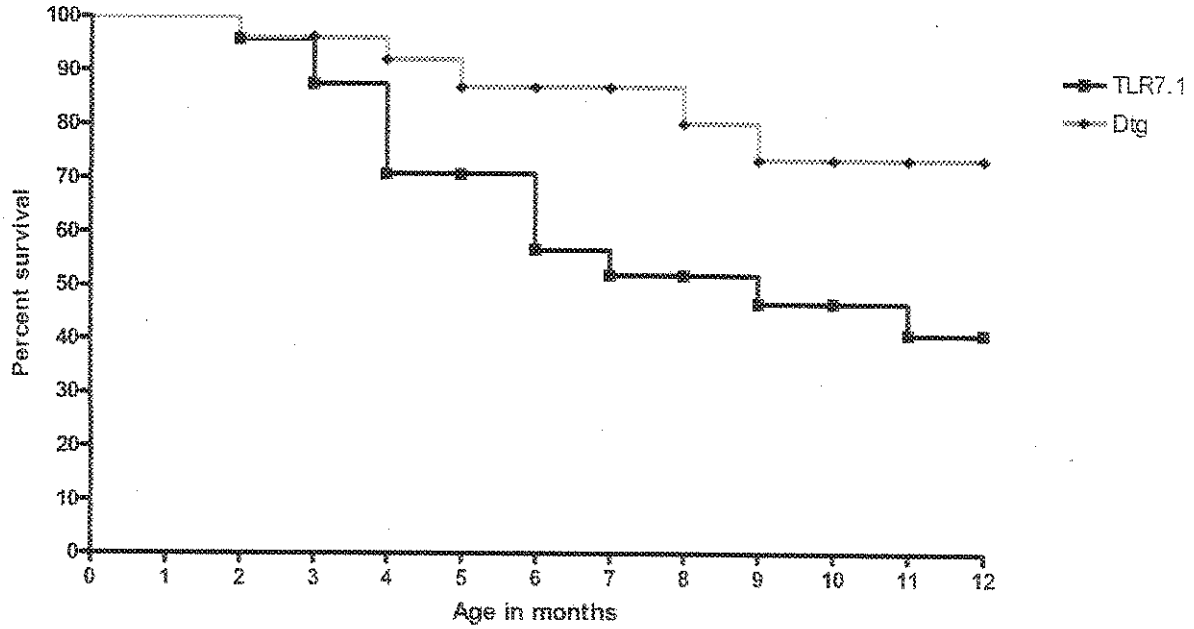


Figure 7

Reduction in Interferon Stimulated Genes (ISG's)
In the Double Transgenic Mice

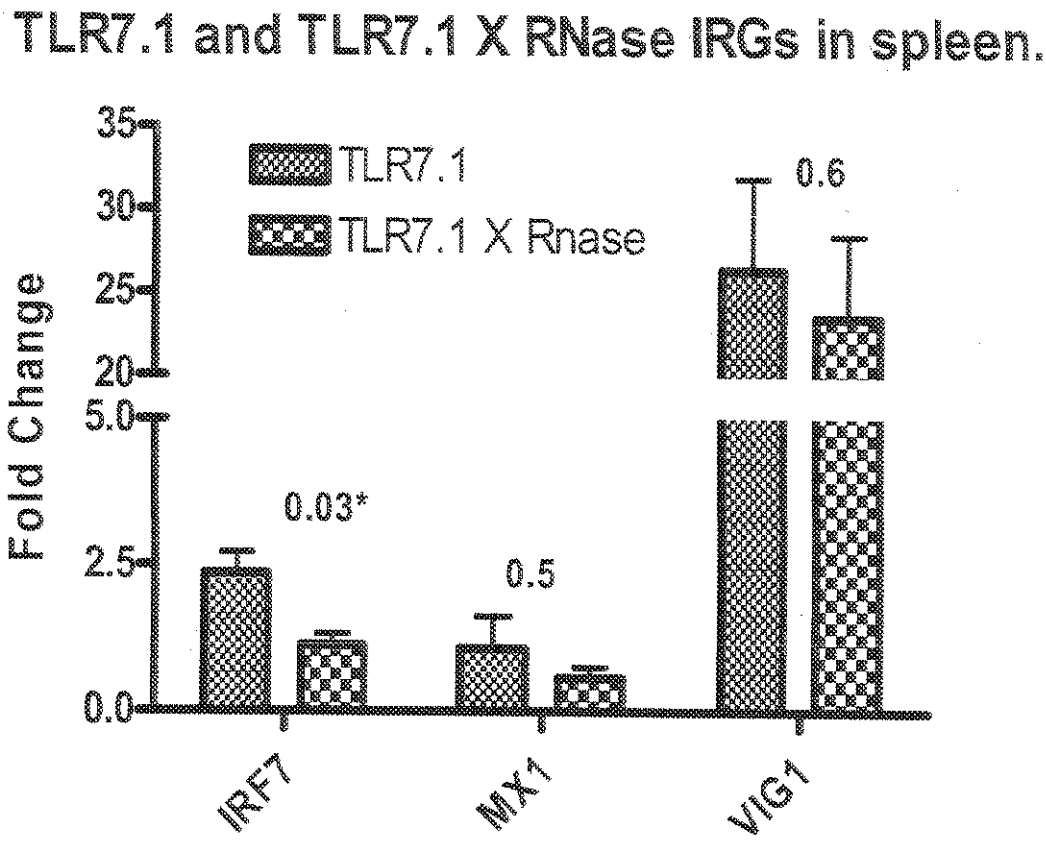


Figure 8

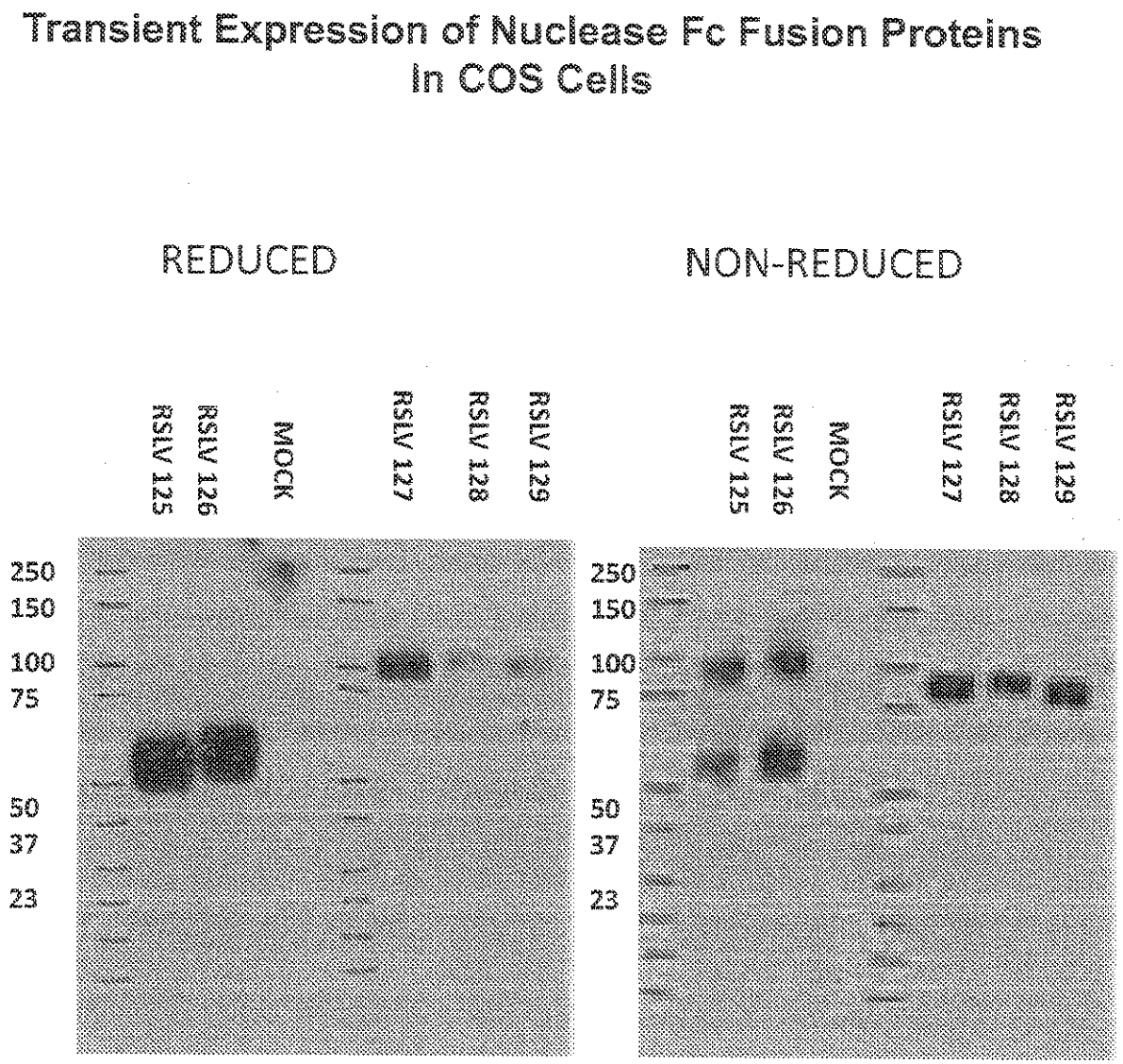

Transient Expression of Nuclease Fc Fusion Proteins In COS Cells
REDUCED
NON-REDUCED
RSLV 125
RSLV 126
MOCK
RSLV 127
RSLV 128
RSLV 129
250
150
100
75
50
37
23

Figure 9

RNase Enzymatic Activity of Protein-A Purified Nuclease Fc Fusion Proteins Using SRED Assay

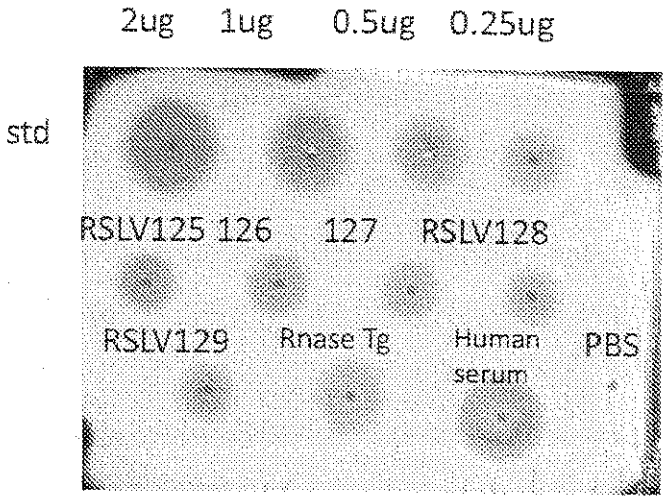


Figure 10

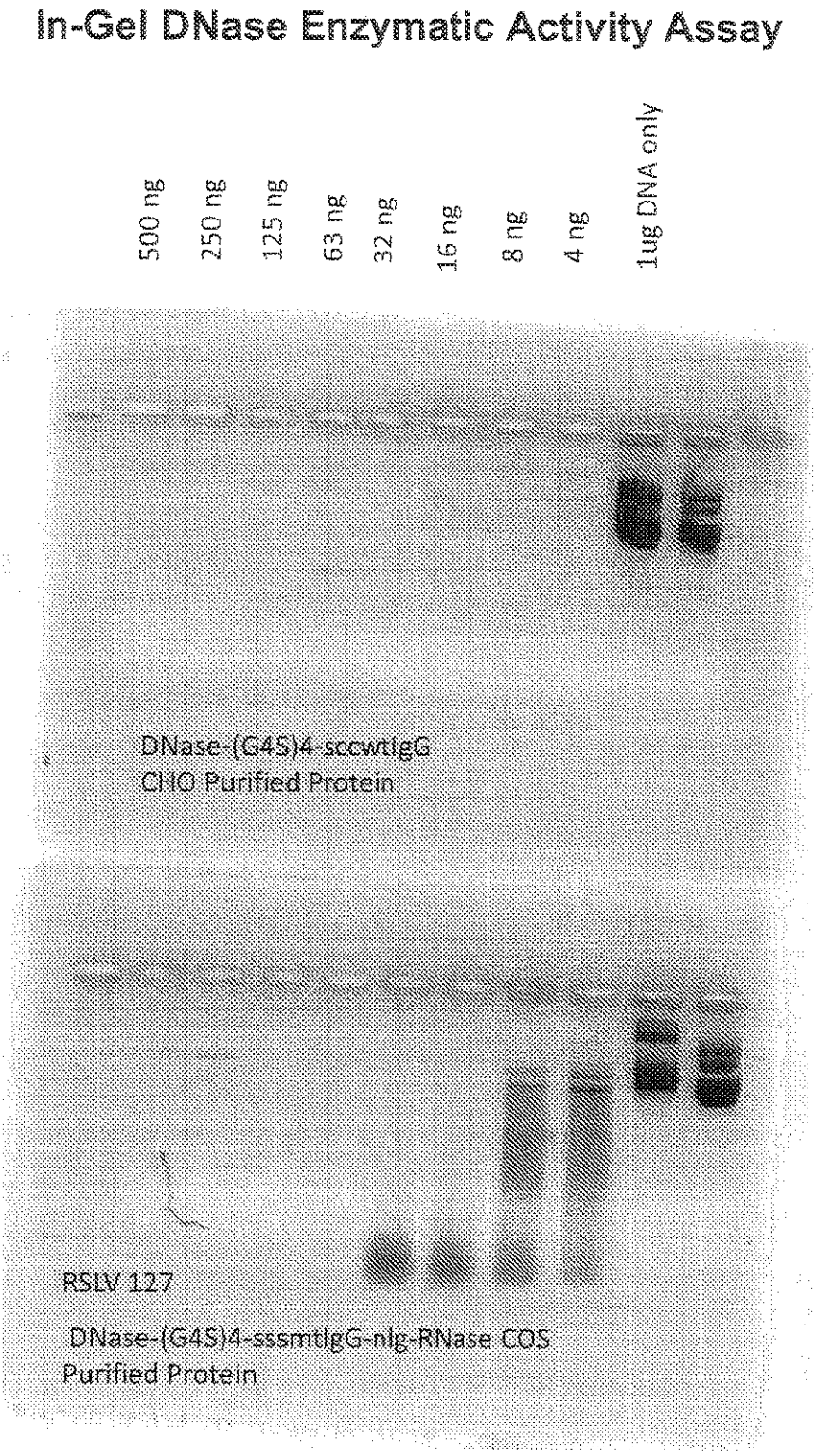

In-Gel DNase Enzymatic Activity Assay
500 ng
250 ng
125 ng
63 ng
32 ng
16 ng
8 ng
4 ng
1ug DNA only
DNase-(G4S)4-sccwtIgG
CHO Purified Protein
RSLV 127
DNase-(G4S)4-sssmtIgG-nIg-RNase COS
Purified Protein

Figure 11a

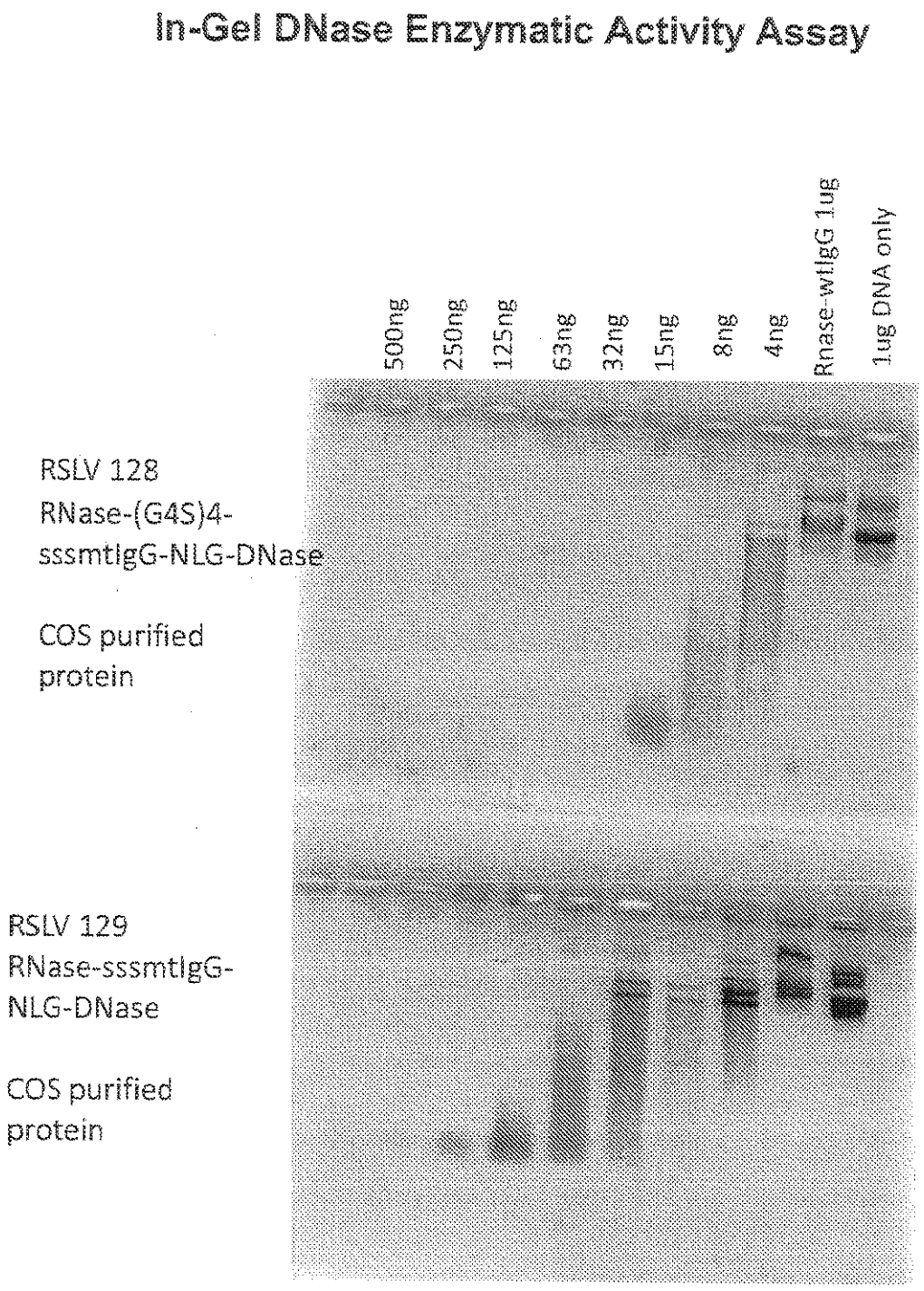

In-Gel DNase Enzymatic Activity Assay
500ng
250ng
125ng
63ng
32ng
15ng
8ng
4ng
Rnase-wtIgG 1ug
1ug DNA only
RSLV 128
RNase-(G4S)4-
sssmtIgG-NLG-DNase
COS purified
protein
RSLV 129
RNase-sssmtIgG-
NLG-DNase
COS purified
protein

Figure 11b

In-Gel DNase Enzymatic Activity Assay

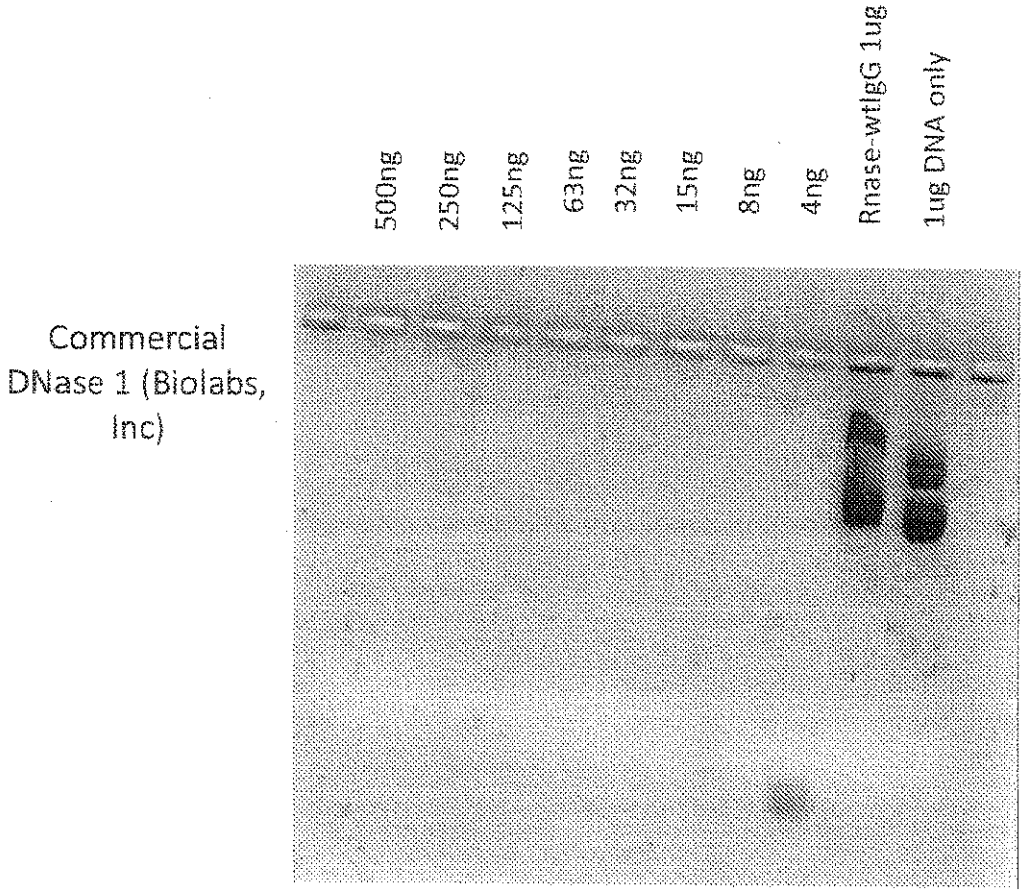


Figure 11c

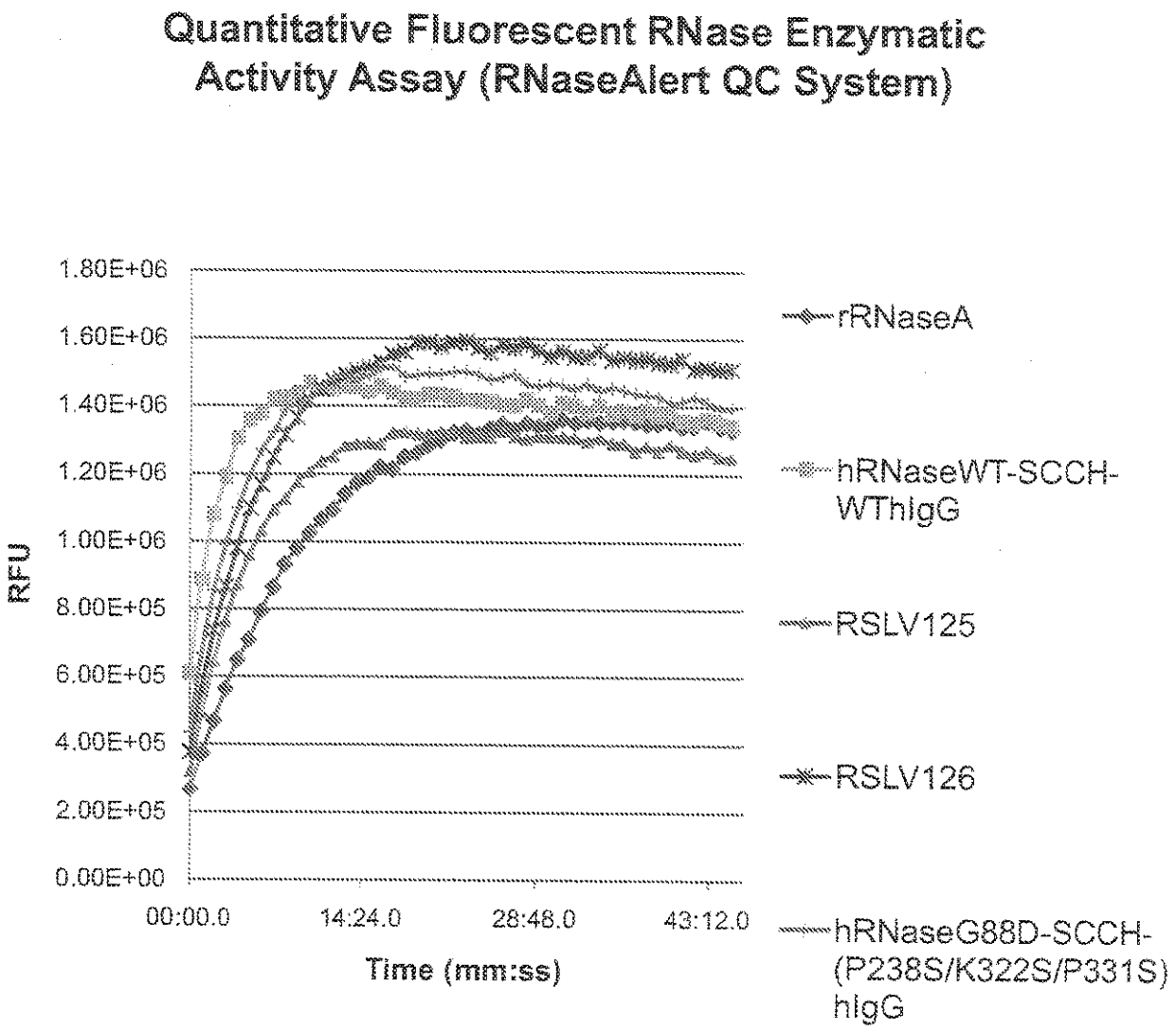

Quantitative Fluorescent RNase Enzymatic Activity Assay (RNaseAlert QC System)
1.80E+06
1.60E+06
1.40E+06
1.20E+06
1.00E+06
8.00E+05
6.00E+05
4.00E+05
2.00E+05
0.00E+00
RFU
00:00.0
14:24.0
28:48.0
43:12.0
Time (mm:ss)
rRNaseA
hRNaseWT-SCCH-WThIgG
RSLV125
RSLV126
hRNaseG88D-SCCH-(P238S/K322S/P331S) hIgG

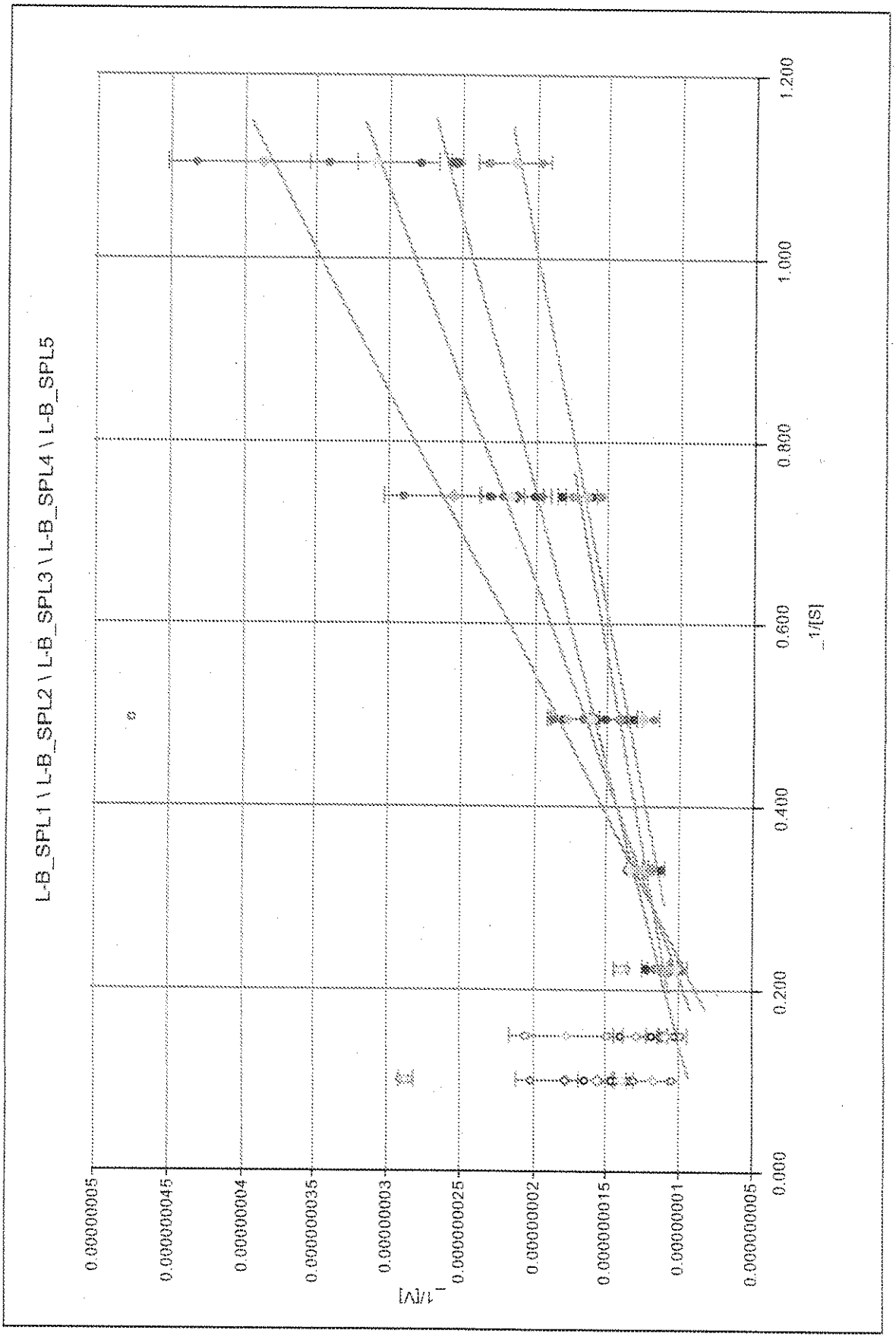
Figure 12

Enzyme Kinetic Analysis of RNase Activity of RNase Fc Fusion Proteins

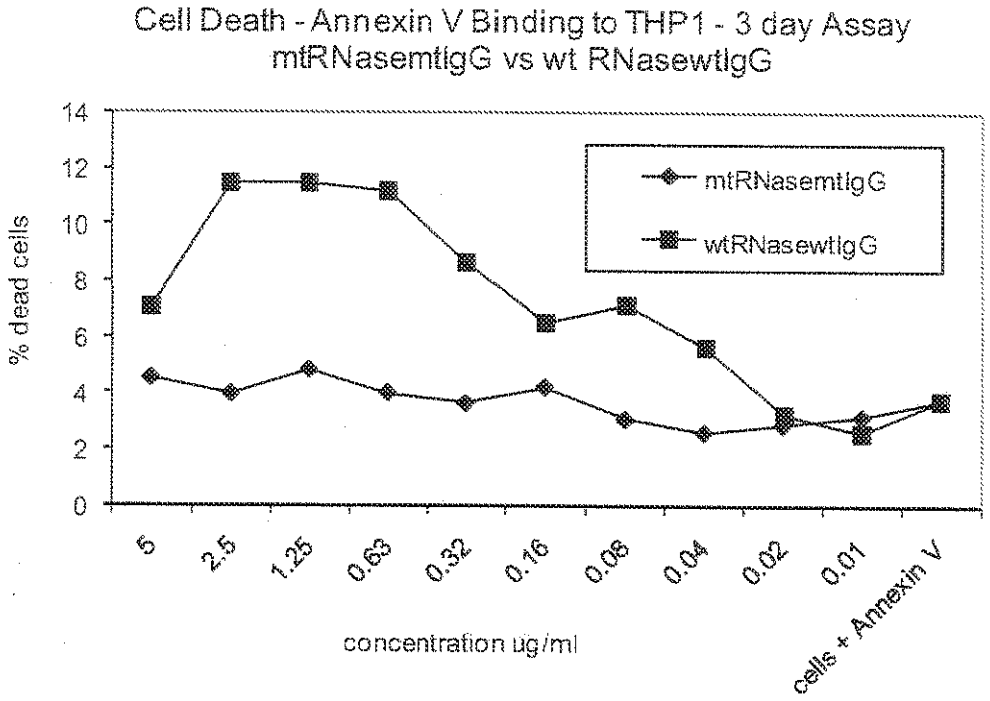
L-B Fitting Results

Curve Name	Well ID	Curve Formula	A	B	R2	Vmax	Km
L-B_SPL1	rRNaseA	Y=A*X+B	1.17E-08	8.41E-09	0.988	1.2E+08	1.3912
L-B_SPL2	hRNaseWT-SCCH-WTIgG	Y=A*X+B	1.26E-08	7.34E-09	0.966	1.4E+08	1.71662
L-B_SPL3	RSLV125	Y=A*X+B	3.22E-08	2.45E-09	0.994	4.1E+08	13.1429
L-B_SPL4	RSLV126	Y=A*X+B	2.31E-08	5.08E-09	0.996	2E+08	4.54724
L-B_SPL5	hRNaseG88D-SCCH-TMIgG	Y=A*X+B	1.66E-08	7.66E-09	0.975	1.3E+08	2.1671

Figure 13


L-B_SPL1 \ L-B_SPL2 \ L-B_SPL3 \ L-B_SPL4 \ L-B_SPL5
1/[V]
1/[S]
0.000000005
0.00000000
0.000000045
0.00000004
0.000000035
0.00000003
0.000000025
0.00000002
0.000000015
0.00000001
0.000000005
0.000
0.200
0.400
0.600
0.800
1.000
1.200

Analysis of Cytotoxicity in THP1 Cells Treated with RNase Fc Fusion Proteins with Wild Type and Mutant Fc Domains



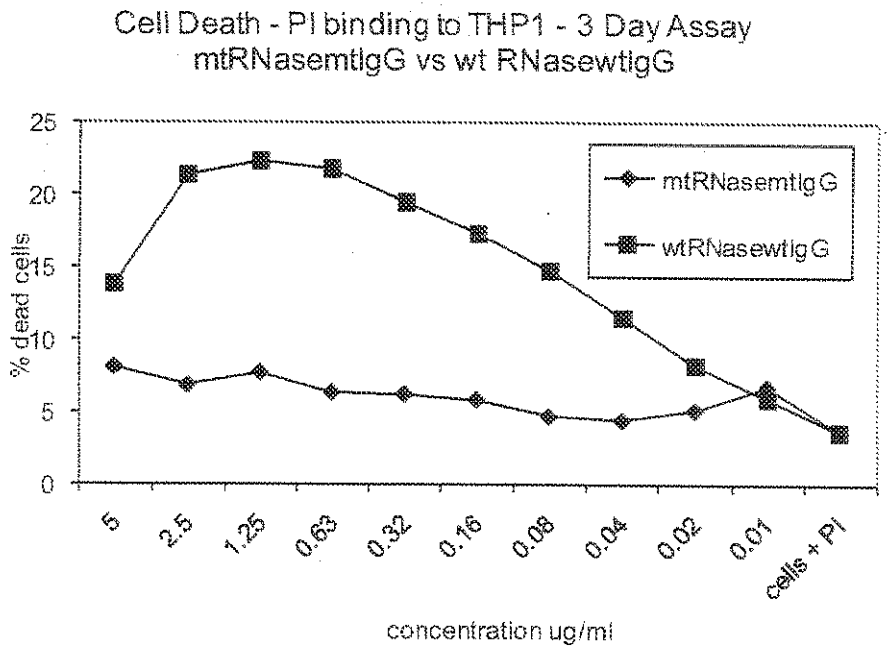


Figure 14

FACS Profile of Cells Treated with RNase Fc Fusion Proteins with WT and Mutant Fc Domains

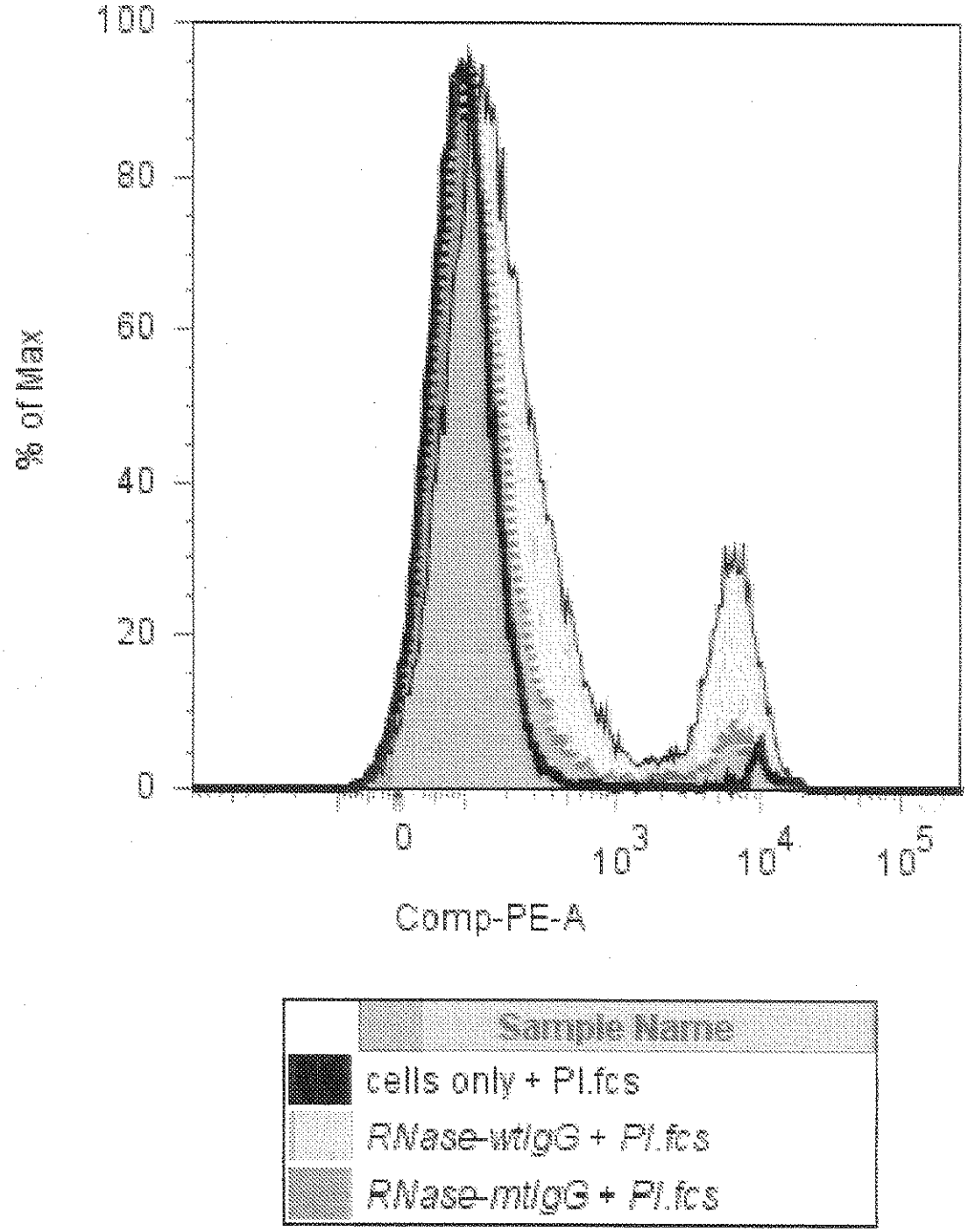


Figure 15

RSLV-132 Potently Inhibits IFN Production Induced by SLE Patient IC's

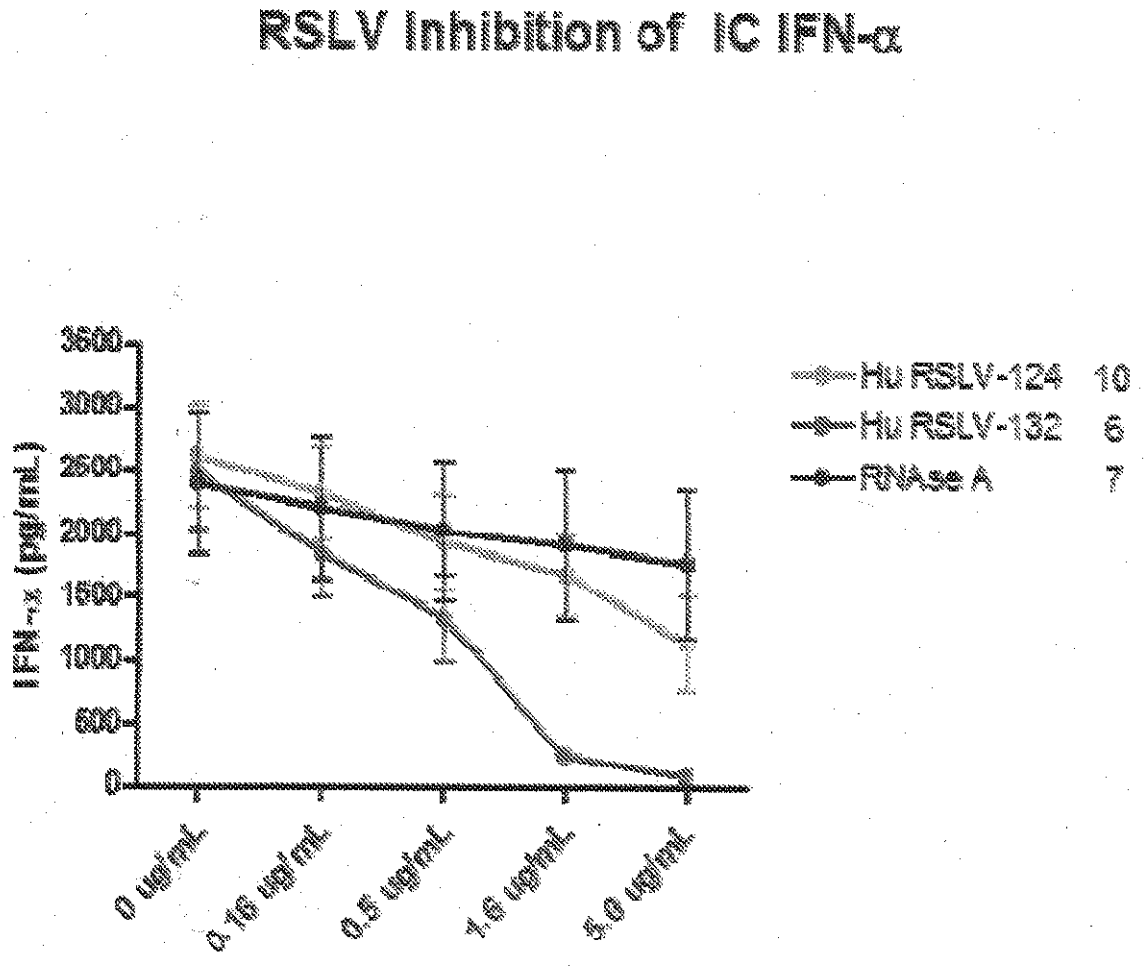


Figure 16

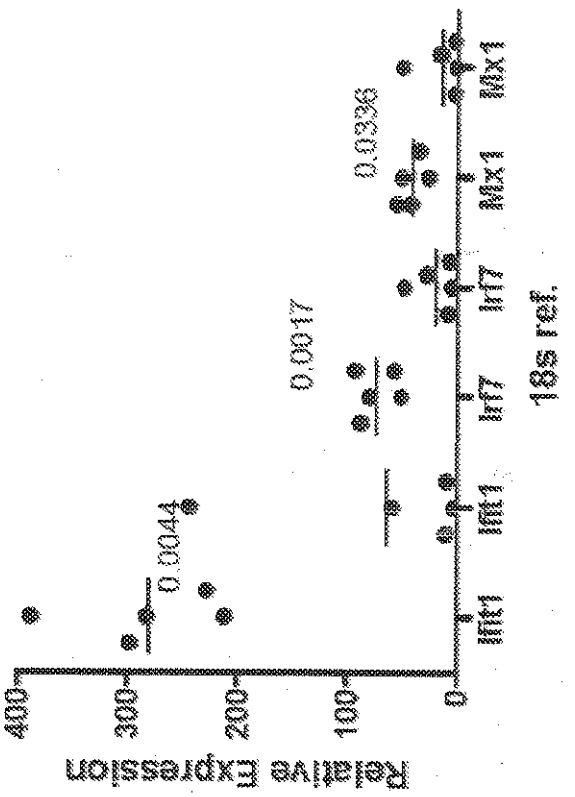

RSLV-132 Effectively Inhibits RNA-Induced IFN Production In Vivo
Relative Expression
400
300
200
100
0
0.0044
0.0017
0.0336
Ifit1
Ifit3
Irf7
Mx1
18s ref.
18s ref. White: IVIG, Black: RSLV 132

Figure 17

RSLV-132 is a Highly Active RNase

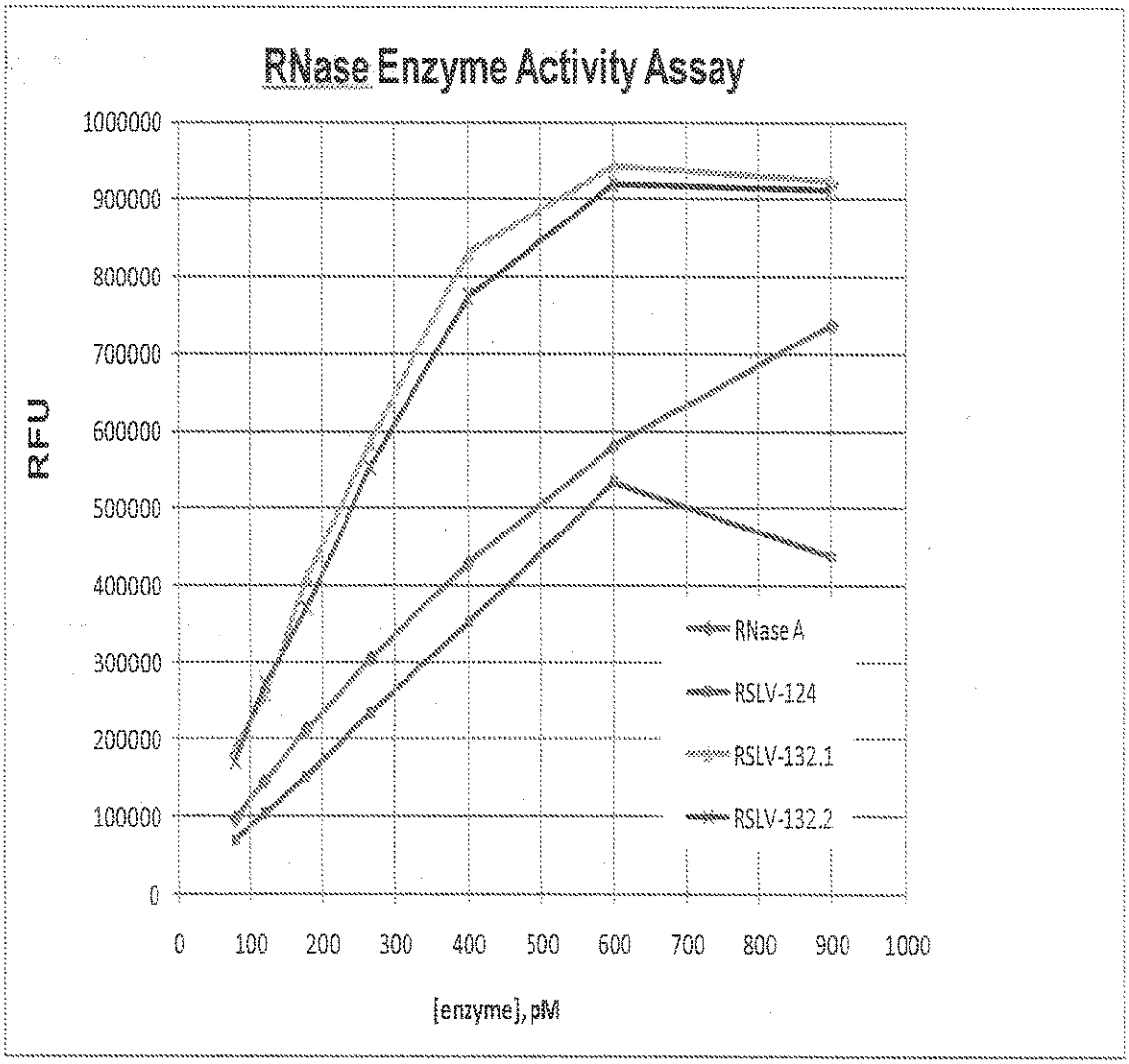
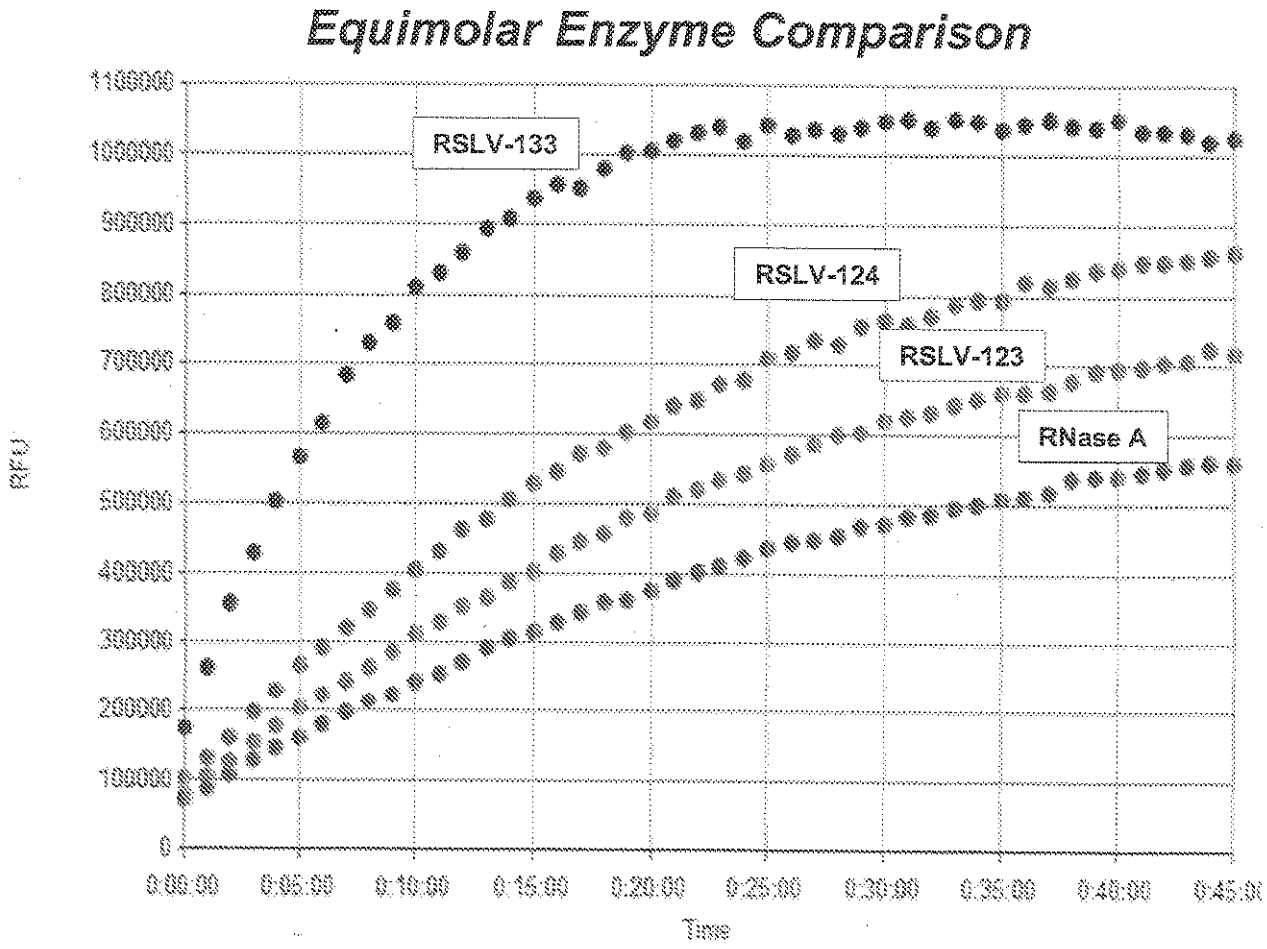


Figure 18

RSLV-133 Demonstrates A Highly Active RNase Moiety



Fluorometric assay: Ex_{485nm}, Em_{528nm}

Figure 19

RSLV-133 Demonstrates A Highly Active DNase Moiety

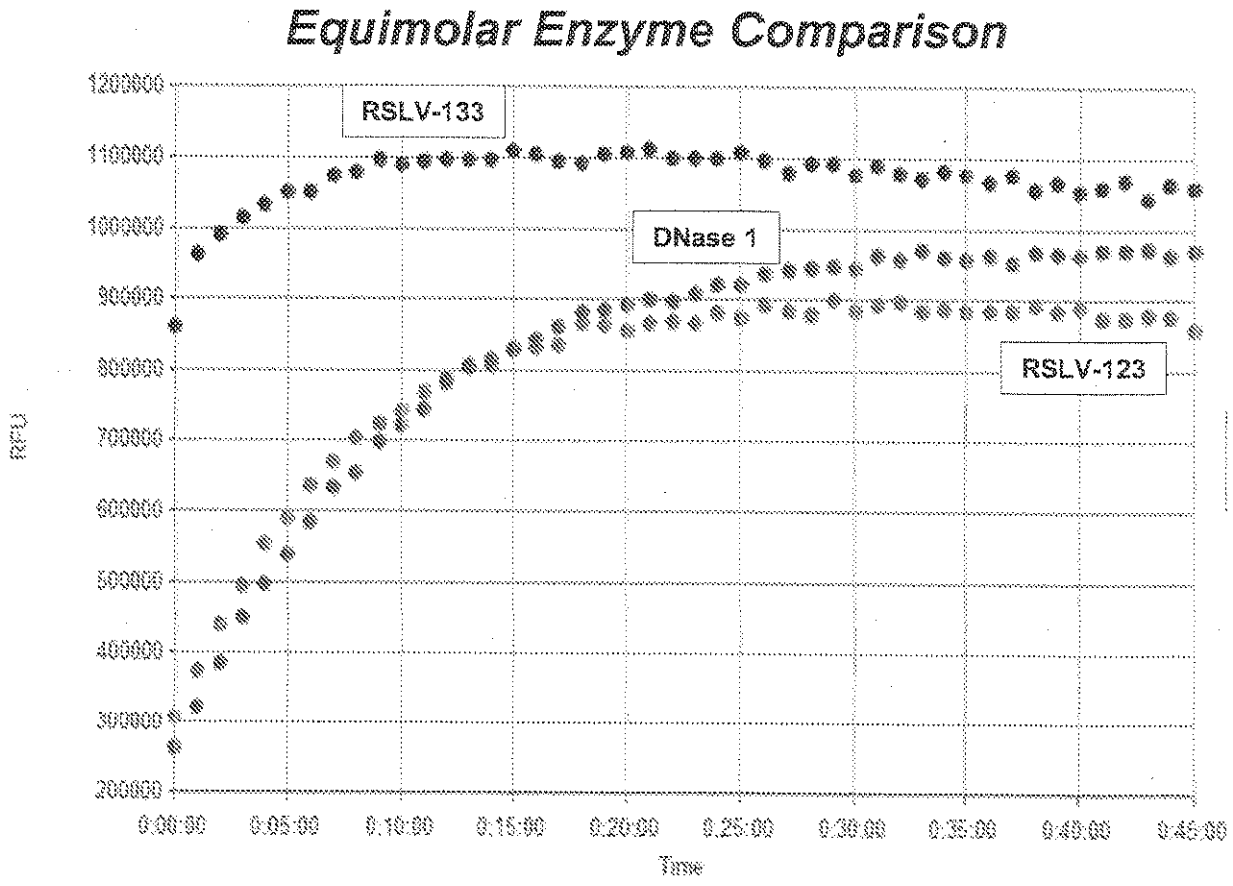


Figure 20

RSLV-133 Is a Highly Contains a Highly Active DNase Moelty

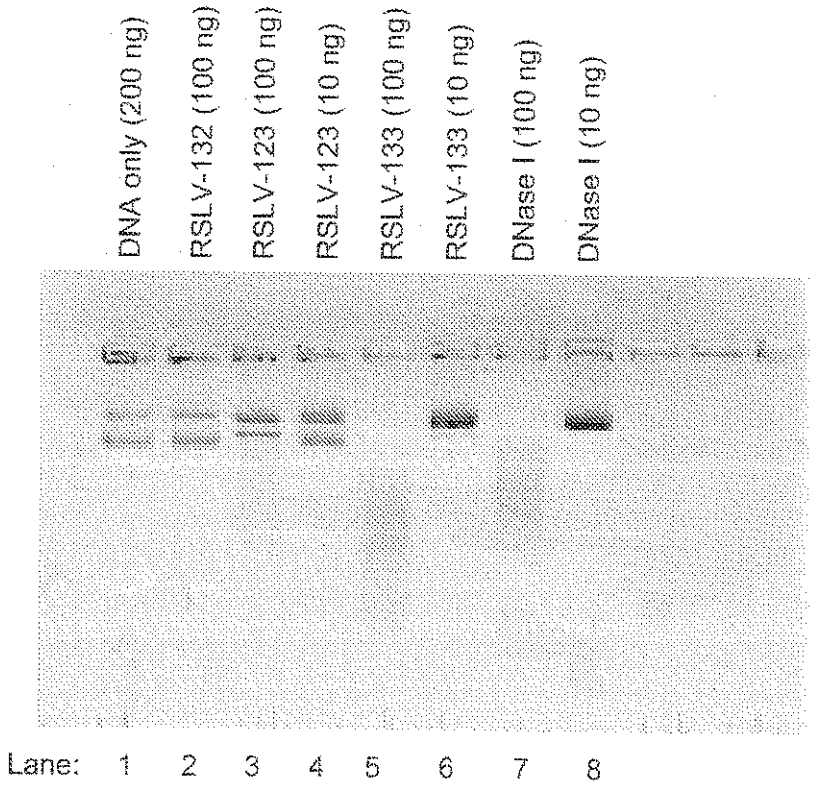


Figure 21

RSLV-132 Demonstrates Decreased Fc Receptor Binding

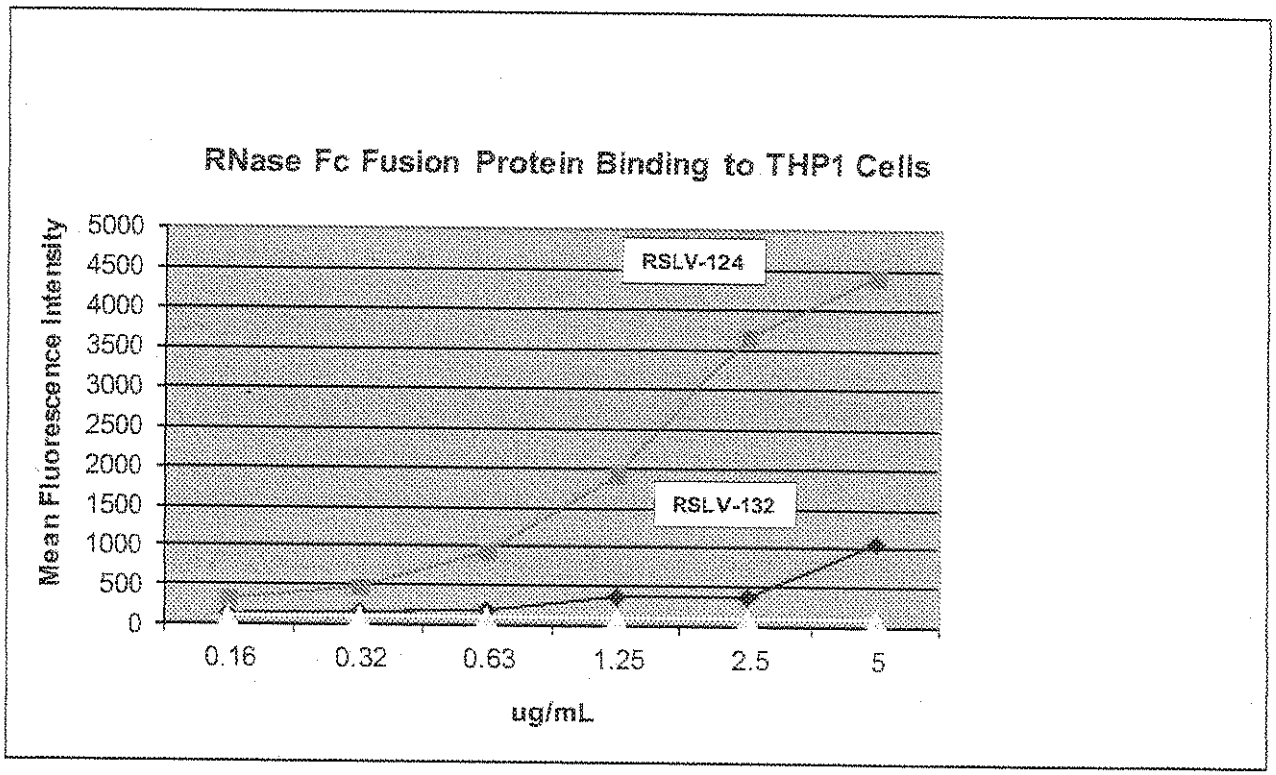


Figure 22