Figure 1

IFNbeta (pg/mL)
2500
2000
1500
1000
500
0
nLUC G0
nLUC G2
nLUC G5
hEPO G0
hEPO G2
hEPO G5
Growth Factor 1
Growth Factor 2
L2K
Surrogate RNA 1 EQ feed
Surrogate RNA 1 alpha feed
Surrogate RNA 1 EQ UF
Surrogate RNA 1 alpha UF
Surrogate RNA 1 EQ dT
Surrogate RNA 1 alpha dT

Figure 2

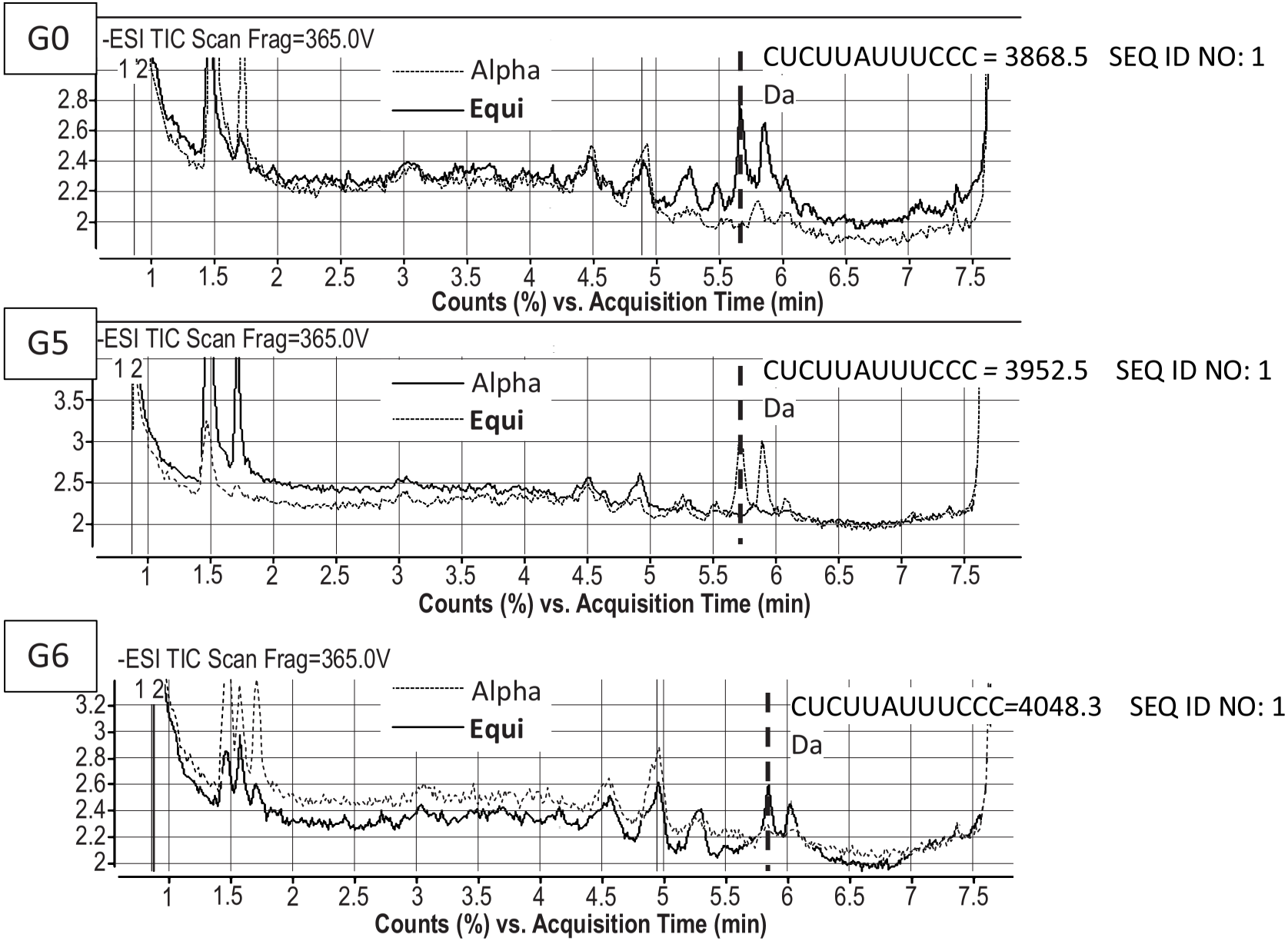

G0
-ESI TIC Scan Frag=365.0V
Alpha
Equi
CUCUUAUUUCCC = 3868.5 SEQ ID NO: 1
Da
Counts (%) vs. Acquisition Time (min)
G5
-ESI TIC Scan Frag=365.0V
Alpha
Equi
CUCUUAUUUCCC = 3952.5 SEQ ID NO: 1
Da
Counts (%) vs. Acquisition Time (min)
G6
-ESI TIC Scan Frag=365.0V
Alpha
Equi
CUCUUAUUUCCC=4048.3 SEQ ID NO: 1
Da
Counts (%) vs. Acquisition Time (min)

Figure 2 (continued)

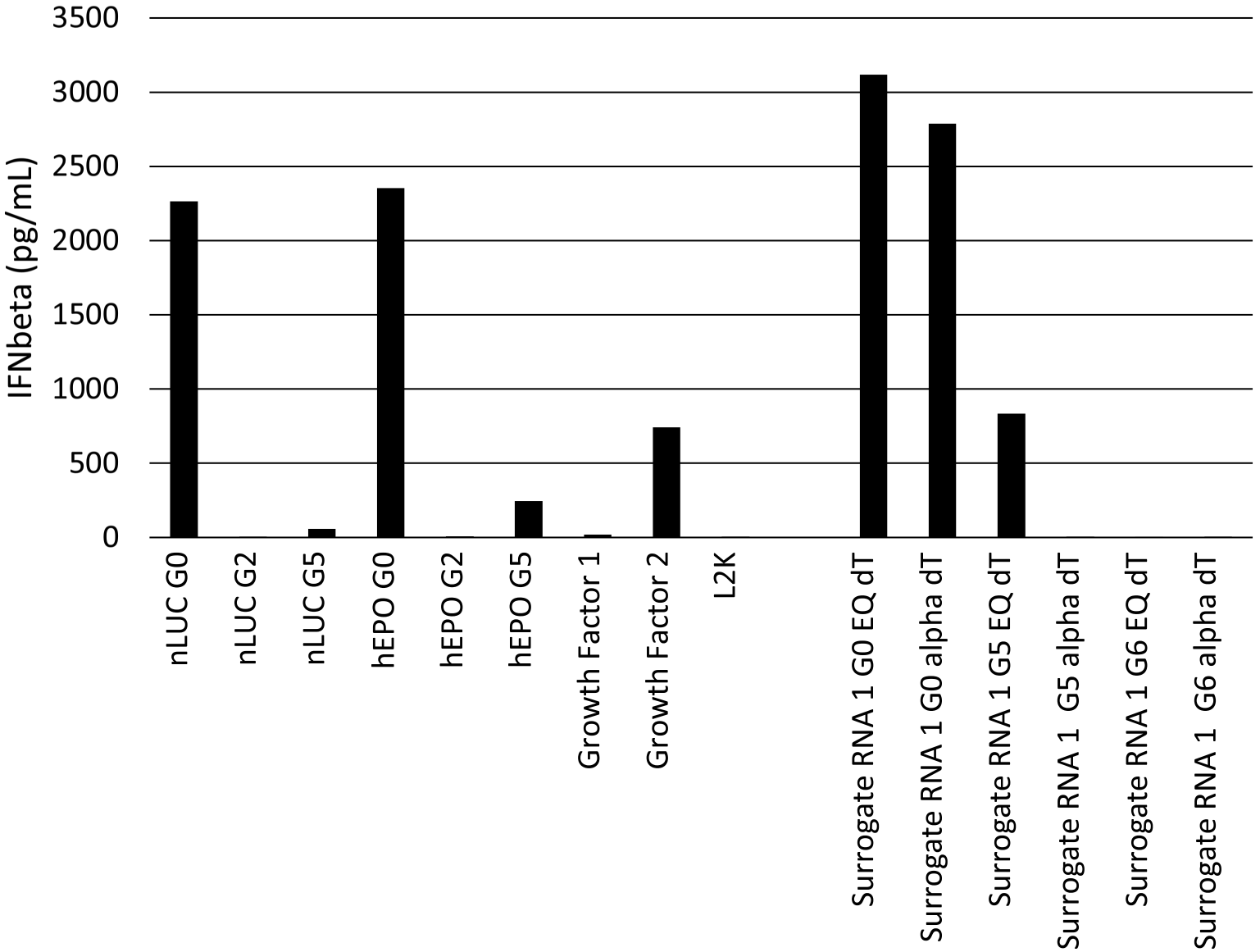


Figure 3

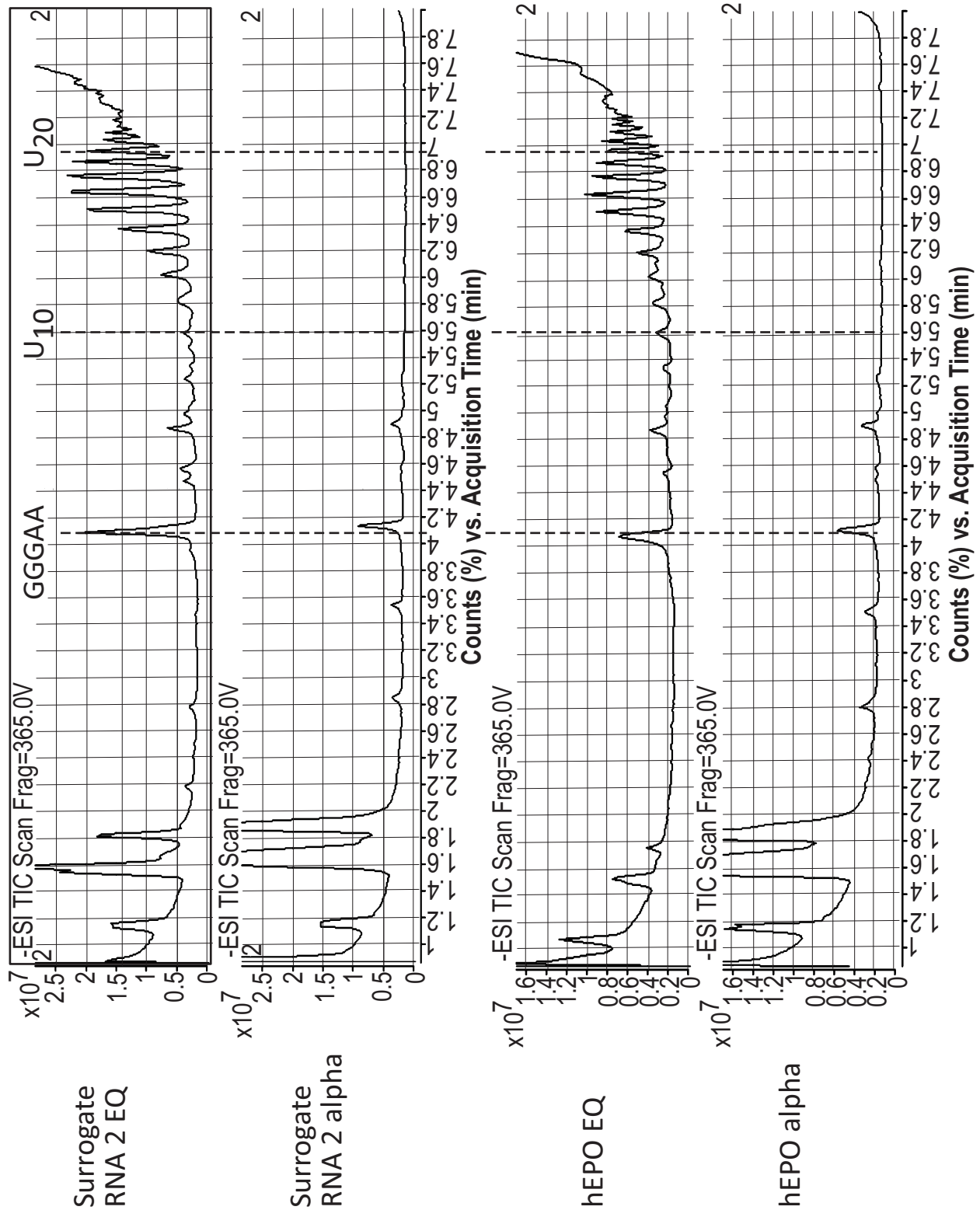

Surrogate RNA 2 EQ
Surrogate RNA 2 alpha
hEPO EQ
hEPO alpha
-ESI TIC Scan Frag=365.0V
GGGAA
U10
U20
Counts (%) vs. Acquisition Time (min)

Figure 4

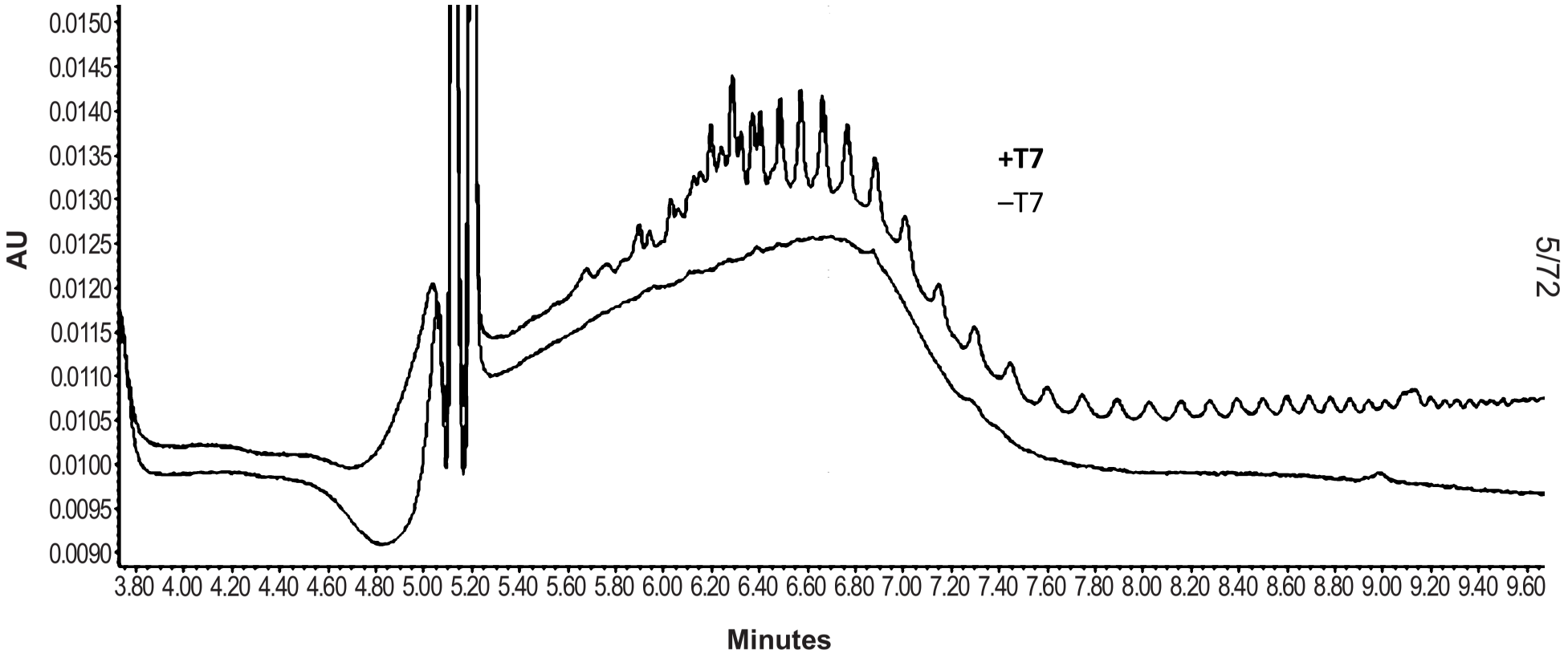

0.0150
0.0145
0.0140
0.0135
0.0130
0.0125
0.0120
0.0115
0.0110
0.0105
0.0100
0.0095
0.0090
AU
+T7
–T7
5/72
3.80 4.00 4.20 4.40 4.60 4.80 5.00 5.20 5.40 5.60 5.80 6.00 6.20 6.40 6.60 6.80 7.00 7.20 7.40 7.60 7.80 8.00 8.20 8.40 8.60 8.80 9.00 9.20 9.40 9.60
Minutes

Figure 4 (continued)

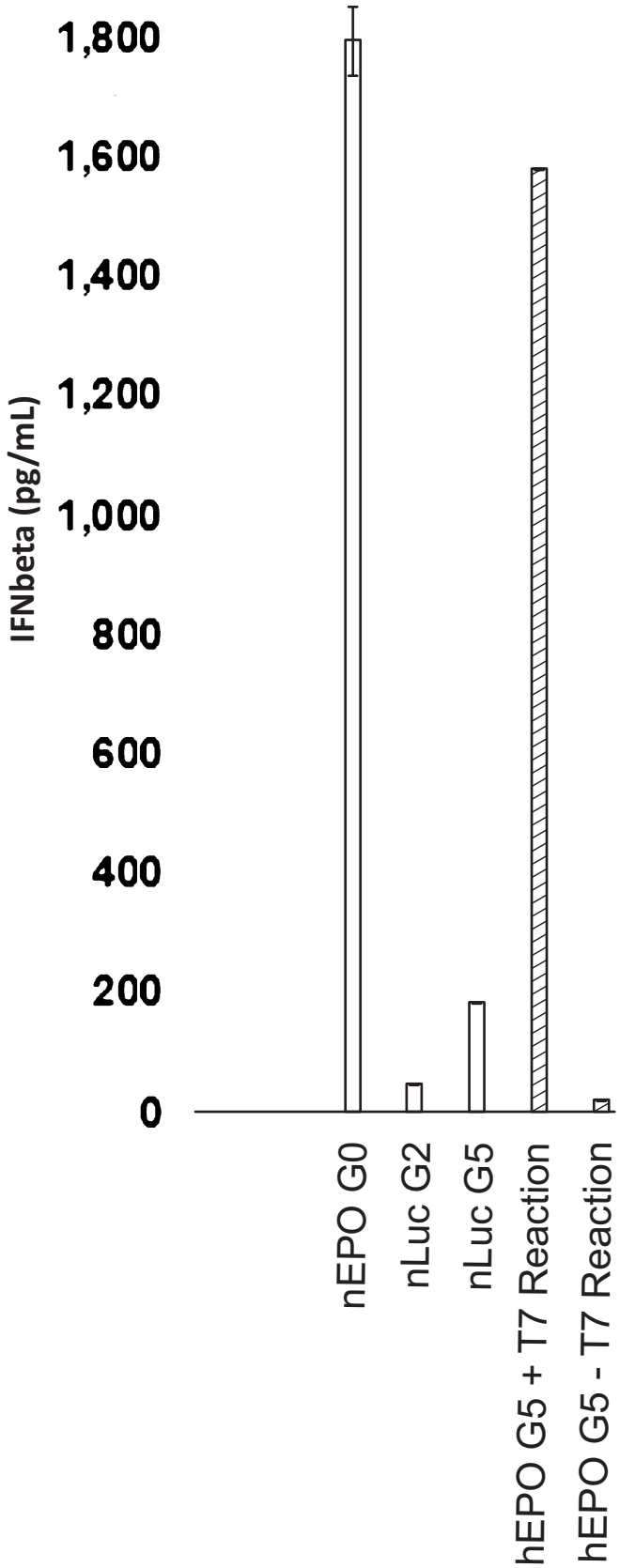


Figure 5A

Appearance of lower MW species in ------trace (+RNase III) correlates with amout of dsRNA impurity in sample

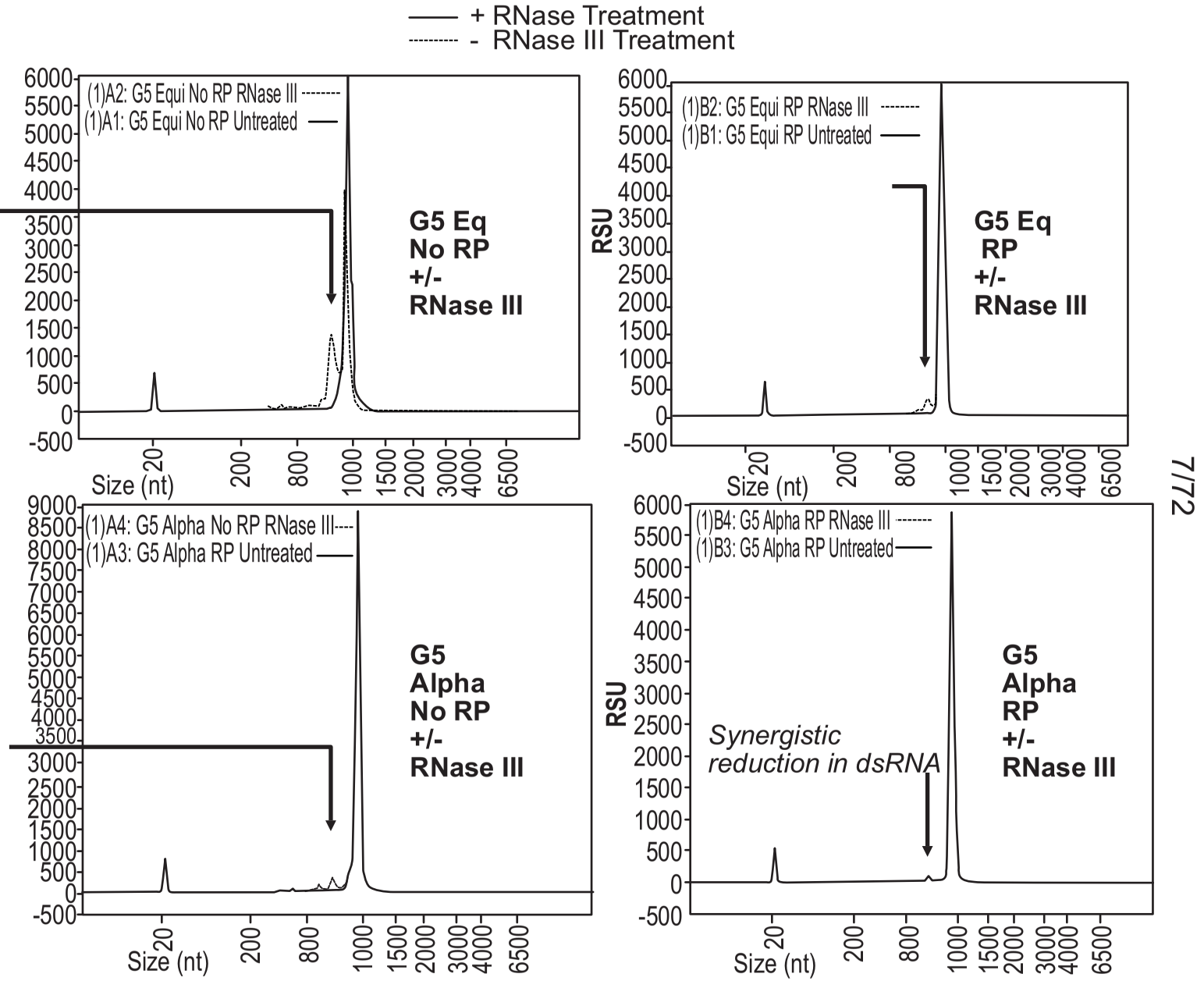
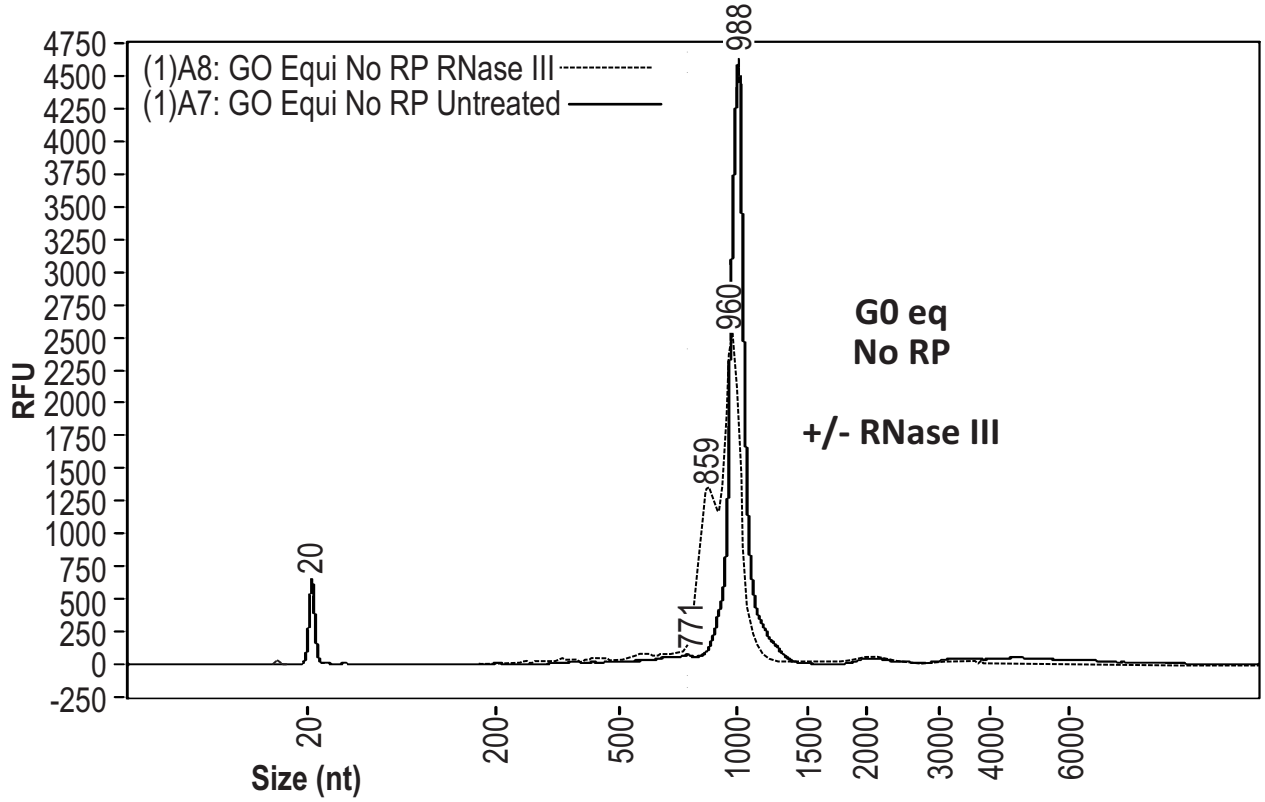


Figure 5B


(1)A8: GO Equi No RP RNase III
(1)A7: GO Equi No RP Untreated
RFU
Size (nt)
988
960
859
771
20
G0 eq
No RP
+/- RNase III

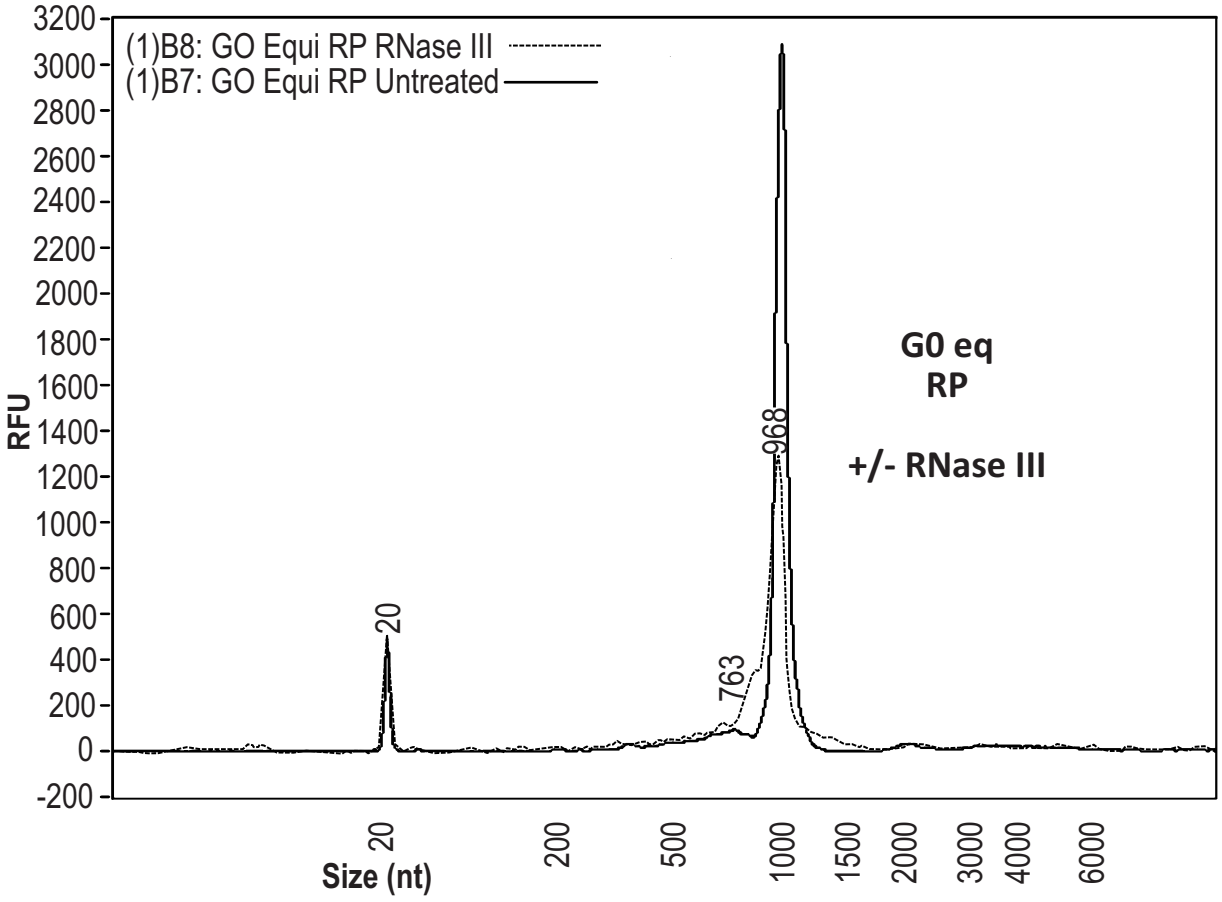

(1)B8: GO Equi RP RNase III
(1)B7: GO Equi RP Untreated
RFU
Size (nt)
968
763
20
G0 eq
RP
+/- RNase III

Figure 6

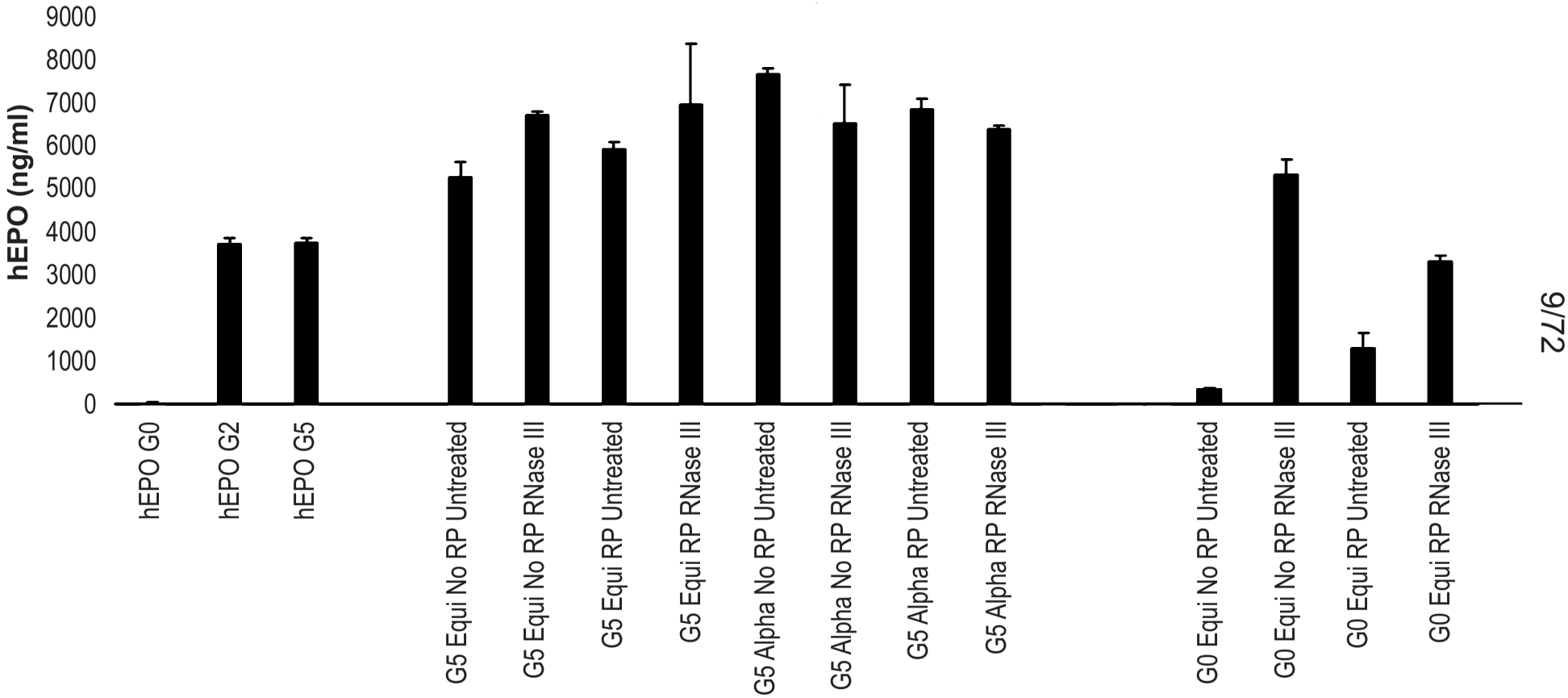

hEPO (ng/ml)
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
hEPO G0
hEPO G2
hEPO G5
G5 Equi No RP Untreated
G5 Equi No RP RNase III
G5 Equi RP Untreated
G5 Equi RP RNase III
G5 Alpha No RP Untreated
G5 Alpha No RP RNase III
G5 Alpha RP Untreated
G5 Alpha RP RNase III
G0 Equi No RP Untreated
G0 Equi No RP RNase III
G0 Equi RP Untreated
G0 Equi RP RNase III

Figure 6 (continued)

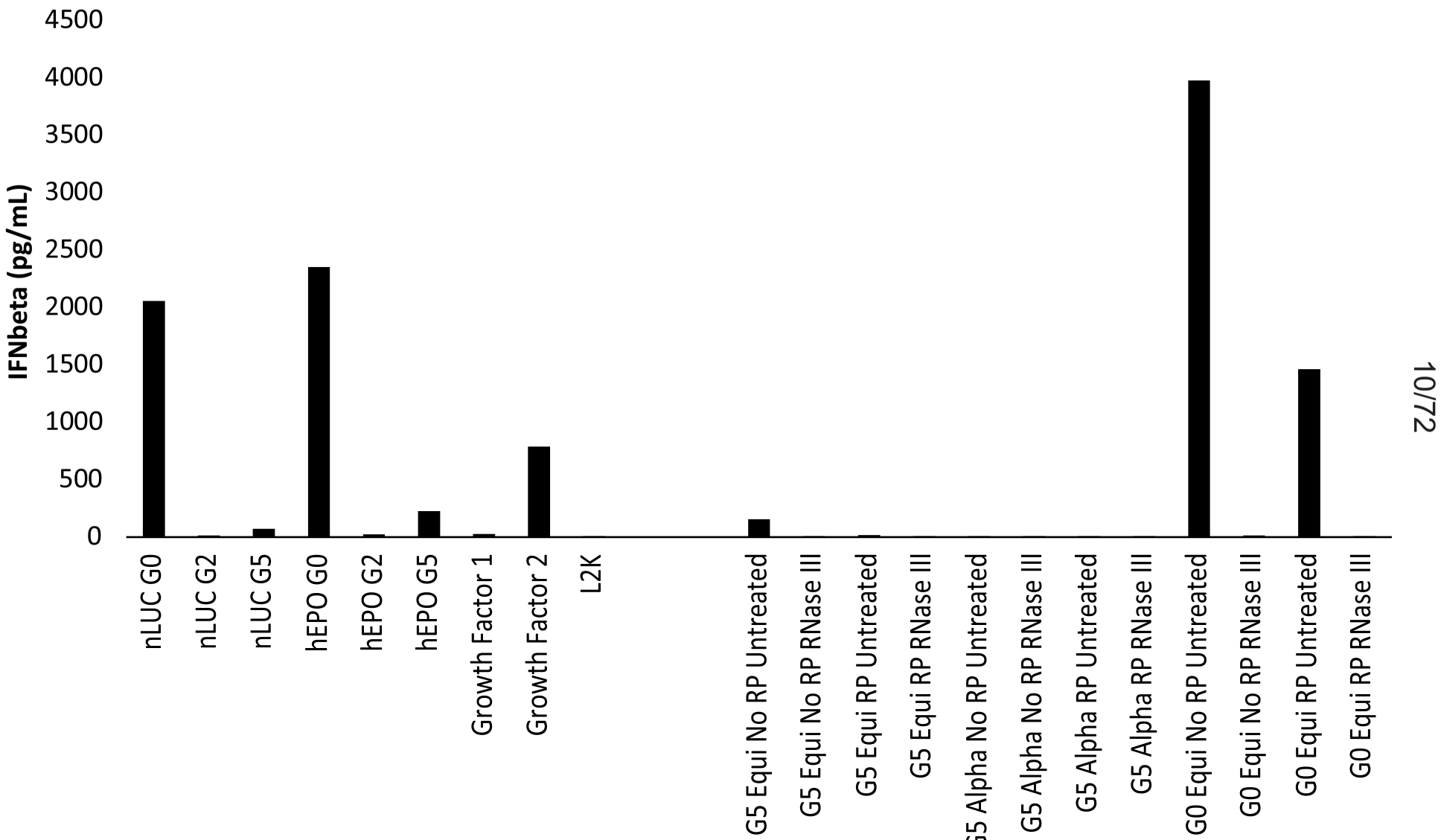


Figure 7

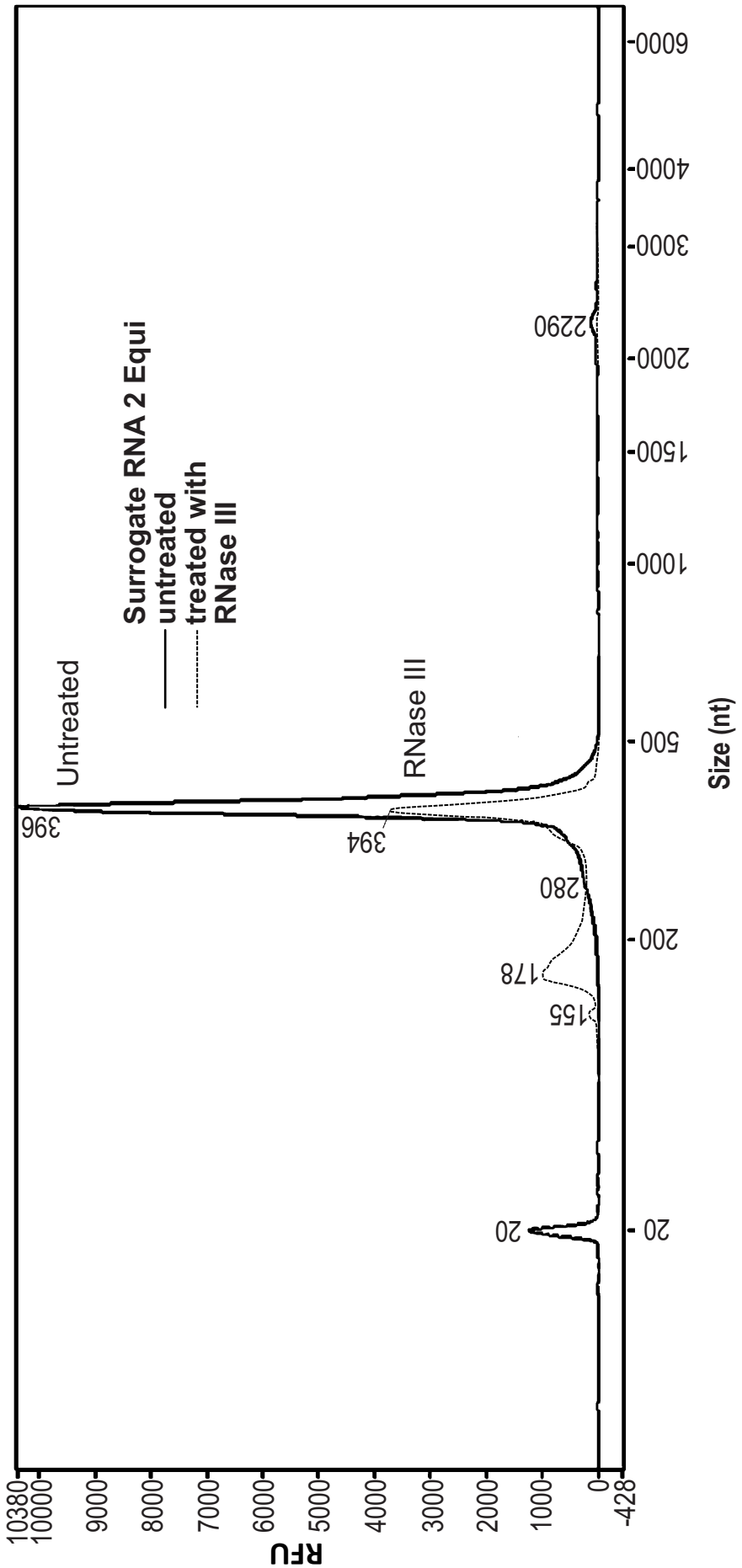

Surrogate RNA 2 Equi
untreated
treated with RNase III
Untreated
RNase III
RFU
Size (nt)
10380
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-428
6000
4000
3000
2000
1500
1000
500
200
20
2290
396
394
280
178
155
20

Figure 7 (continued)

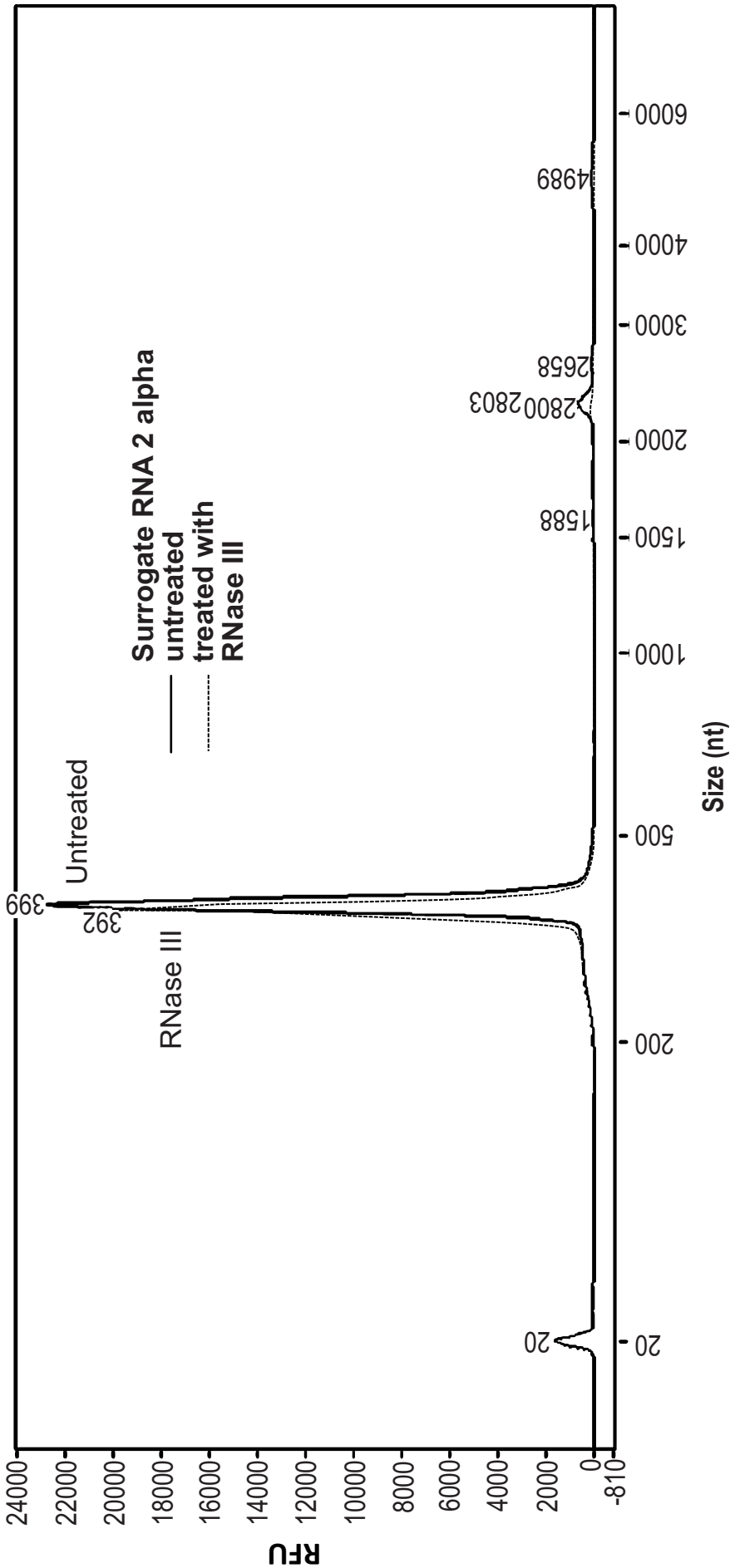


Figure 8A

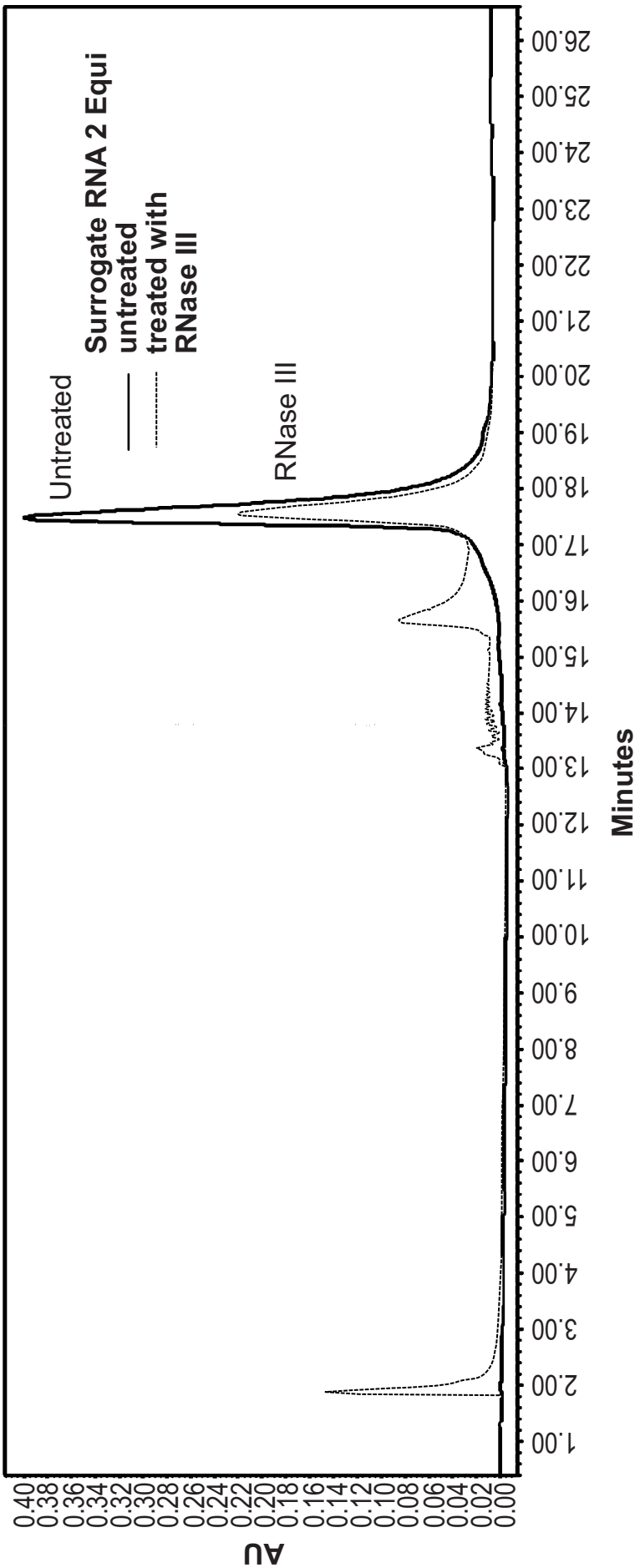

Surrogate RNA 2 Equi
untreated
treated with
RNase III
Untreated
RNase III
AU
Minutes
0.40
0.38
0.36
0.34
0.32
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
10.00
11.00
12.00
13.00
14.00
15.00
16.00
17.00
18.00
19.00
20.00
21.00
22.00
23.00
24.00
25.00
26.00

Figure 8B

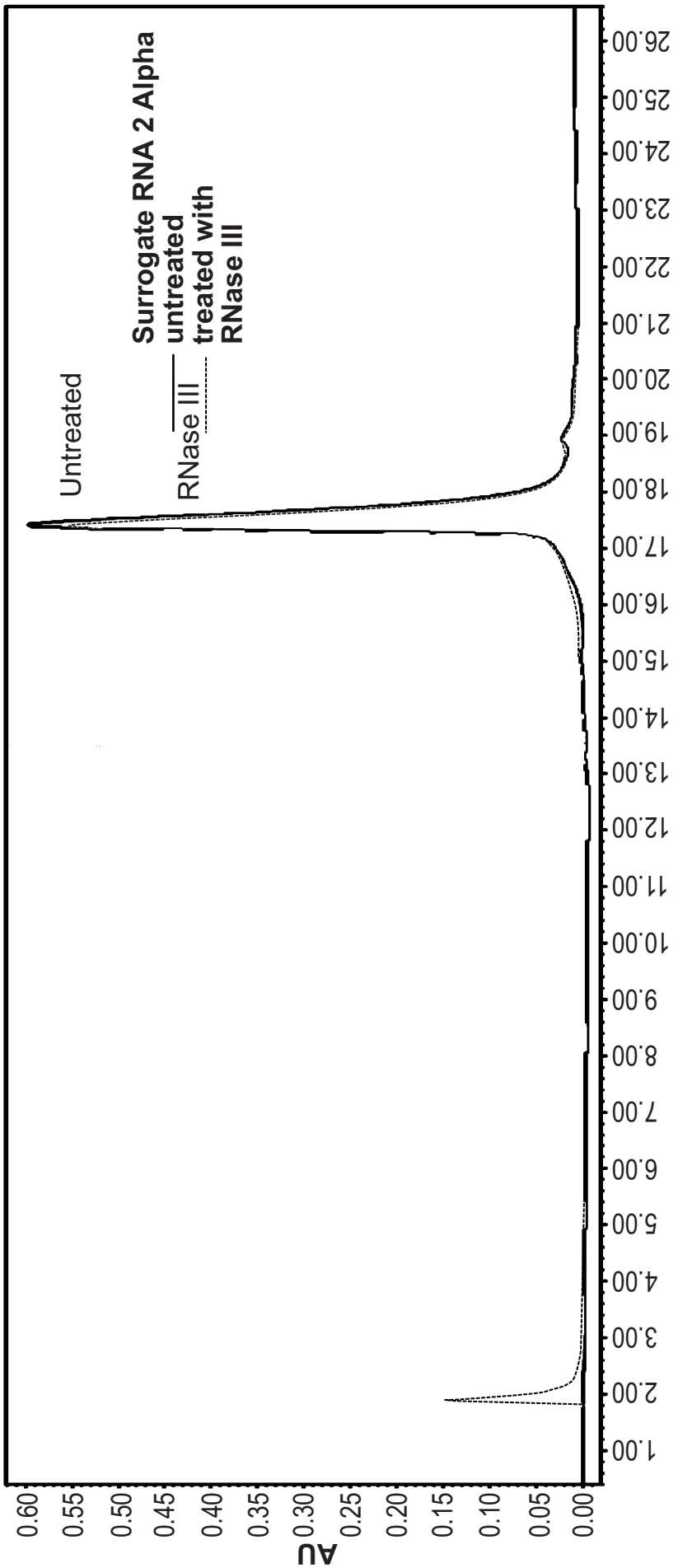

Surrogate RNA 2 Alpha
untreated
treated with
RNase III
RNase III
Untreated
AU
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
10.00
11.00
12.00
13.00
14.00
15.00
16.00
17.00
18.00
19.00
20.00
21.00
22.00
23.00
24.00
25.00
26.00

Figure 9

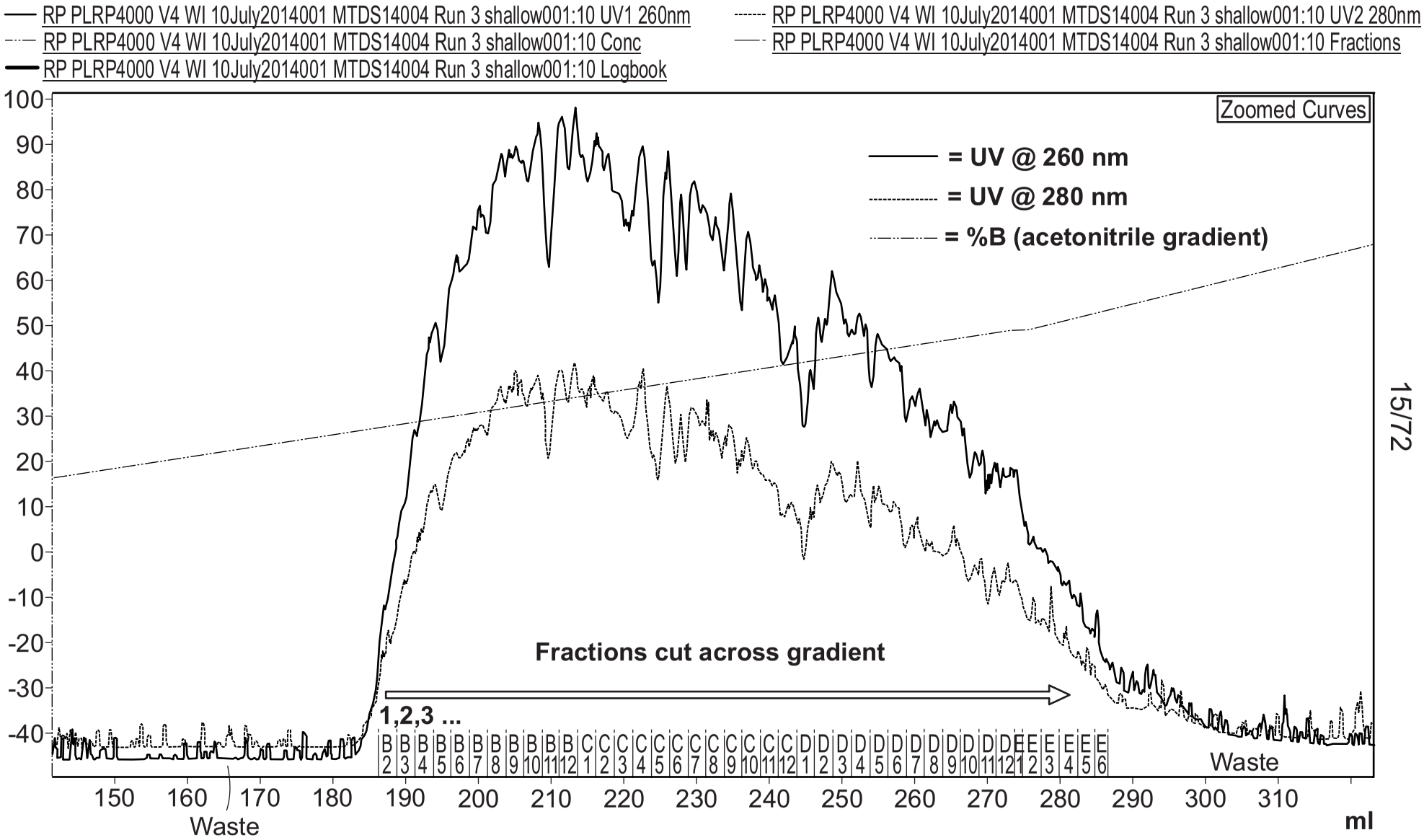

RP PLRP4000 V4 WI 10July2014001 MTDS14004 Run 3 shallow001:10 UV1 260nm
RP PLRP4000 V4 WI 10July2014001 MTDS14004 Run 3 shallow001:10 UV2 280nm
RP PLRP4000 V4 WI 10July2014001 MTDS14004 Run 3 shallow001:10 Conc
RP PLRP4000 V4 WI 10July2014001 MTDS14004 Run 3 shallow001:10 Fractions
RP PLRP4000 V4 WI 10July2014001 MTDS14004 Run 3 shallow001:10 Logbook
Zoomed Curves
= UV @ 260 nm
= UV @ 280 nm
= %B (acetonitrile gradient)
Fractions cut across gradient
1,2,3 ...
Waste
Waste
ml
15/72

Figure 10A

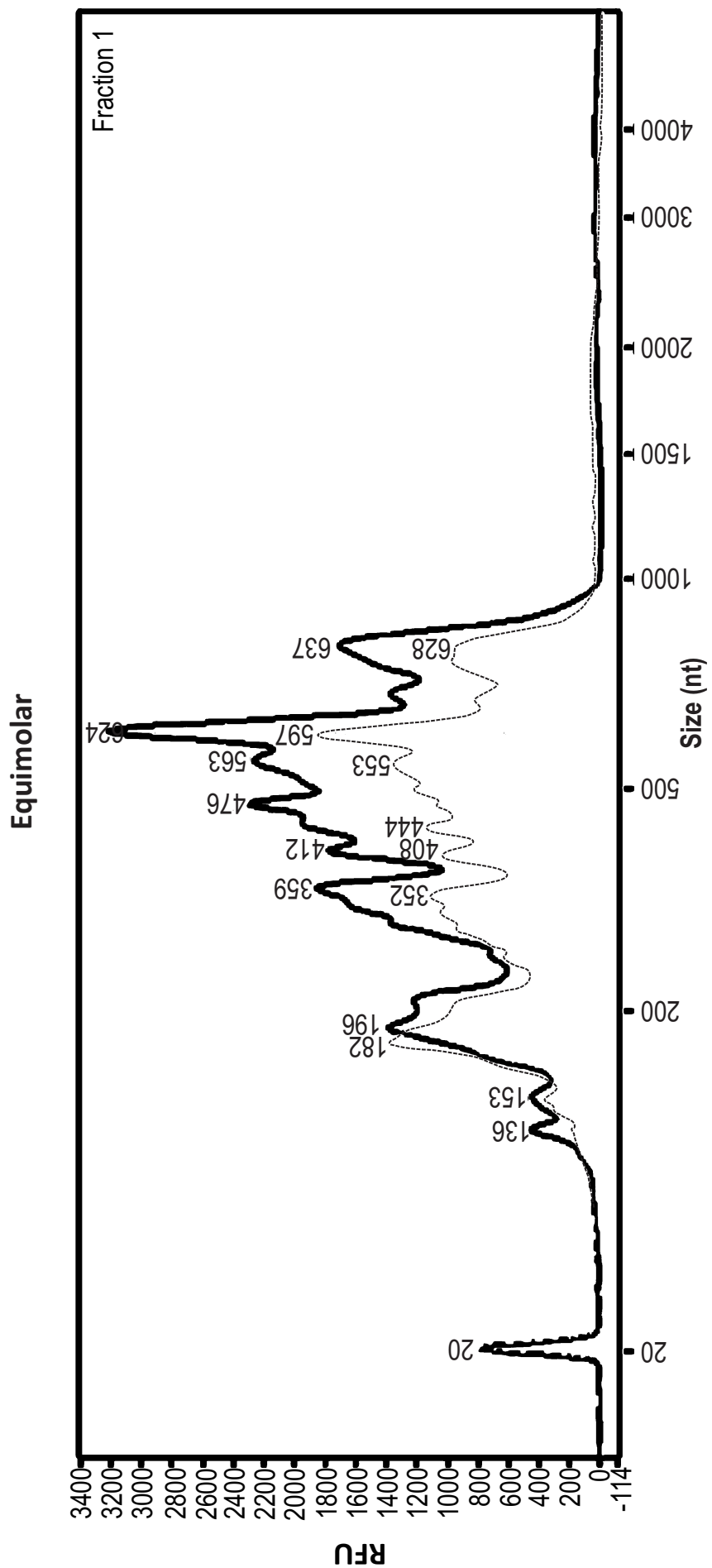

Fraction 1
Equimolar
RFU
Size (nt)
3400
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
-114
20
200
500
1000
1500
2000
3000
4000
20
136
153
182
196
359
352
408
412
444
476
563
597
553
624
637
628

Figure 10A (continued)

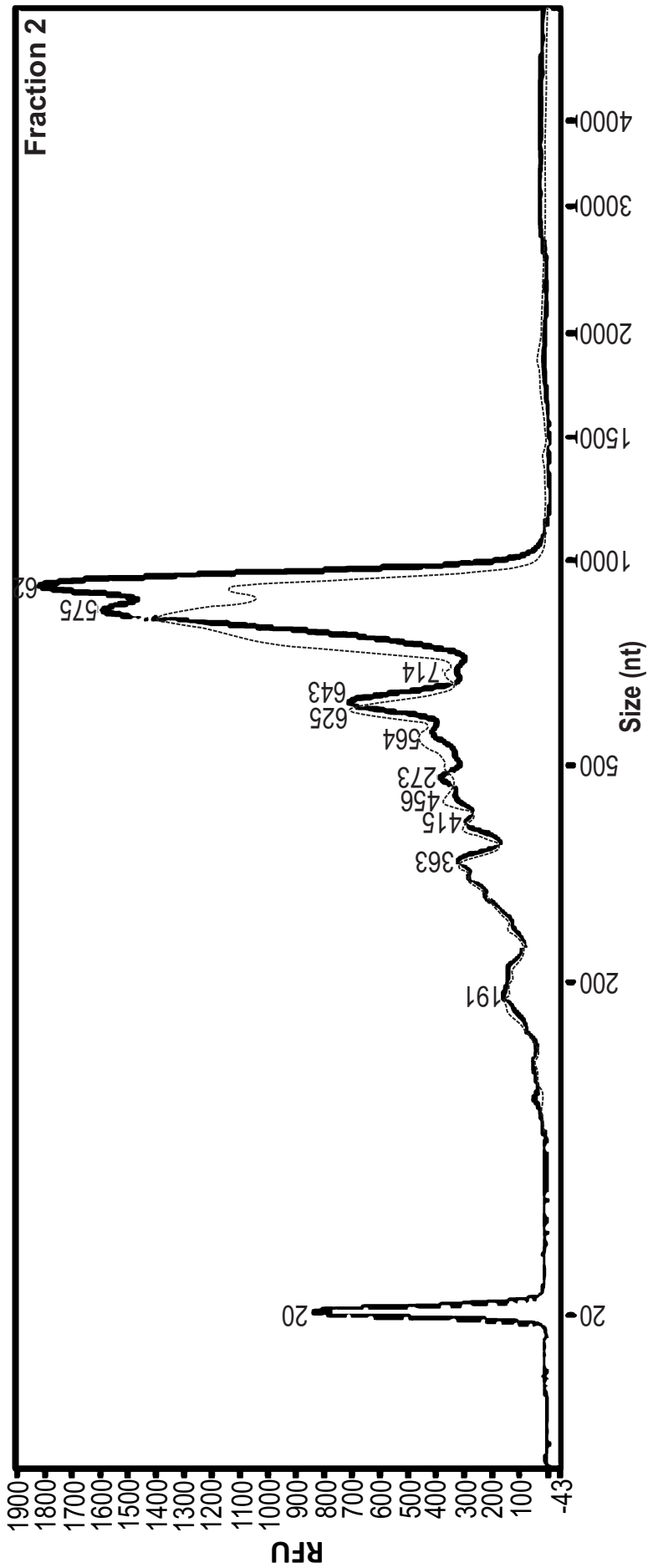


Figure 10A (continued)

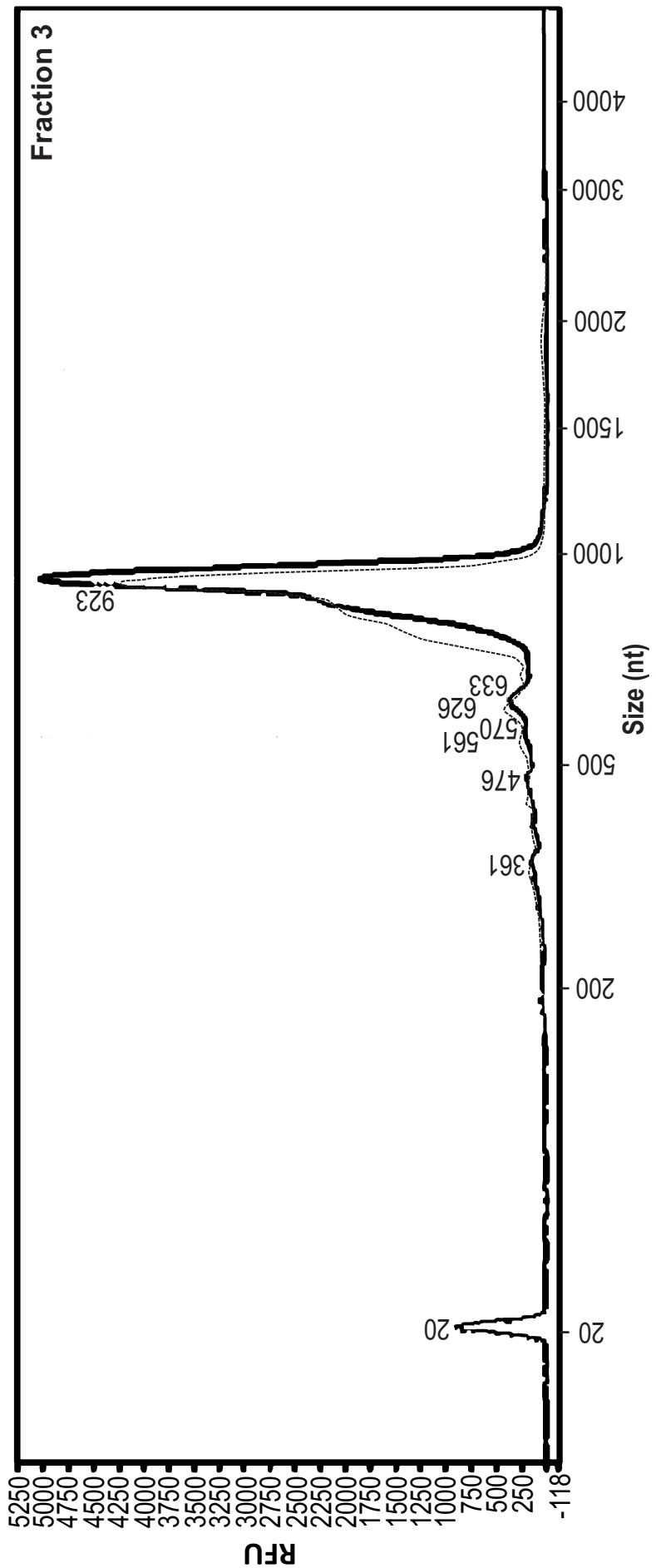


Figure 10B

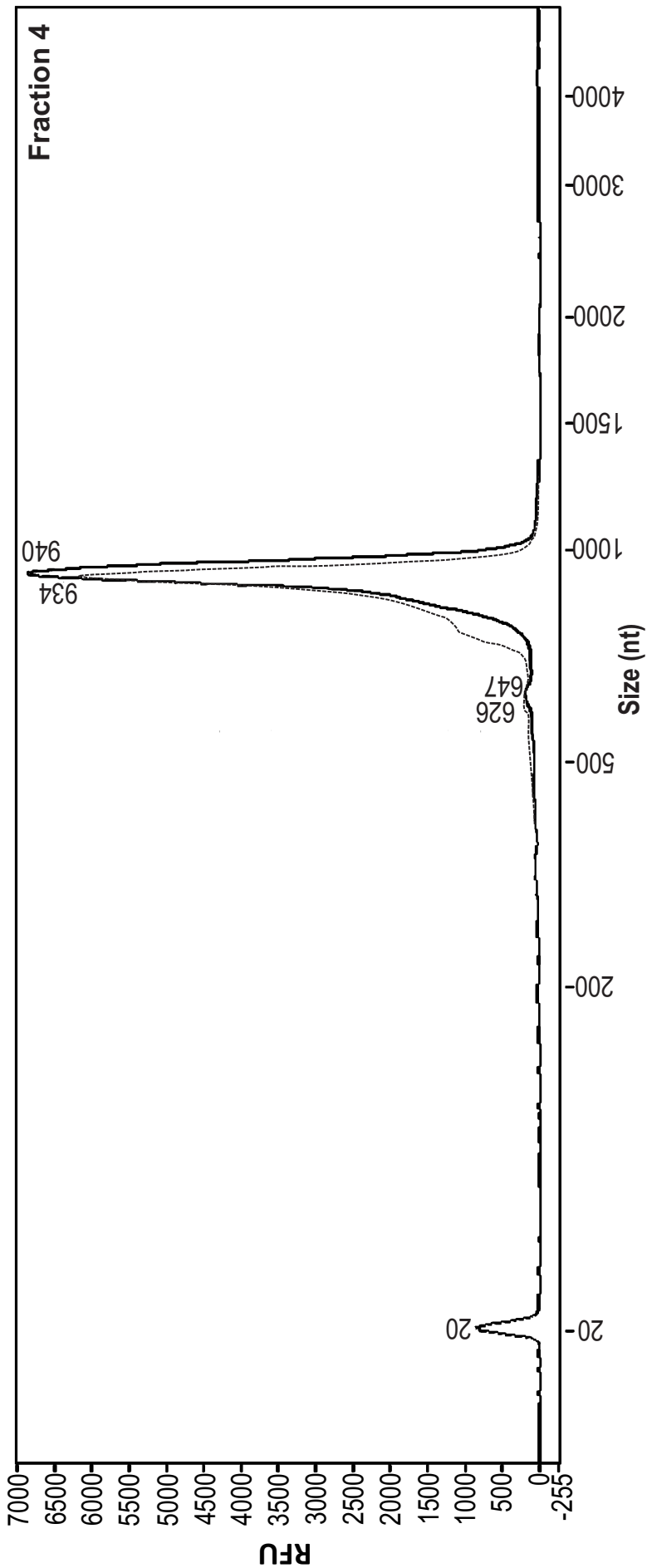

Fraction 4
RFU
7000
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-255
Size (nt)
20
626
647
934
940
20-
200-
500-
1000-
1500-
2000-
3000-
4000-

Figure 10B (continued)

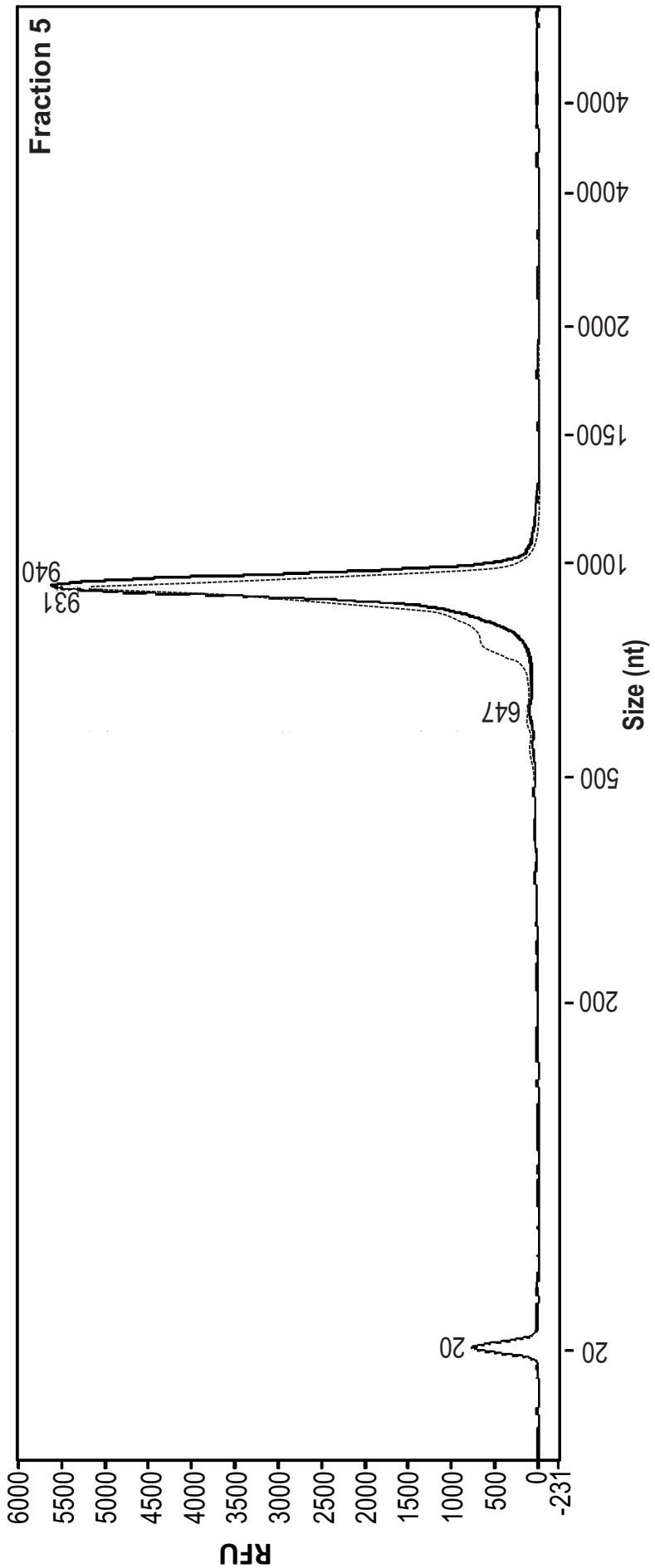


Figure 10B (continued)

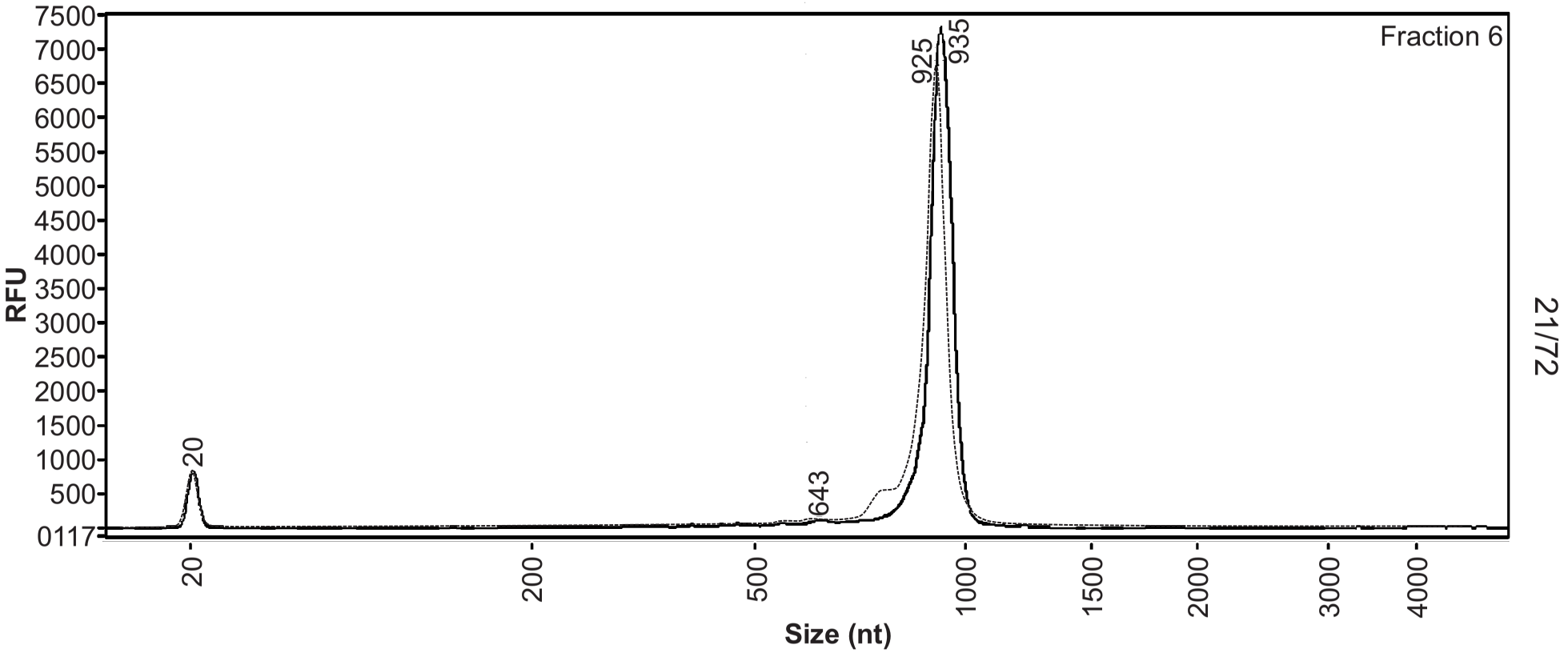


Figure 10C

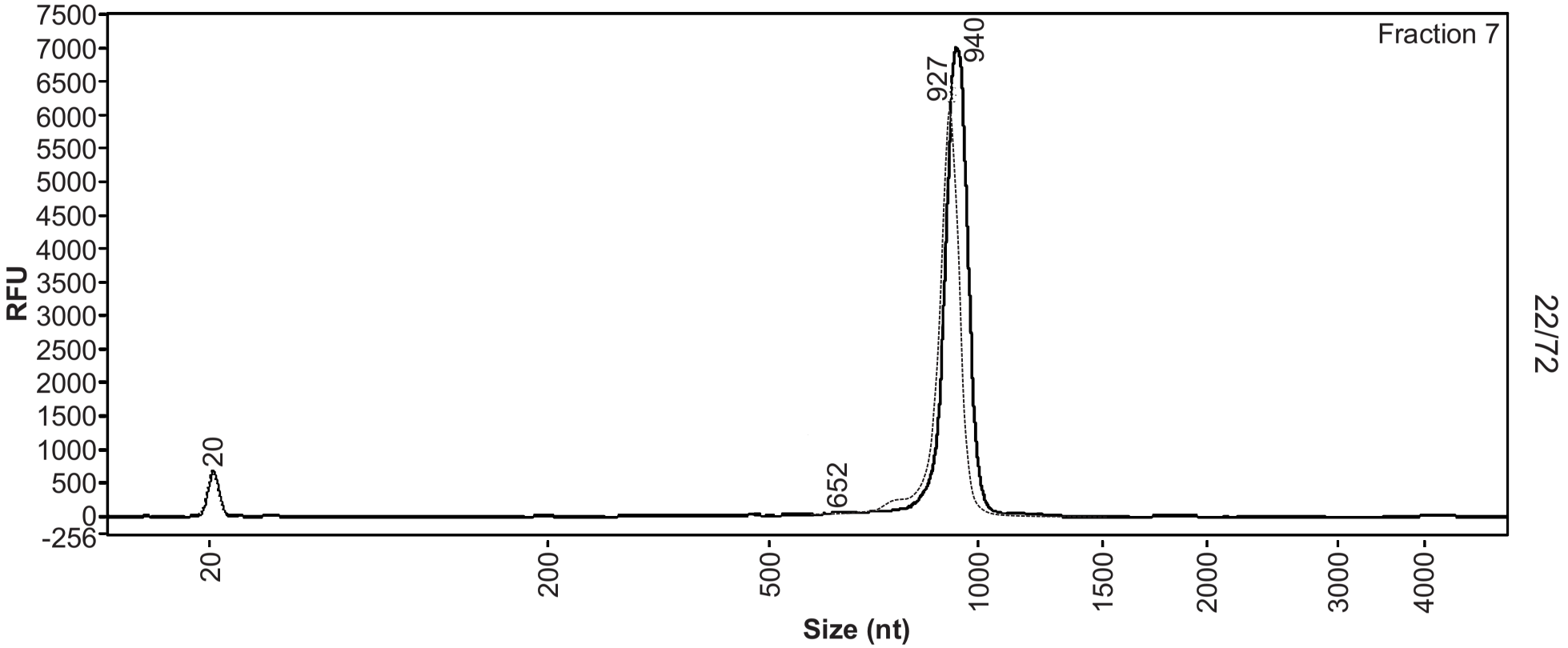

Fraction 7
RFU
7500
7000
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-256
20
652
927
940
20
200
500
1000
1500
2000
3000
4000
Size (nt)

Figure 10C (continued)

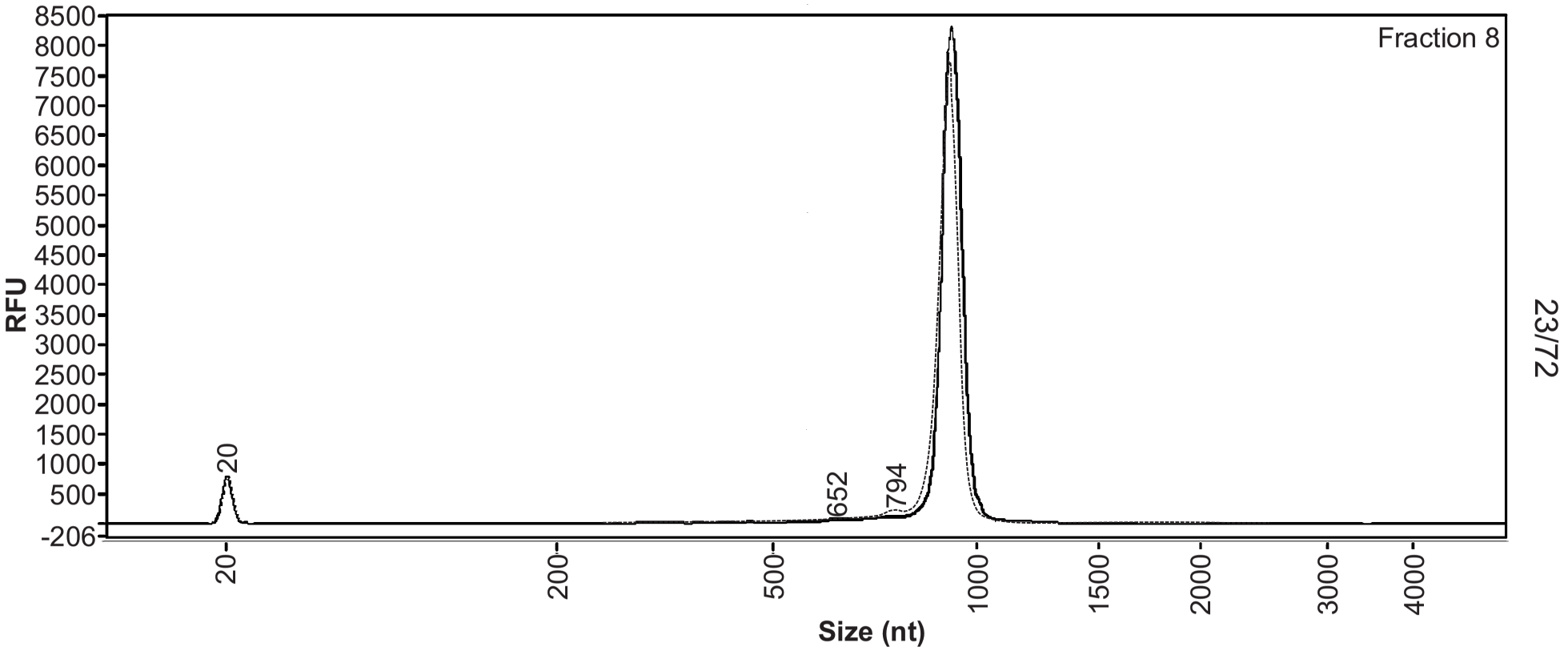


Figure 10C (continued)

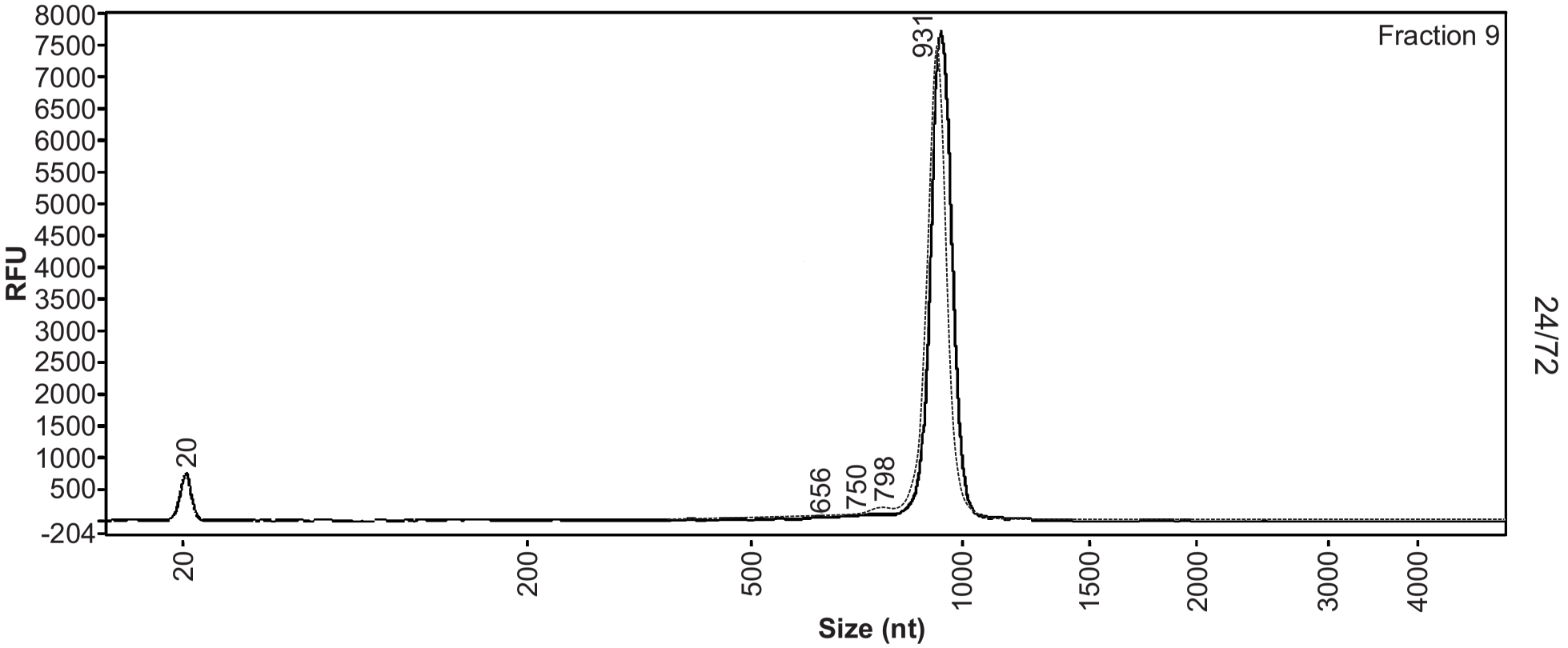


Figure 10D

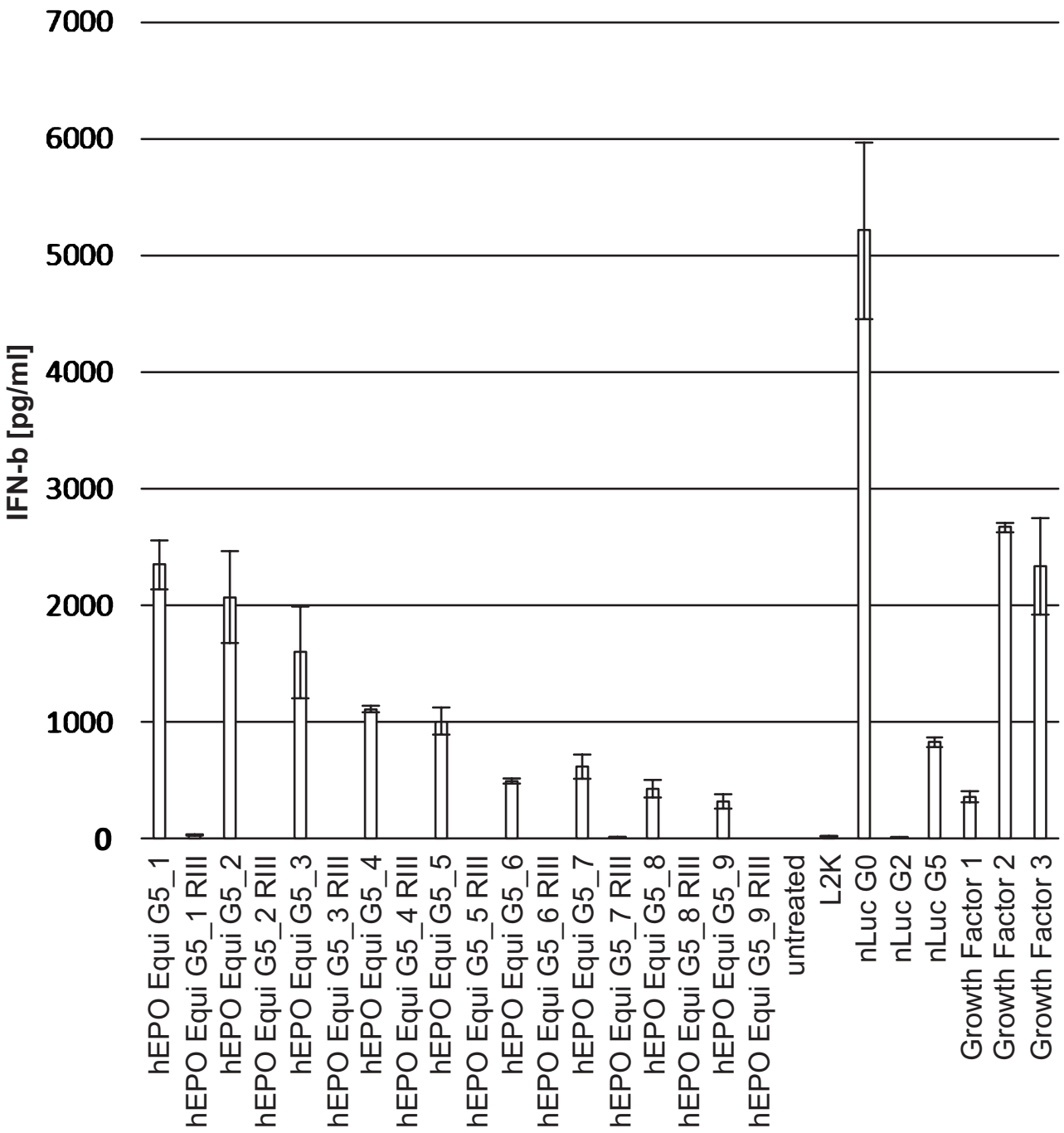

IFN-b [pg/ml]
7000
6000
5000
4000
3000
2000
1000
0
hEPO Equi G5_1
hEPO Equi G5_1 RIII
hEPO Equi G5_2
hEPO Equi G5_2 RIII
hEPO Equi G5_3
hEPO Equi G5_3 RIII
hEPO Equi G5_4
hEPO Equi G5_4 RIII
hEPO Equi G5_5
hEPO Equi G5_5 RIII
hEPO Equi G5_6
hEPO Equi G5_6 RIII
hEPO Equi G5_7
hEPO Equi G5_7 RIII
hEPO Equi G5_8
hEPO Equi G5_8 RIII
hEPO Equi G5_9
hEPO Equi G5_9 RIII
untreated
L2K
nLuc G0
nLuc G2
nLuc G5
Growth Factor 1
Growth Factor 2
Growth Factor 3

Figure 11A

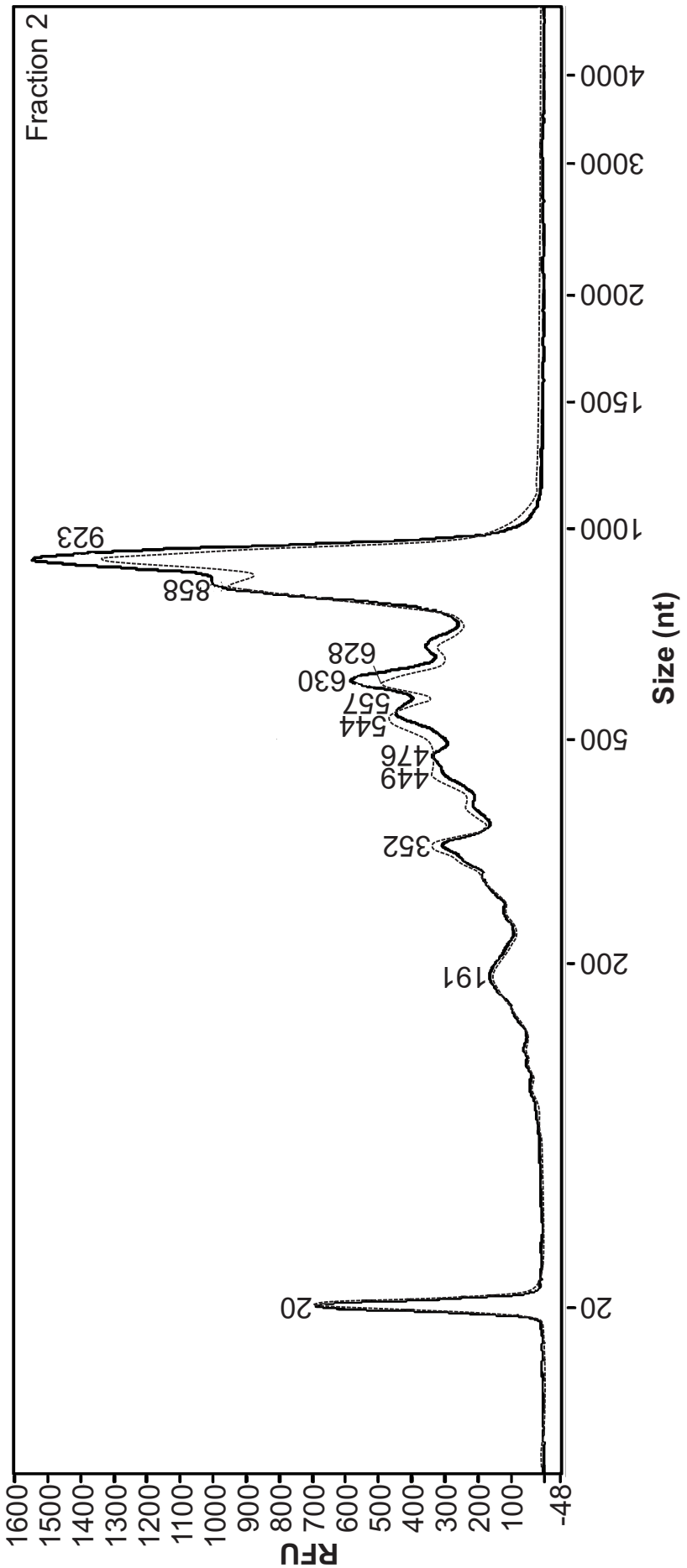

Fraction 2
RFU
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
-48
20
191
352
449
476
544
557
630
628
858
923
20
200
500
1000
1500
2000
3000
4000
Size (nt)

Figure 11A (continued)

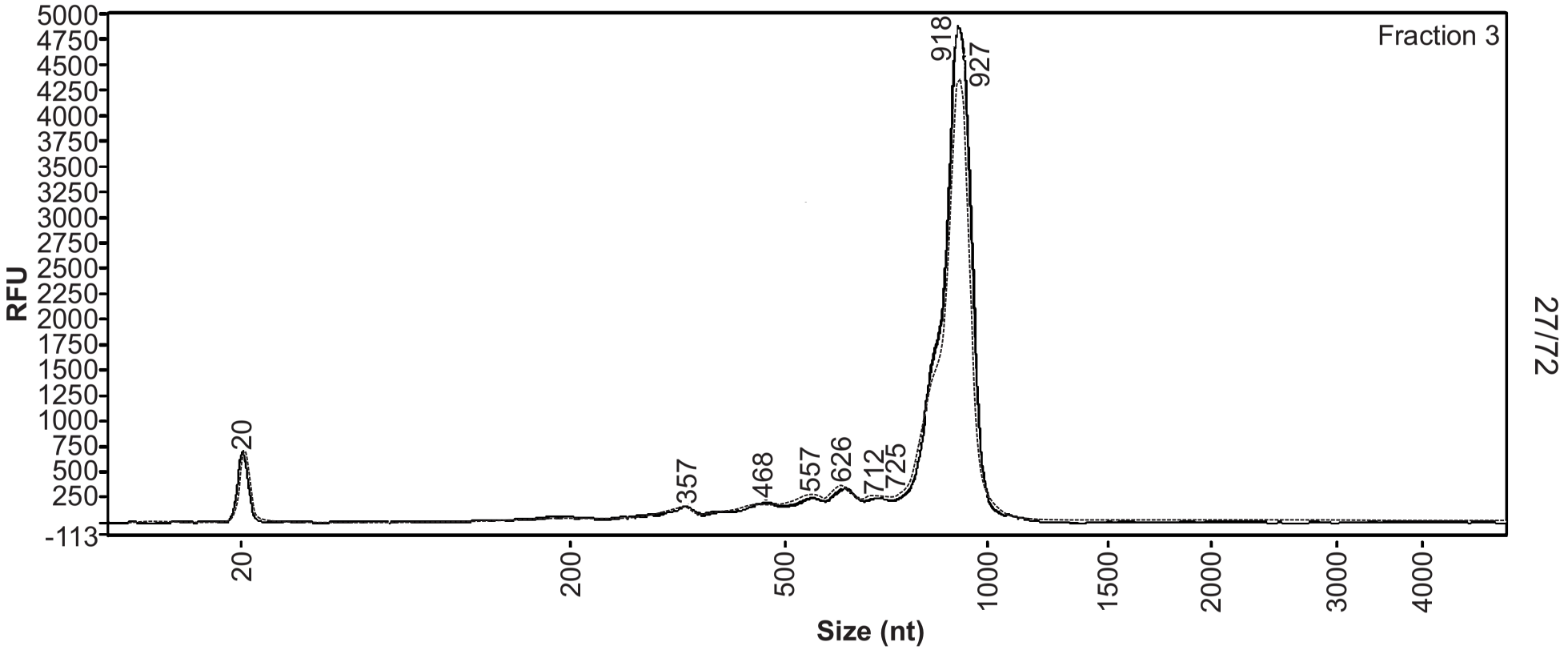


Figure 11A (continued)

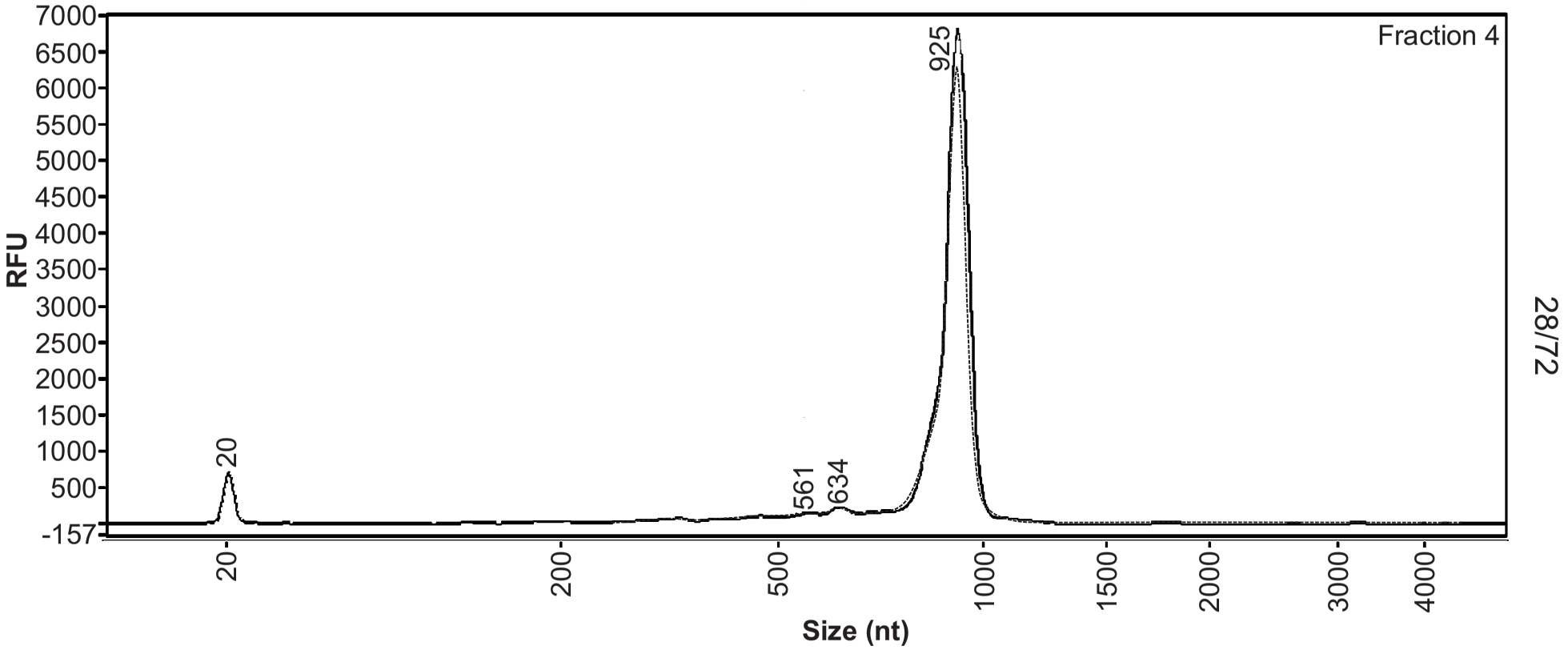


Figure 11B

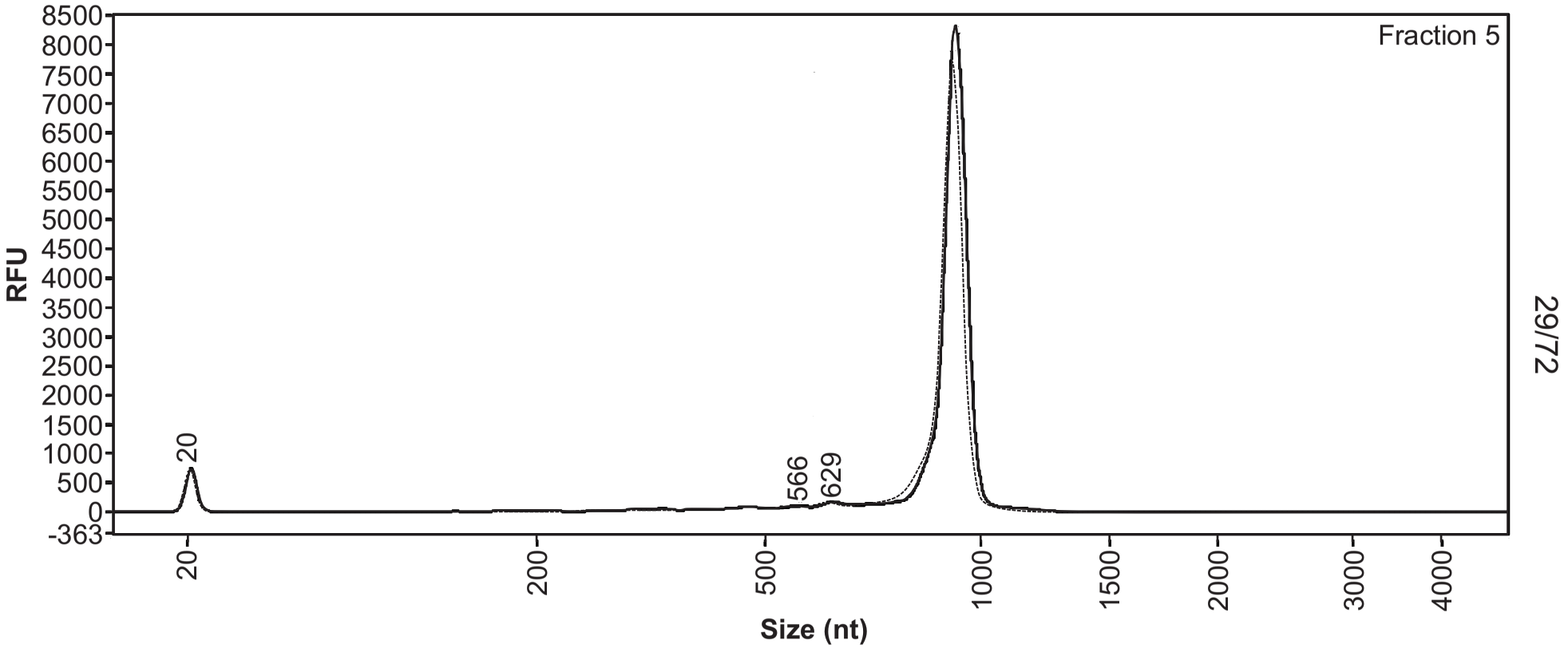

Fraction 5
RFU
8500
8000
7500
7000
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-363
20
566
629
20
200
500
1000
1500
2000
3000
4000
Size (nt)

Figure 11B (continued)

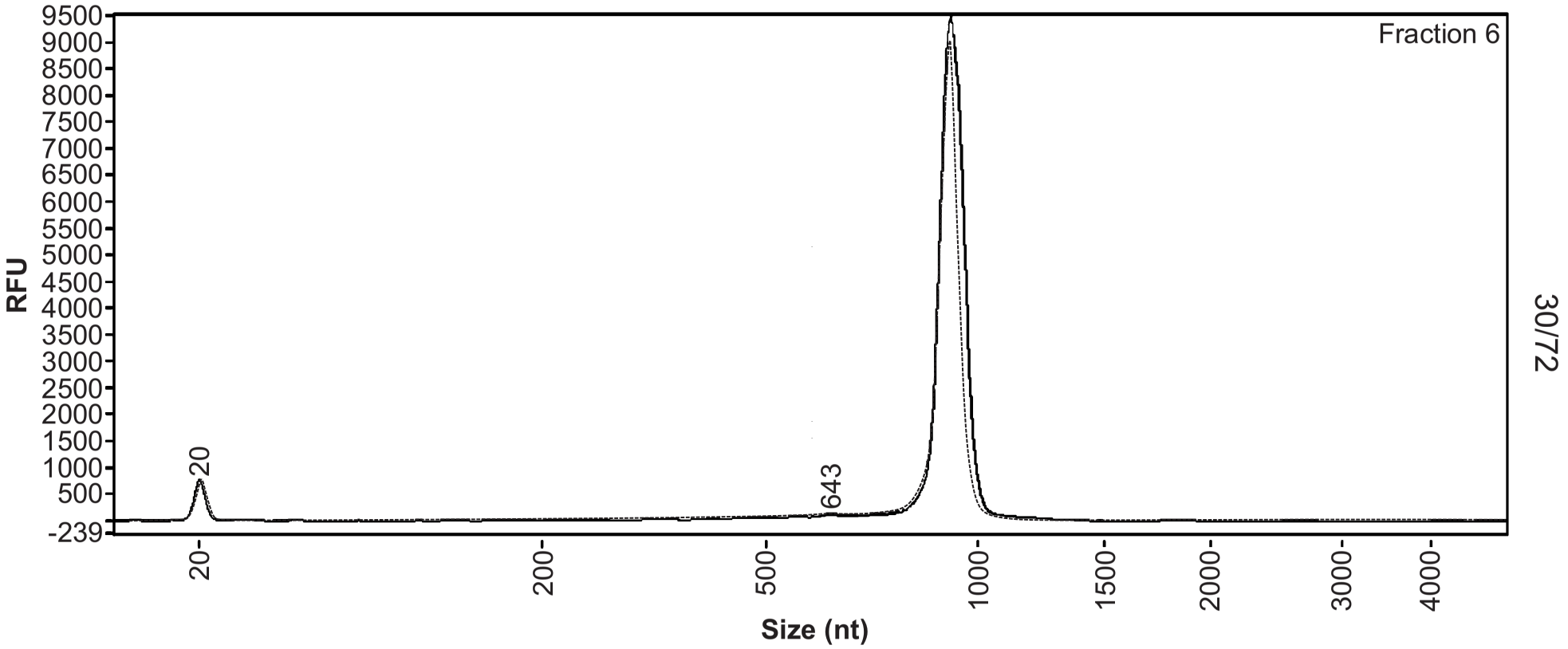


Figure 11B (continued)

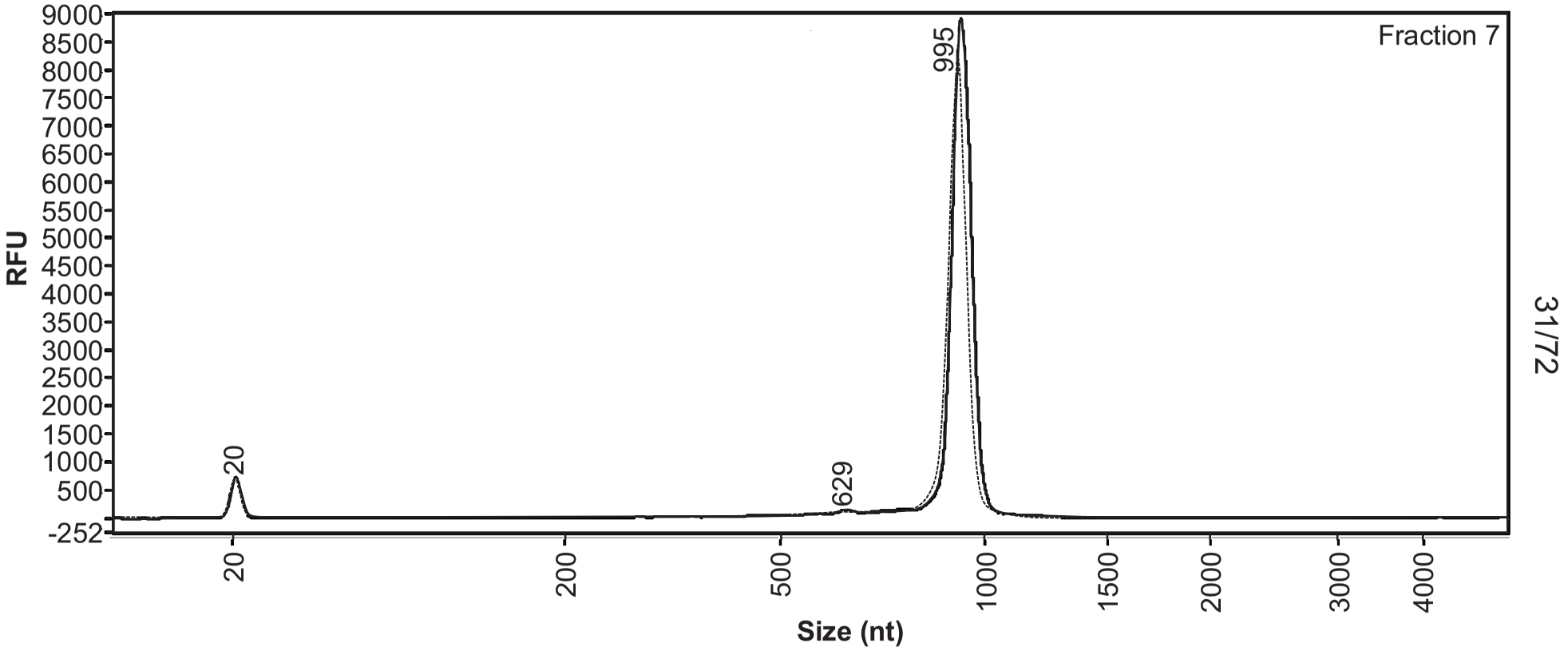


Figure 11C

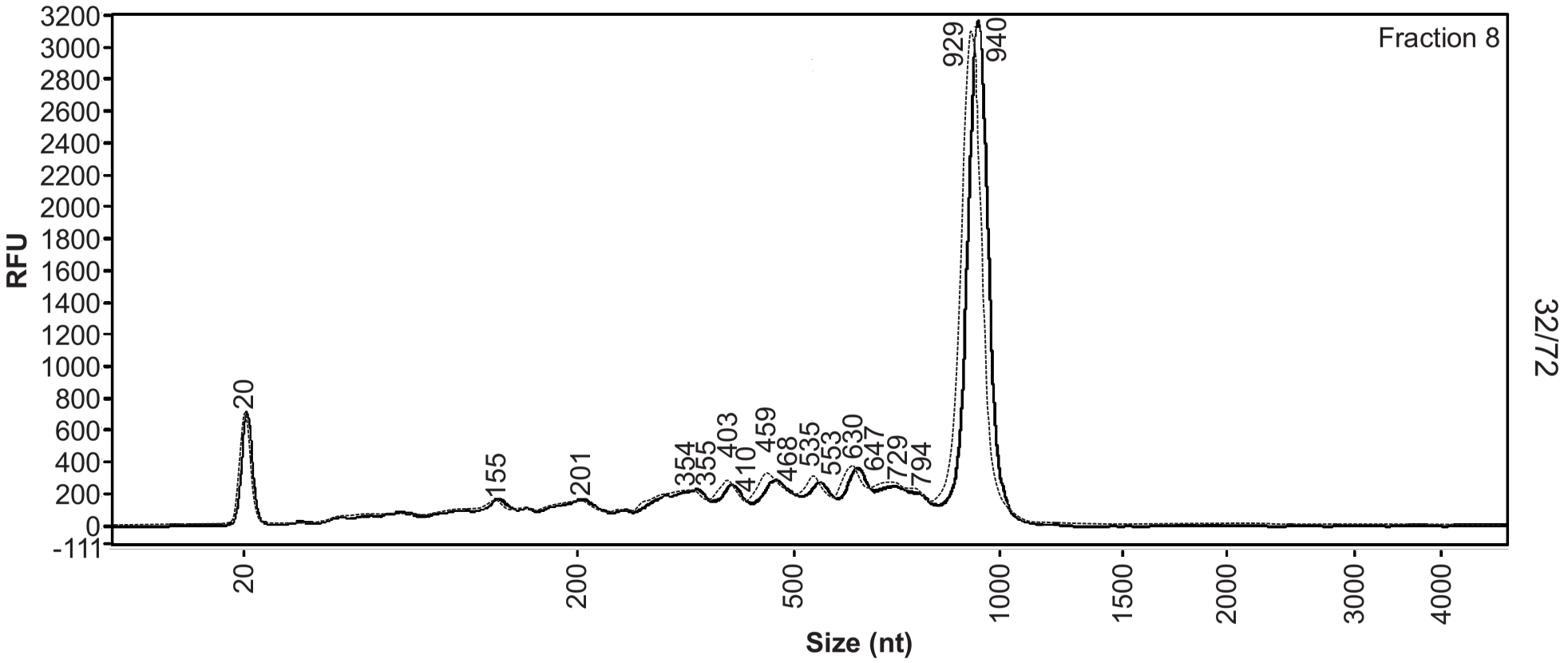

Fraction 8
RFU
Size (nt)
20
155
201
354
355
403
410
459
468
535
553
630
647
729
794
929
940
32/72

Figure 11C (continued)

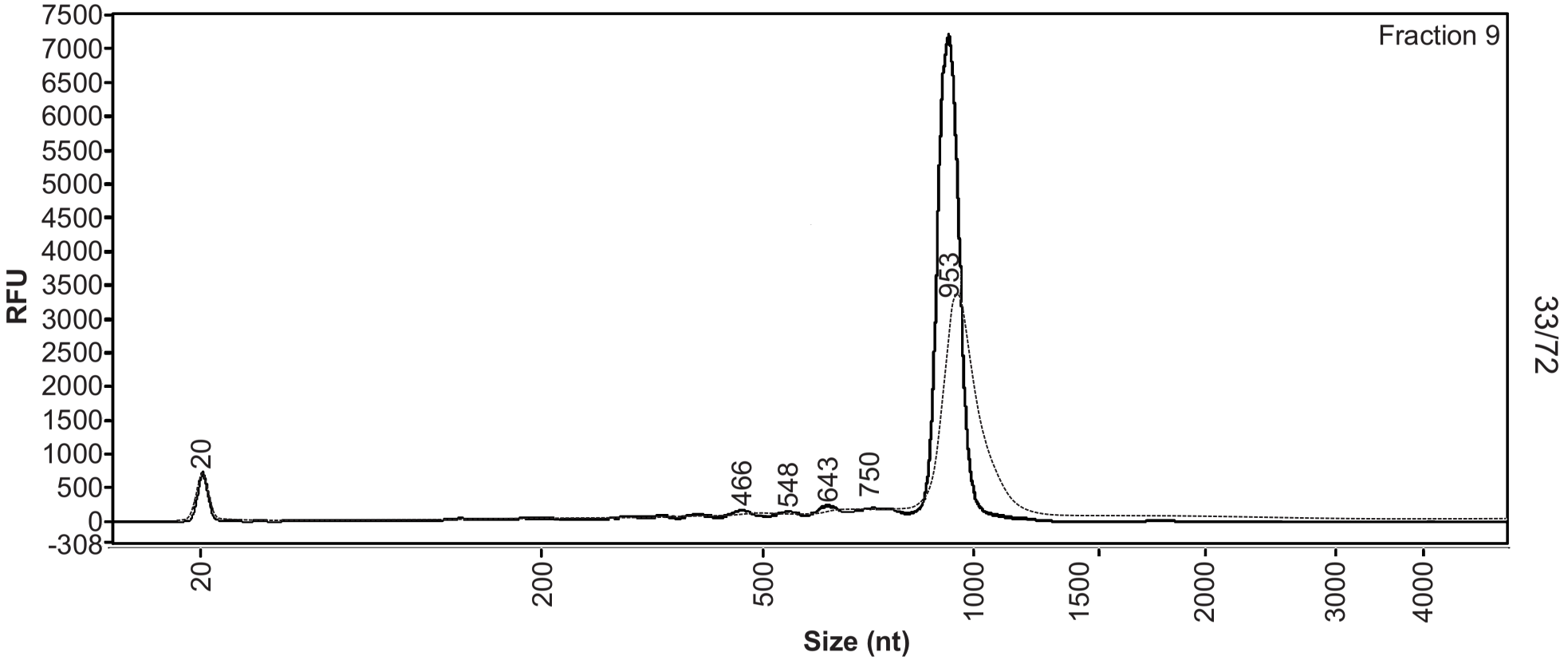


Figure 11C (continued)

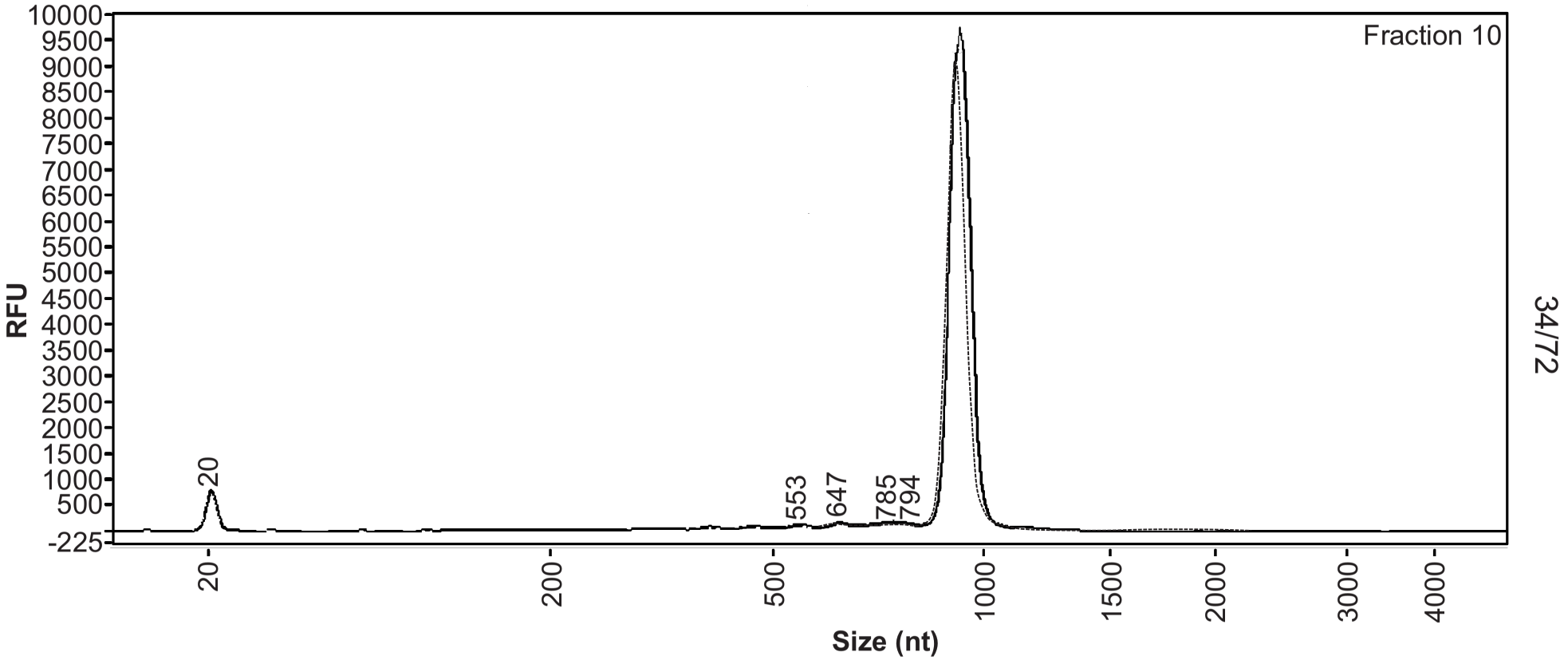


Figure 11D

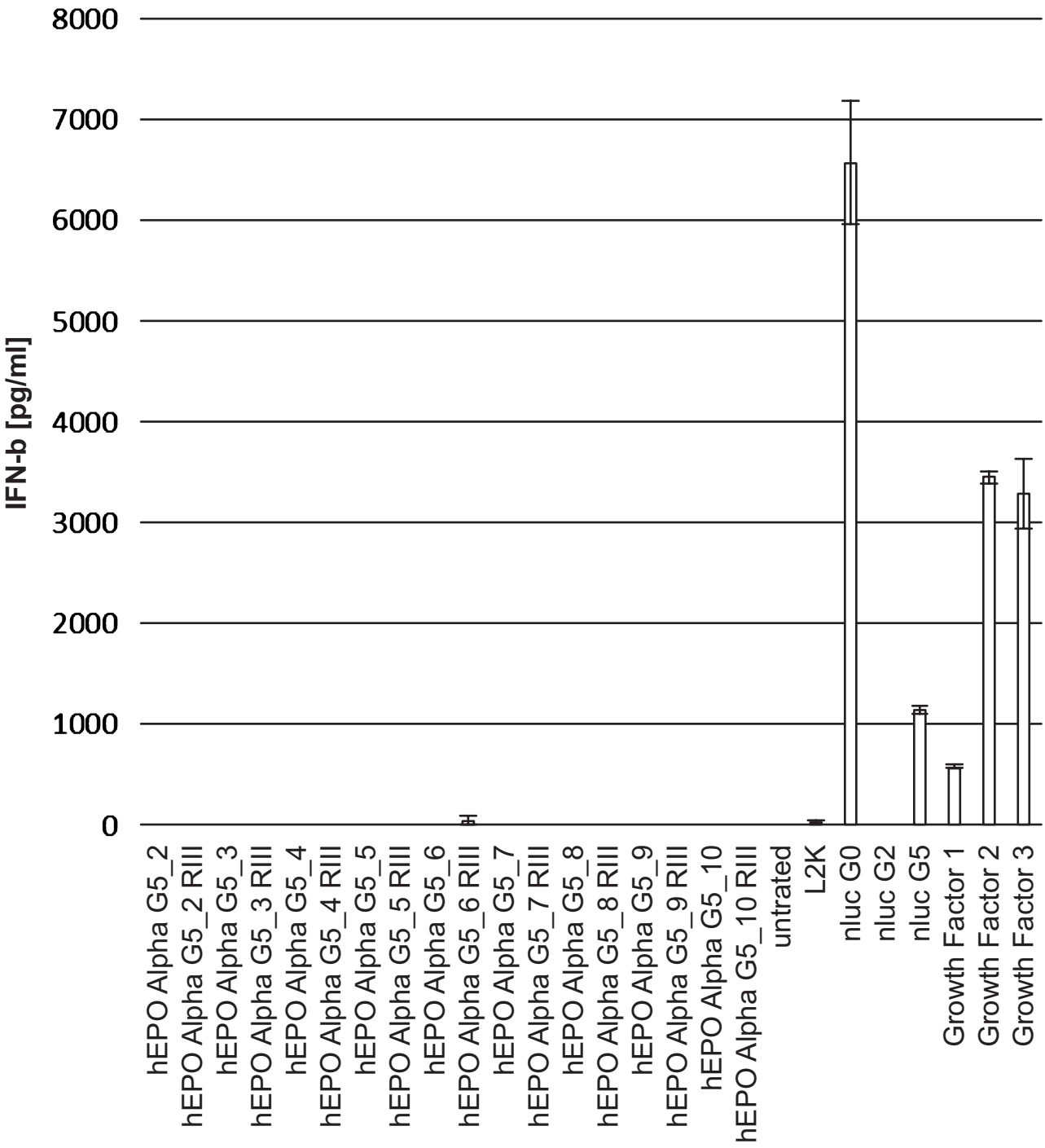

8000
7000
6000
5000
4000
3000
2000
1000
0
IFN-b [pg/ml]
hEPO Alpha G5_2
hEPO Alpha G5_2 RIII
hEPO Alpha G5_3
hEPO Alpha G5_3 RIII
hEPO Alpha G5_4
hEPO Alpha G5_4 RIII
hEPO Alpha G5_5
hEPO Alpha G5_5 RIII
hEPO Alpha G5_6
hEPO Alpha G5_6 RIII
hEPO Alpha G5_7
hEPO Alpha G5_7 RIII
hEPO Alpha G5_8
hEPO Alpha G5_8 RIII
hEPO Alpha G5_9
hEPO Alpha G5_9 RIII
hEPO Alpha G5_10
hEPO Alpha G5_10 RIII
untrated
L2K
nluc G0
nluc G2
nluc G5
Growth Factor 1
Growth Factor 2
Growth Factor 3

Figure 12

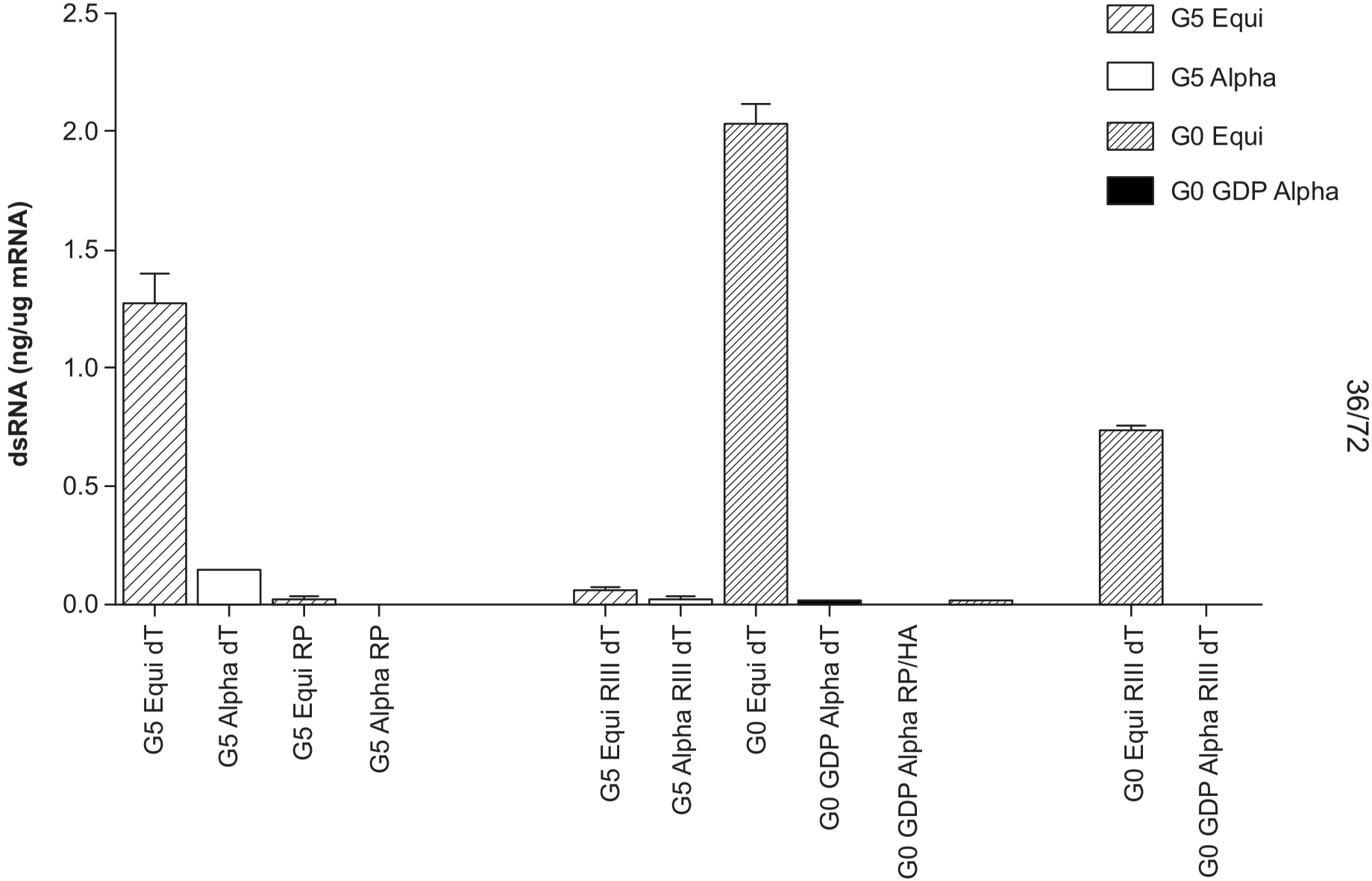

G5 Equi
G5 Alpha
G0 Equi
G0 GDP Alpha
dsRNA (ng/ug mRNA)
2.5
2.0
1.5
1.0
0.5
0.0
G5 Equi dT
G5 Alpha dT
G5 Equi RP
G5 Alpha RP
G5 Equi RIII dT
G5 Alpha RIII dT
G0 Equi dT
G0 GDP Alpha dT
G0 GDP Alpha RP/HA
G0 Equi RIII dT
G0 GDP Alpha RIII dT

Figure 13

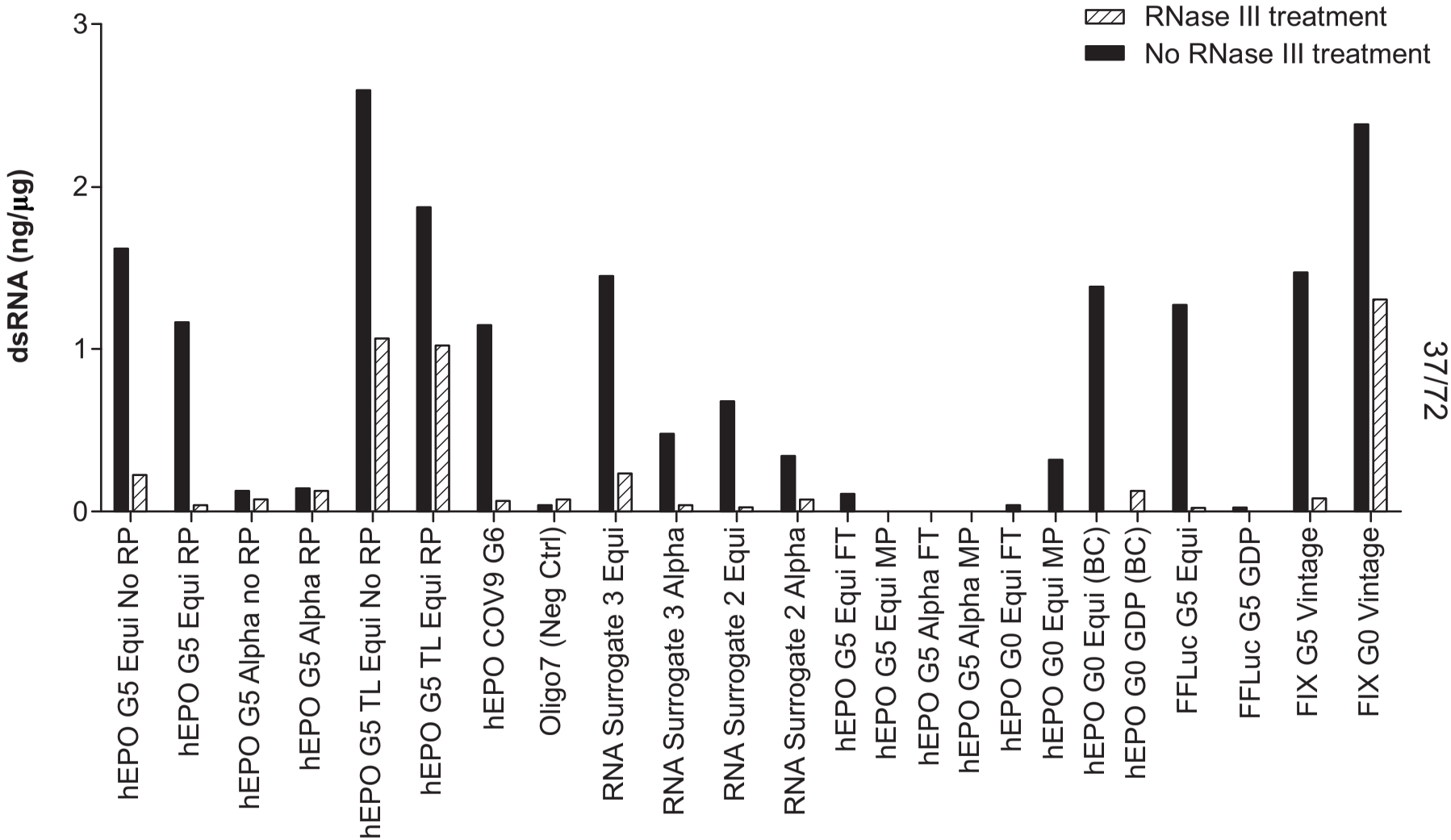

RNase III treatment
No RNase III treatment
dsRNA (ng/µg)
3
2
1
0
hEPO G5 Equi No RP
hEPO G5 Equi RP
hEPO G5 Alpha no RP
hEPO G5 Alpha RP
hEPO G5 TL Equi No RP
hEPO G5 TL Equi RP
hEPO COV9 G6
Oligo7 (Neg Ctrl)
RNA Surrogate 3 Equi
RNA Surrogate 3 Alpha
RNA Surrogate 2 Equi
RNA Surrogate 2 Alpha
hEPO G5 Equi FT
hEPO G5 Equi MP
hEPO G5 Alpha FT
hEPO G5 Alpha MP
hEPO G0 Equi FT
hEPO G0 Equi MP
hEPO G0 Equi (BC)
hEPO G0 GDP (BC)
FFLuc G5 Equi
FFLuc G5 GDP
FIX G5 Vintage
FIX G0 Vintage

Figure 14

IFNbeta [pg/ml]
0
500
1000
1500
2000
2500
3000
3500
4000
GDP 37C 2hr capped
GDP 25C 6hr capped
GDP 25C O/N capped
GTP 37C 2hr capped
GTP 25C 6hr capped
GTP 25C O/N capped
G5 Std 37C 2hr capped
G5 Std 25C 6hr capped
G5 Std 25C O/N capped
G0 Std 37C 2hr capped
G0 Std 25C 6hr capped
untreated
L2K
nLuc G0
nLuc G2
nLuc G5
Growth Factor 1
Growth Factor 2
Growth Factor 3

Figure 15

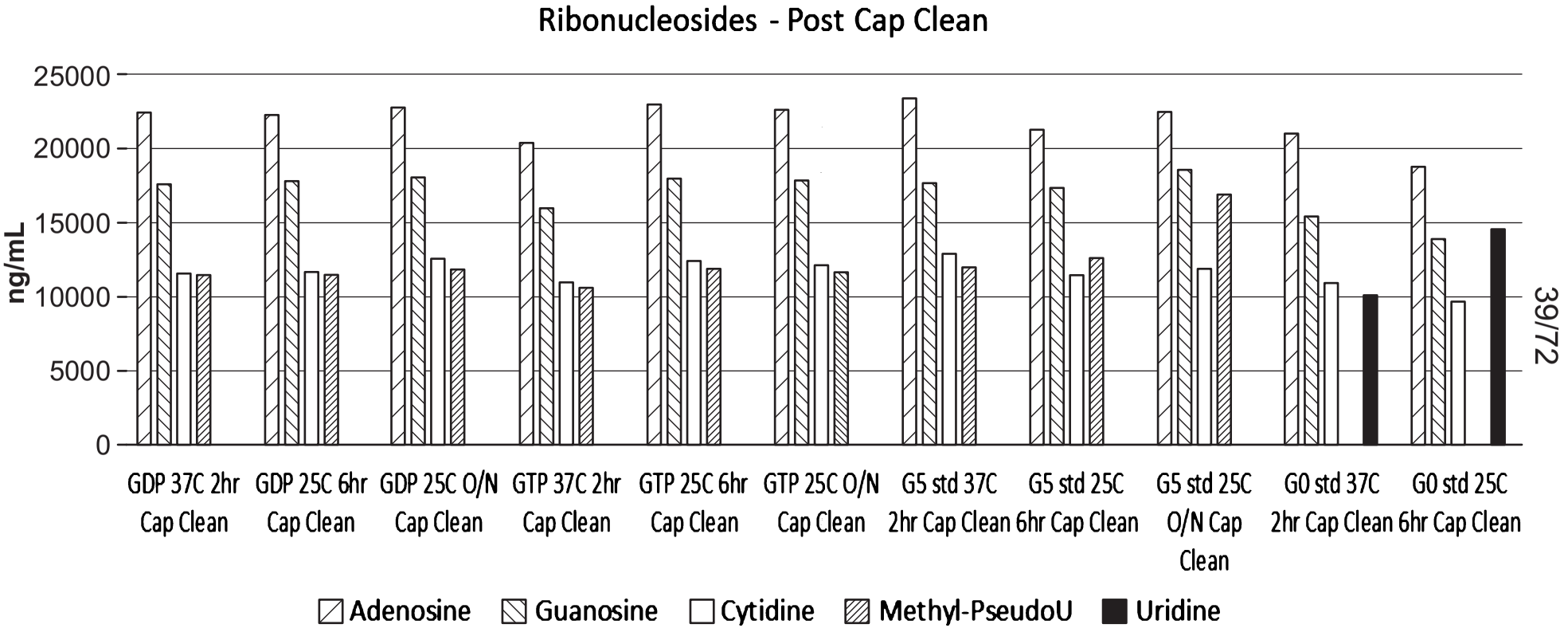

Ribonucleosides - Post Cap Clean
25000
20000
15000
10000
5000
0
ng/mL
GDP 37C 2hr Cap Clean
GDP 25C 6hr Cap Clean
GDP 25C O/N Cap Clean
GTP 37C 2hr Cap Clean
GTP 25C 6hr Cap Clean
GTP 25C O/N Cap Clean
G5 std 37C 2hr Cap Clean
G5 std 25C 6hr Cap Clean
G5 std 25C O/N Cap Clean
G0 std 37C 2hr Cap Clean
G0 std 25C 6hr Cap Clean
Adenosine
Guanosine
Cytidine
Methyl-PseudoU
Uridine

Figure 16A

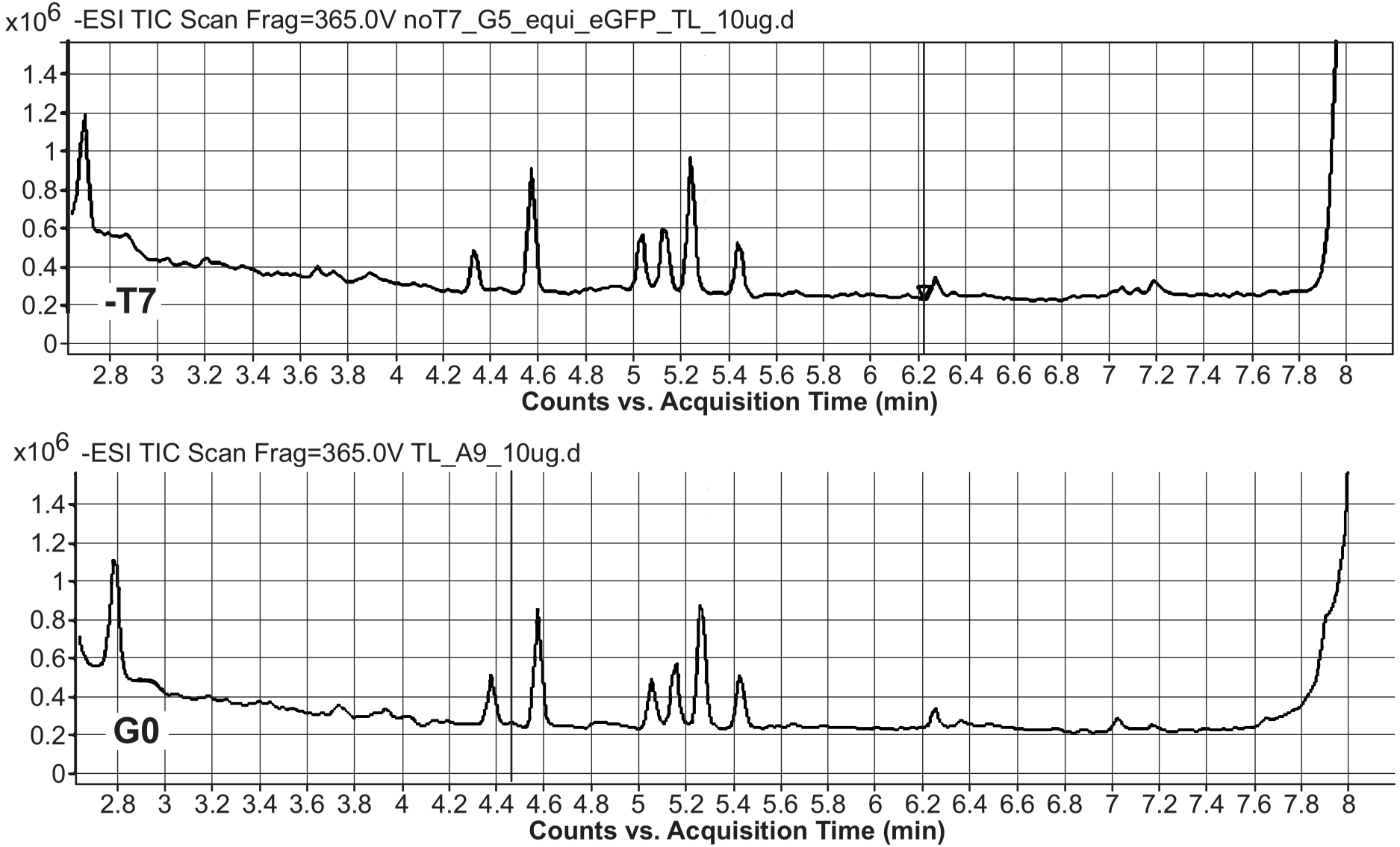

x10^6 -ESI TIC Scan Frag=365.0V noT7_G5_equi_eGFP_TL_10ug.d
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-T7
2.8 3 3.2 3.4 3.6 3.8 4 4.2 4.4 4.6 4.8 5 5.2 5.4 5.6 5.8 6 6.2 6.4 6.6 6.8 7 7.2 7.4 7.6 7.8 8
Counts vs. Acquisition Time (min)
x10^6 -ESI TIC Scan Frag=365.0V TL_A9_10ug.d
1.4
1.2
1
0.8
0.6
0.4
0.2
0
G0
2.8 3 3.2 3.4 3.6 3.8 4 4.2 4.4 4.6 4.8 5 5.2 5.4 5.6 5.8 6 6.2 6.4 6.6 6.8 7 7.2 7.4 7.6 7.8 8
Counts vs. Acquisition Time (min)

Figure 16A (continued)

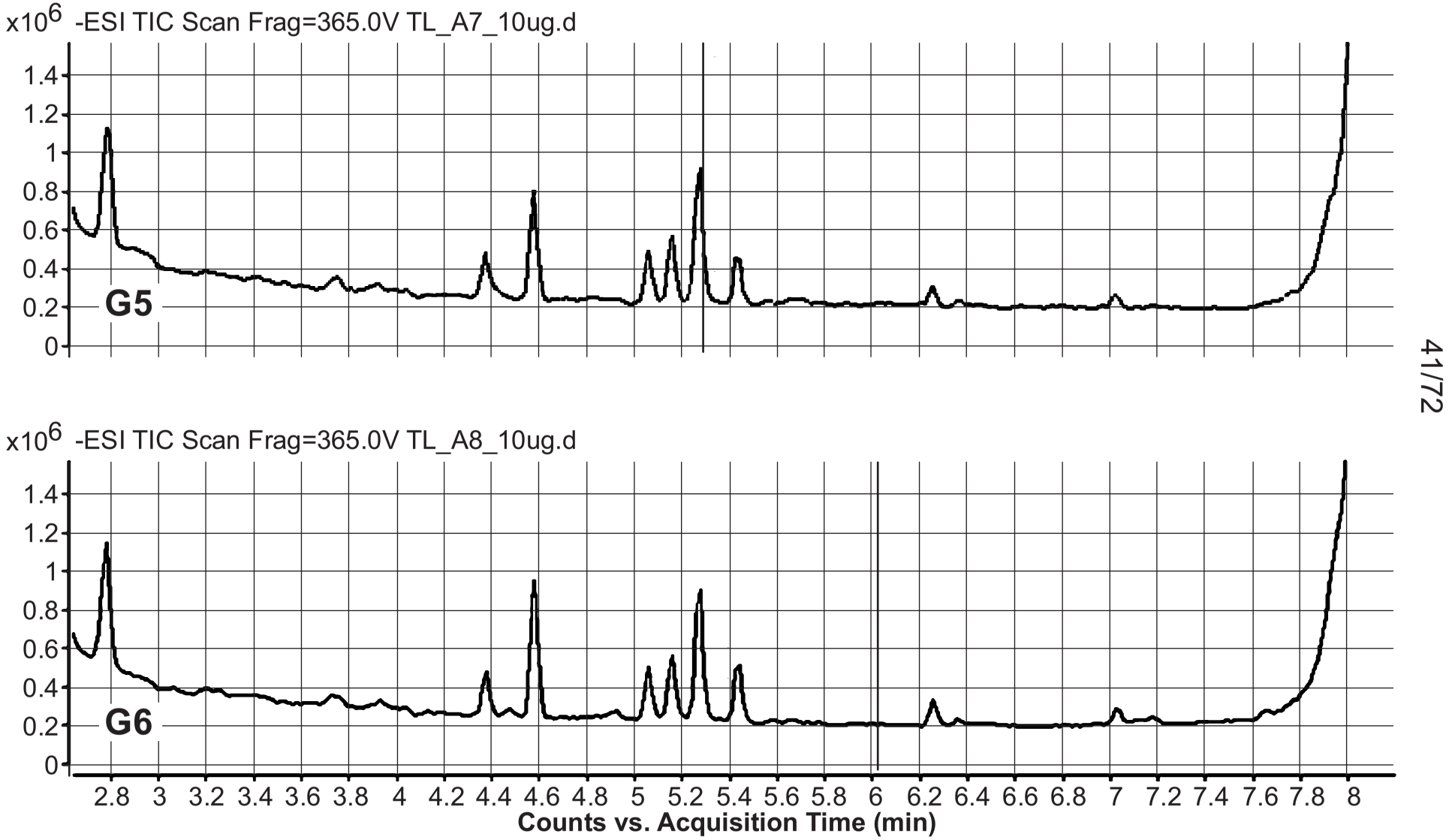
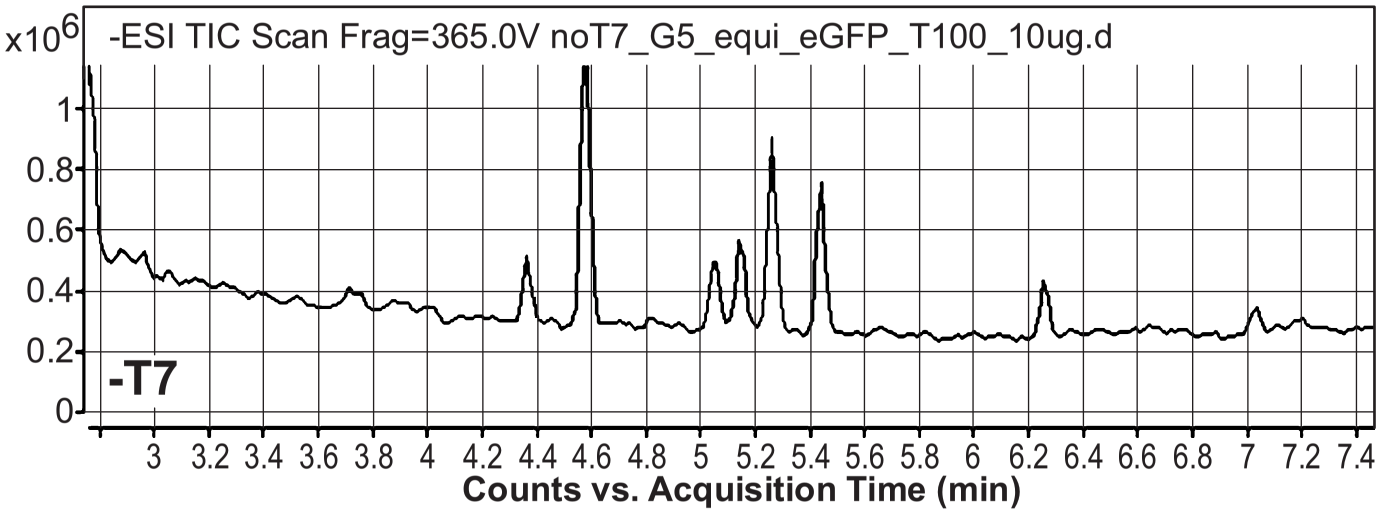
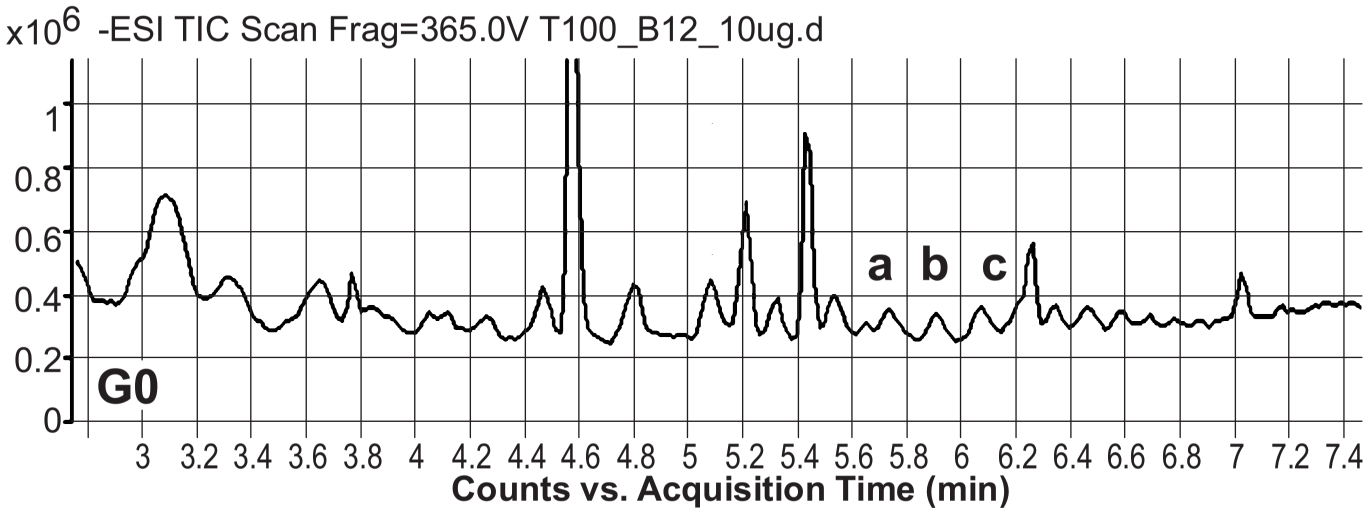


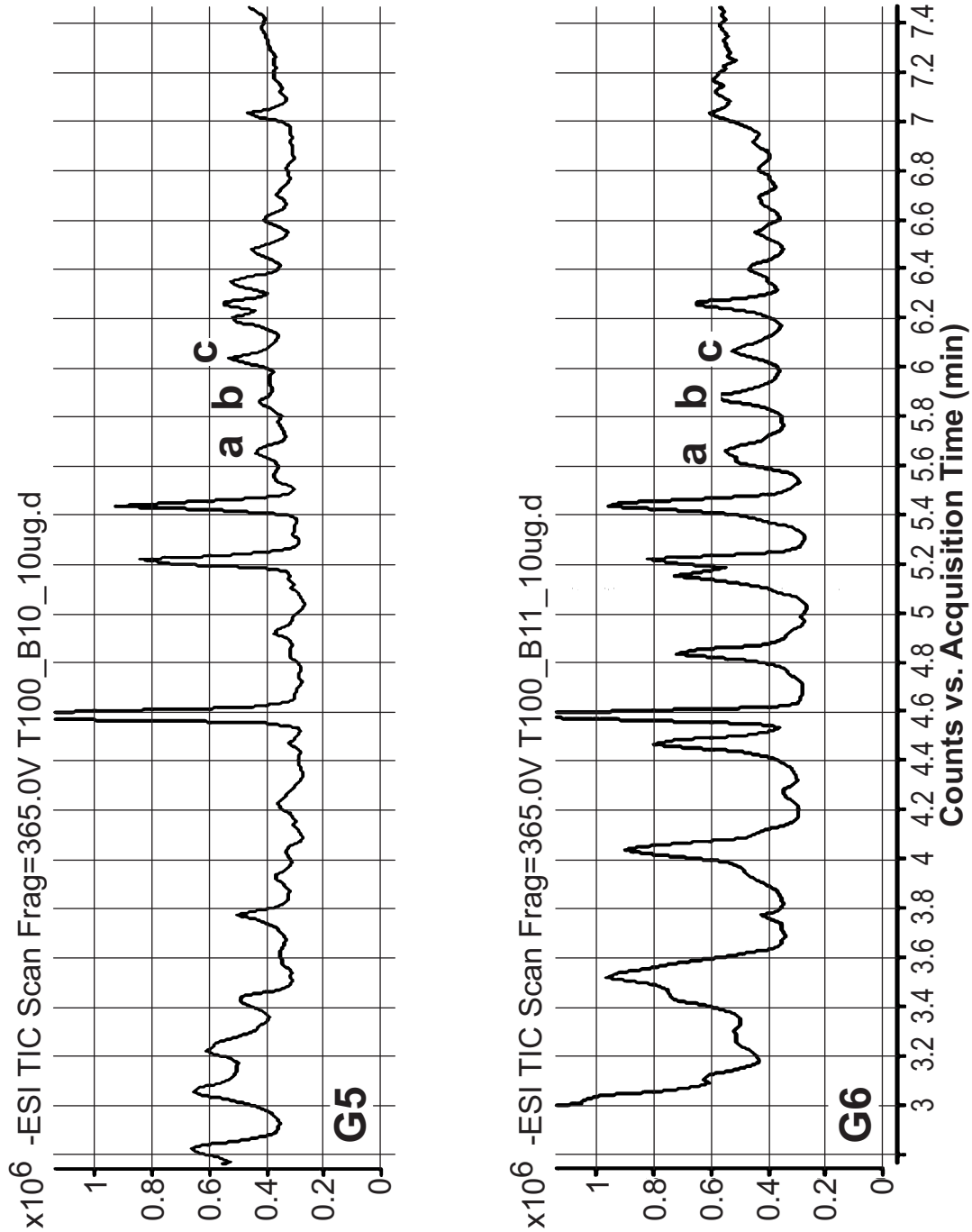
Figure 16B





	ID
-T7	None
G0 (a)	ppp-U_{13}-OH
G0 (b)	ppp-U_{14}-OH
G0 (c)	ppp-U_{15}-OH
G5 (a)	ppp-U_{12}-OH
G5 (b)	ppp-U_{13}-OH
G5 (c)	ppp-U_{14}-OH
G6 (a)	ppp-U_{11}-OH
G6 (b)	ppp-U_{12}-OH
G6 (c)	ppp-U_{13}-OH

Figure 16B (continued)



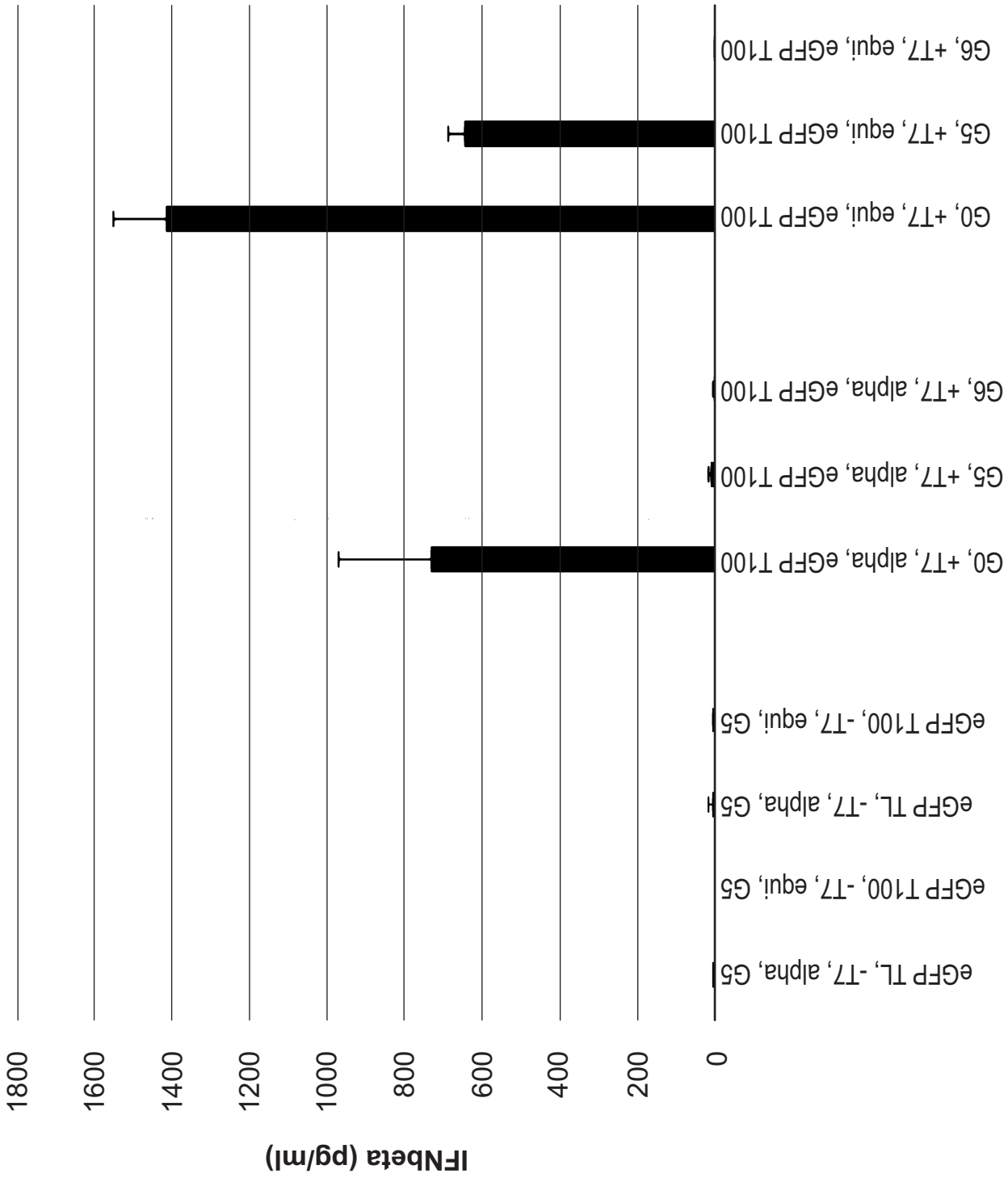

IFNbeta (pg/ml)
0
200
400
600
800
1000
1200
1400
1600
1800
eGFP TL, -T7, alpha, G5
eGFP T100, -T7, equi, G5
eGFP TL, -T7, alpha, G5
eGFP T100, -T7, equi, G5
G0, +T7, alpha, eGFP T100
G5, +T7, alpha, eGFP T100
G6, +T7, alpha, eGFP T100
G0, +T7, equi, eGFP T100
G5, +T7, equi, eGFP T100
G6, +T7, equi, eGFP T100

Figure 17

Figure 18

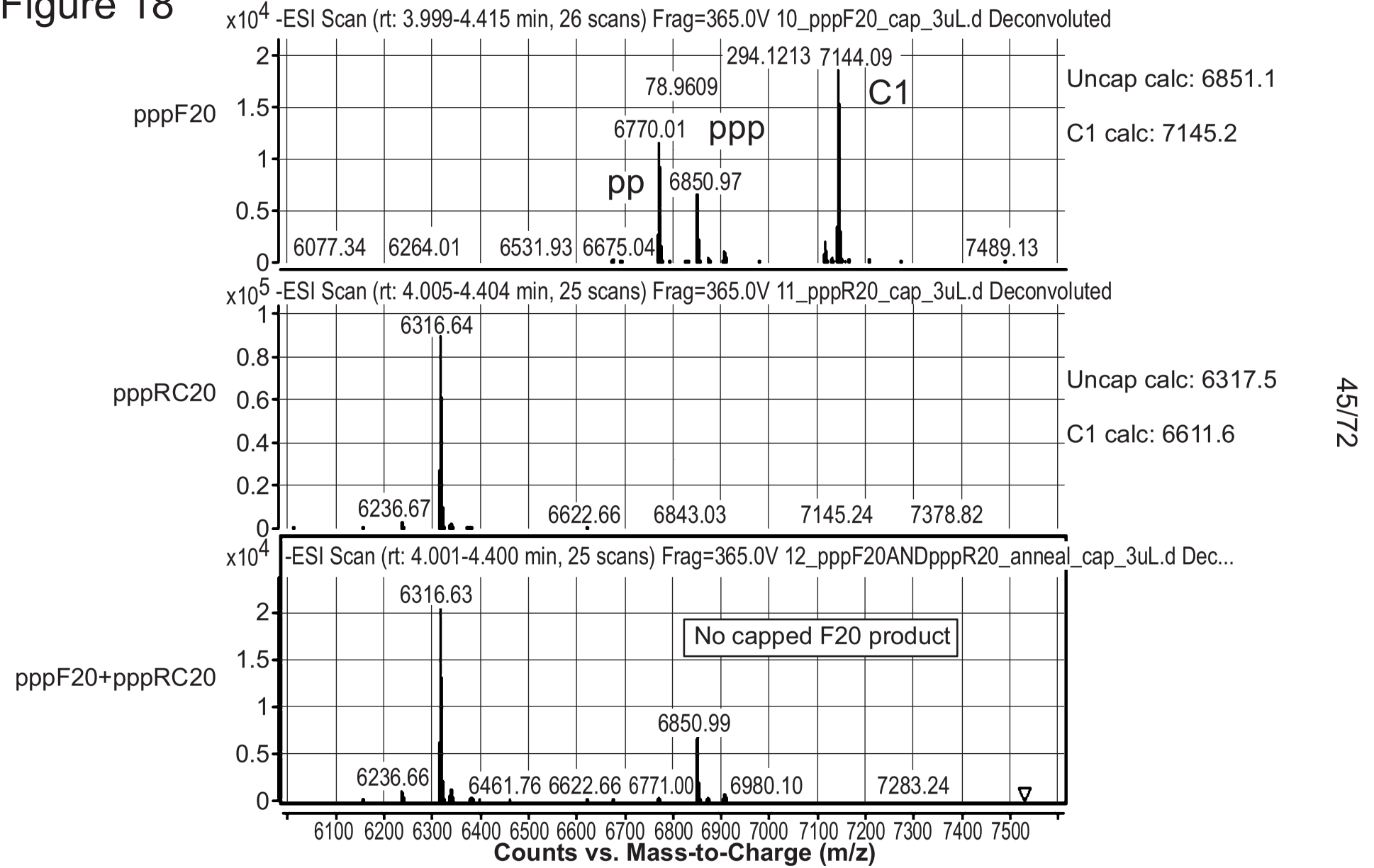

x10^4 -ESI Scan (rt: 3.999-4.415 min, 26 scans) Frag=365.0V 10_pppF20_cap_3uL.d Deconvoluted
pppF20
294.1213
7144.09
78.9609
C1
Uncap calc: 6851.1
C1 calc: 7145.2
6770.01
ppp
pp
6850.97
6077.34
6264.01
6531.93
6675.04
7489.13
x10^5 -ESI Scan (rt: 4.005-4.404 min, 25 scans) Frag=365.0V 11_pppR20_cap_3uL.d Deconvoluted
pppRC20
6316.64
Uncap calc: 6317.5
C1 calc: 6611.6
6236.67
6622.66
6843.03
7145.24
7378.82
x10^4 -ESI Scan (rt: 4.001-4.400 min, 25 scans) Frag=365.0V 12_pppF20ANDpppR20_anneal_cap_3uL.d Dec...
pppF20+pppRC20
6316.63
No capped F20 product
6850.99
6236.66
6461.76
6622.66
6771.00
6980.10
7283.24
6100 6200 6300 6400 6500 6600 6700 6800 6900 7000 7100 7200 7300 7400 7500
Counts vs. Mass-to-Charge (m/z)

Figure 19

ppp-R30 + OH-F20

OH-GGGAAAUAAGAGAGAAAAGA

CCCUUUAUUCUCUCUUUUCUUCUCAUUCUU-ppp

SEQ ID NO: 2

ppp-R30 + OH-F25

OH-GGGAAAUAAGAGAGAAAAGAAGAGU

CCCUUUAUUCUCUCUUUUCUUCUCAUUCUU-ppp

SEQ ID NO: 3

ppp-R30 + OH-F30

OH-GGGAAAUAAGAGAGAAAAGAAGAGUAAGAA

CCCUUUAUUCUCUCUUUUCUUCUCAUUCUU-ppp

SEQ ID NO: 4

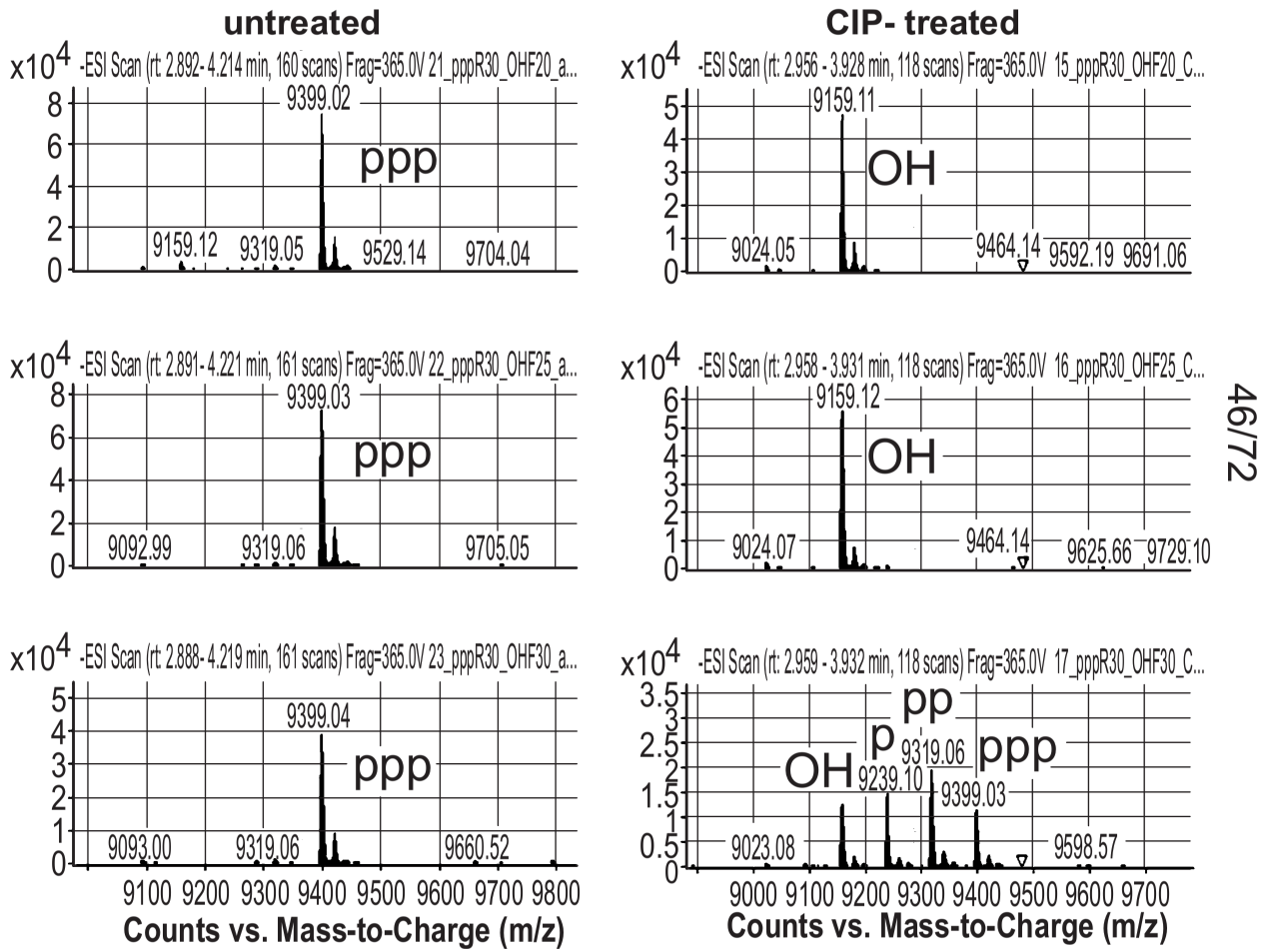


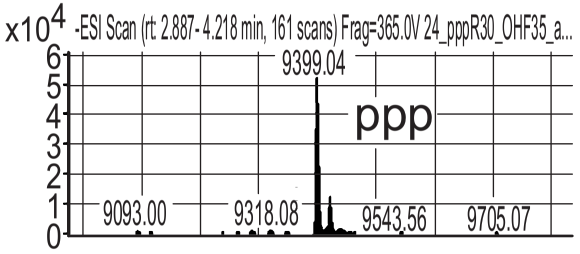
Figure 19 (continued)

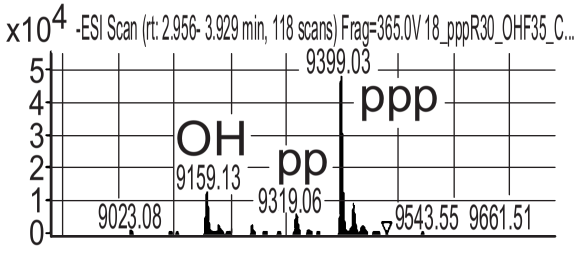
ppp-R30 + OH-F35

OH-GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAU

CCCUUUAUUCUCUCUUUUCUUCUCAUUCUU-ppp

SEQ ID NO: 5



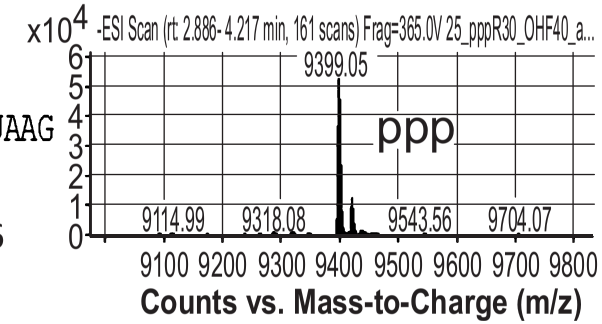


ppp-R30 + OH-F40

OH-GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAG

CCCUUUAUUCUCUCUUUUCUUCUCAUUCUU-ppp

SEQ ID NO: 6



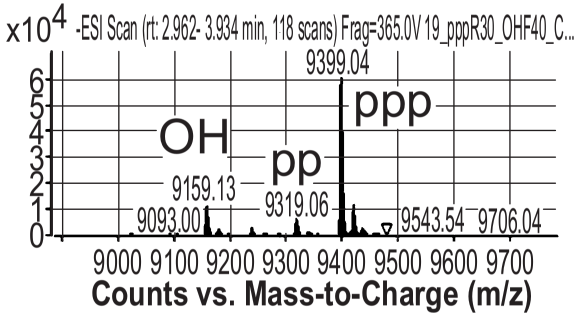
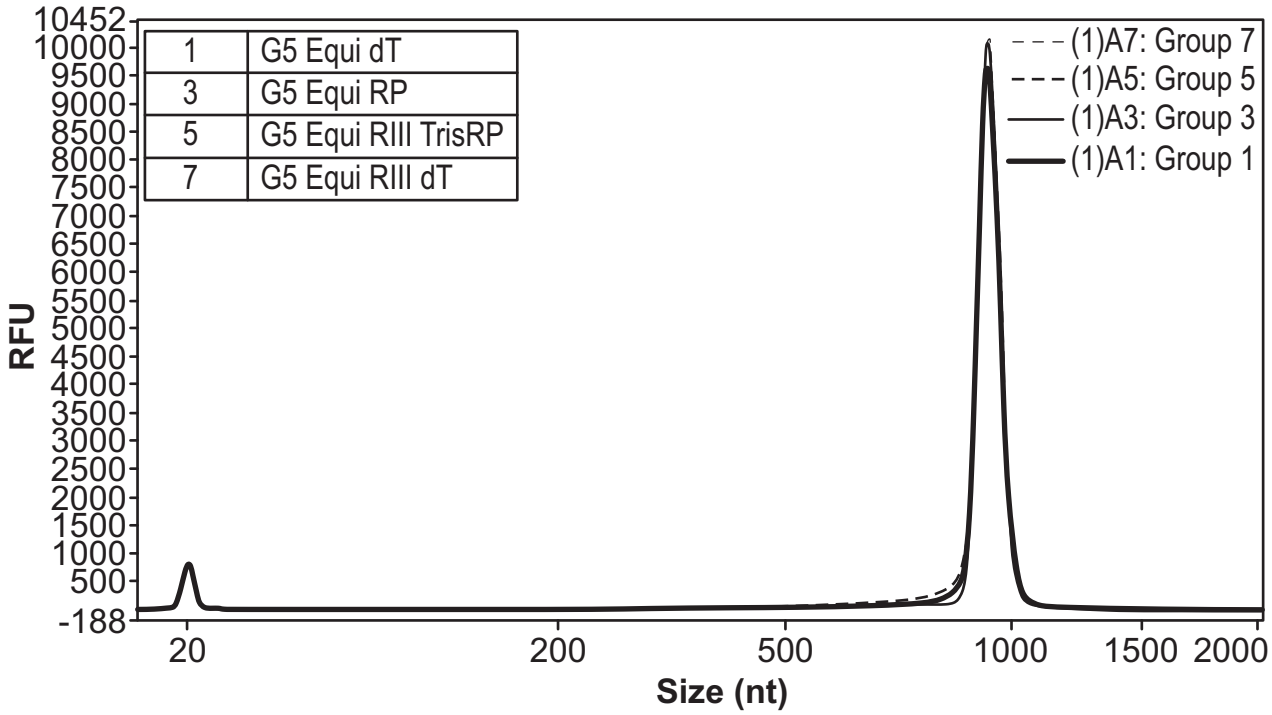


Figure 20


10452
10000
9500
9000
8500
8000
7500
7000
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
-188
RFU
20
200
500
1000
1500
2000
Size (nt)
1 G5 Equi dT
3 G5 Equi RP
5 G5 Equi RIII TrisRP
7 G5 Equi RIII dT
(1)A7: Group 7
(1)A5: Group 5
(1)A3: Group 3
(1)A1: Group 1

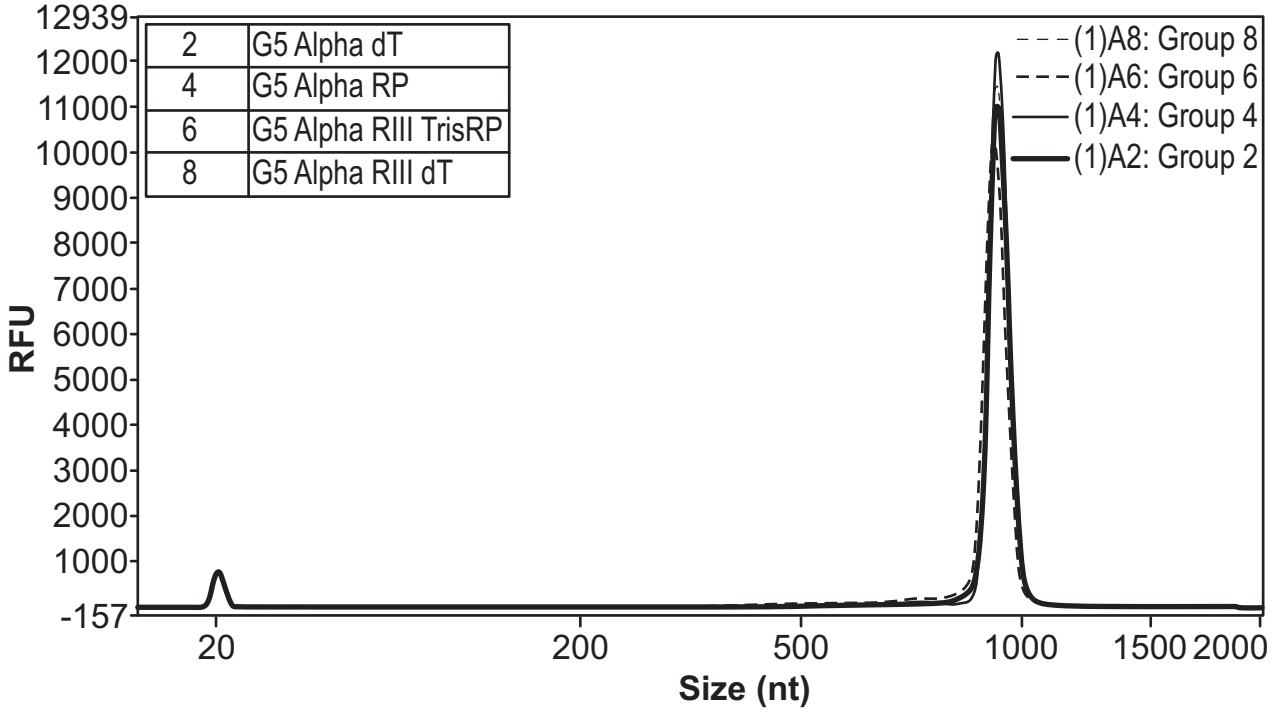
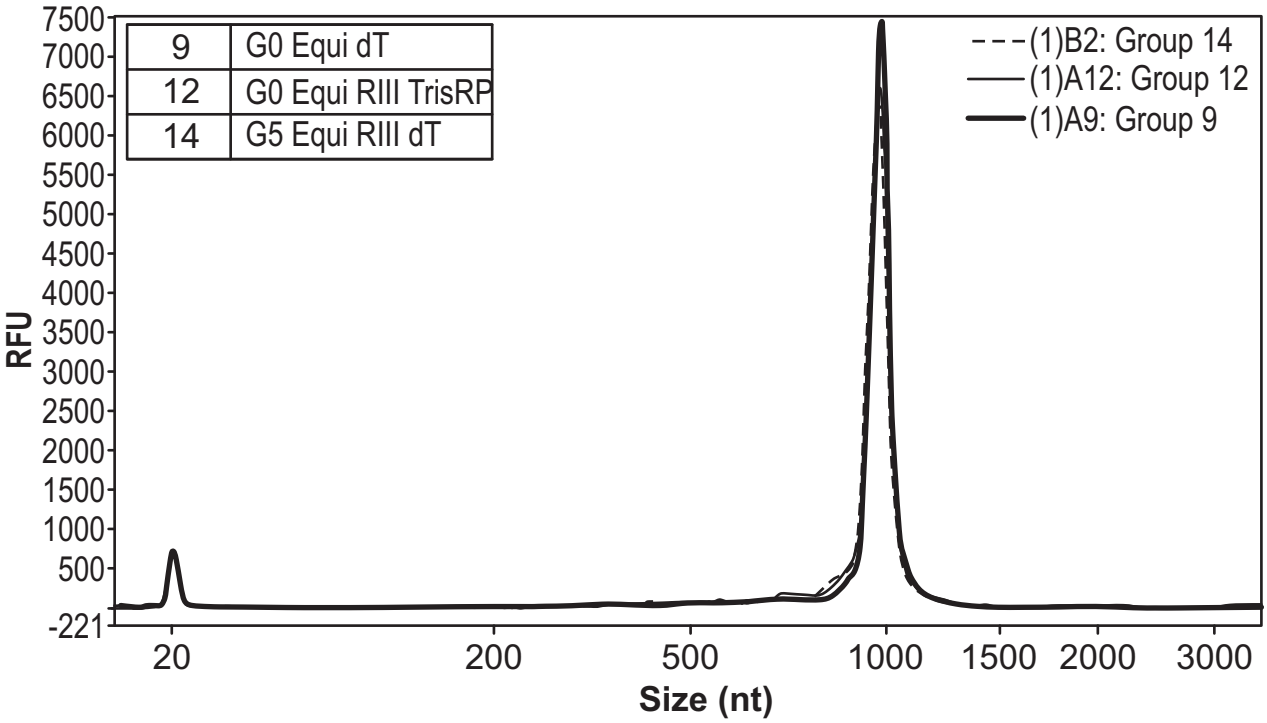

12939
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
-157
RFU
20
200
500
1000
1500
2000
Size (nt)
2 G5 Alpha dT
4 G5 Alpha RP
6 G5 Alpha RIII TrisRP
8 G5 Alpha RIII dT
(1)A8: Group 8
(1)A6: Group 6
(1)A4: Group 4
(1)A2: Group 2

Figure 20 (continued)



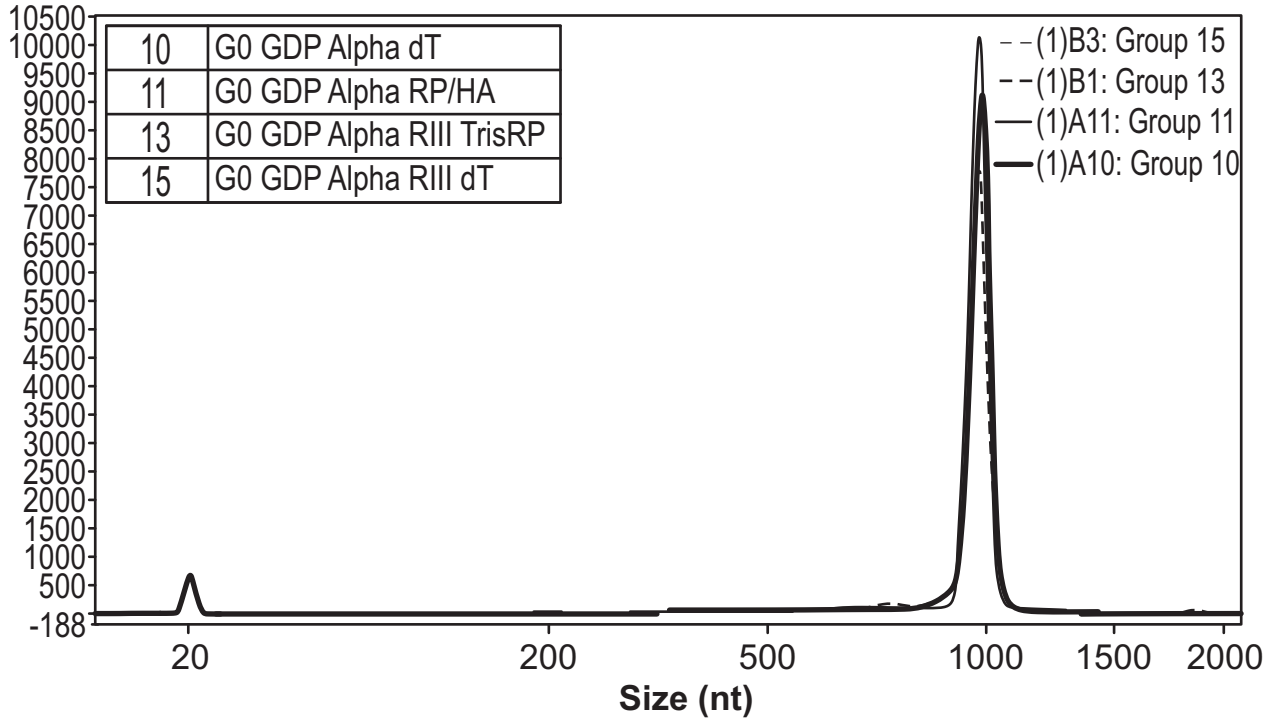


Figure 21

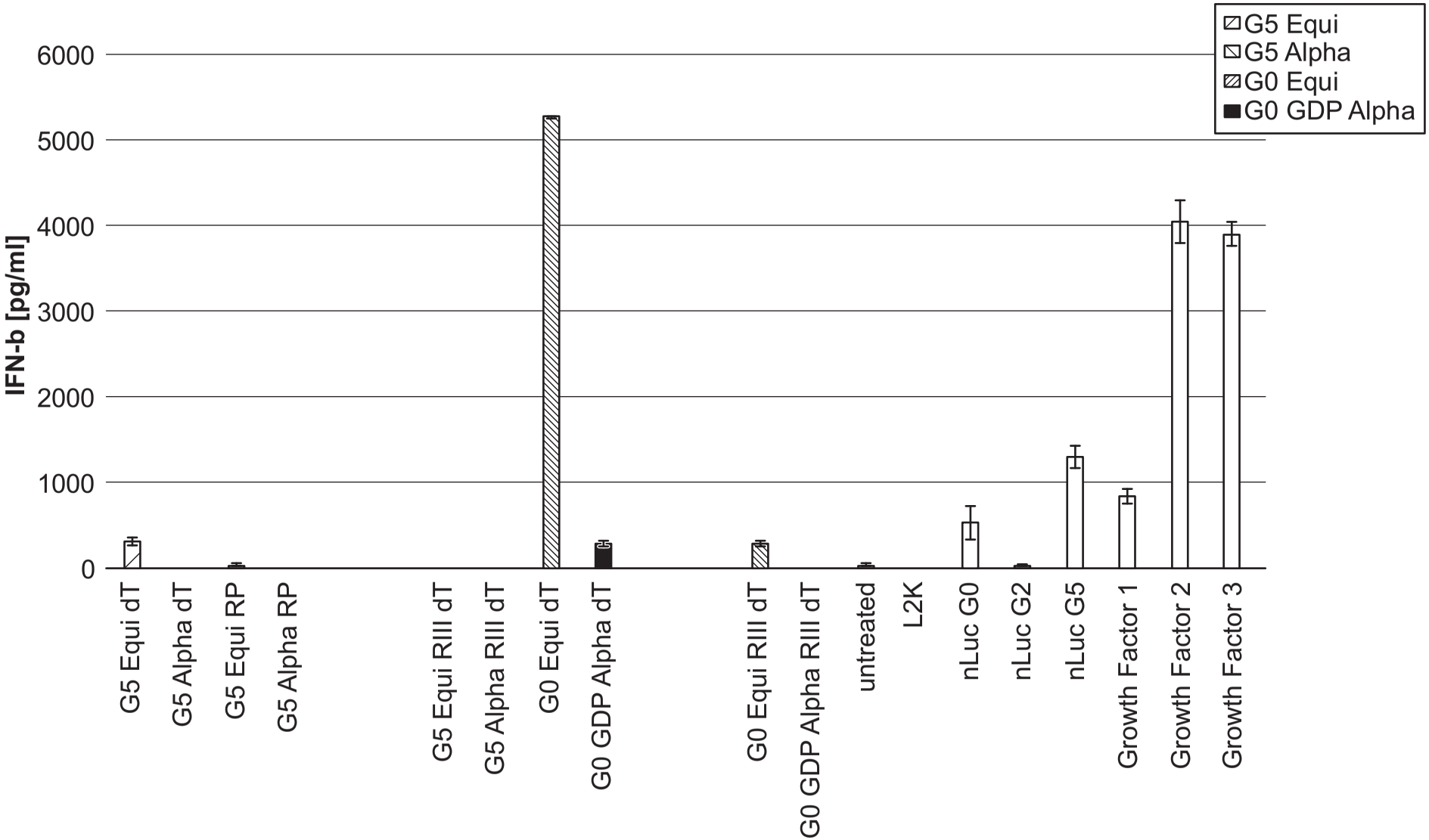

G5 Equi
G5 Alpha
G0 Equi
G0 GDP Alpha
IFN-b [pg/ml]
6000
5000
4000
3000
2000
1000
0
G5 Equi dT
G5 Alpha dT
G5 Equi RP
G5 Alpha RP
G5 Equi RIII dT
G5 Alpha RIII dT
G0 Equi dT
G0 GDP Alpha dT
G0 Equi RIII dT
G0 GDP Alpha RIII dT
untreated
L2K
nLuc G0
nLuc G2
nLuc G5
Growth Factor 1
Growth Factor 2
Growth Factor 3

Figure 22

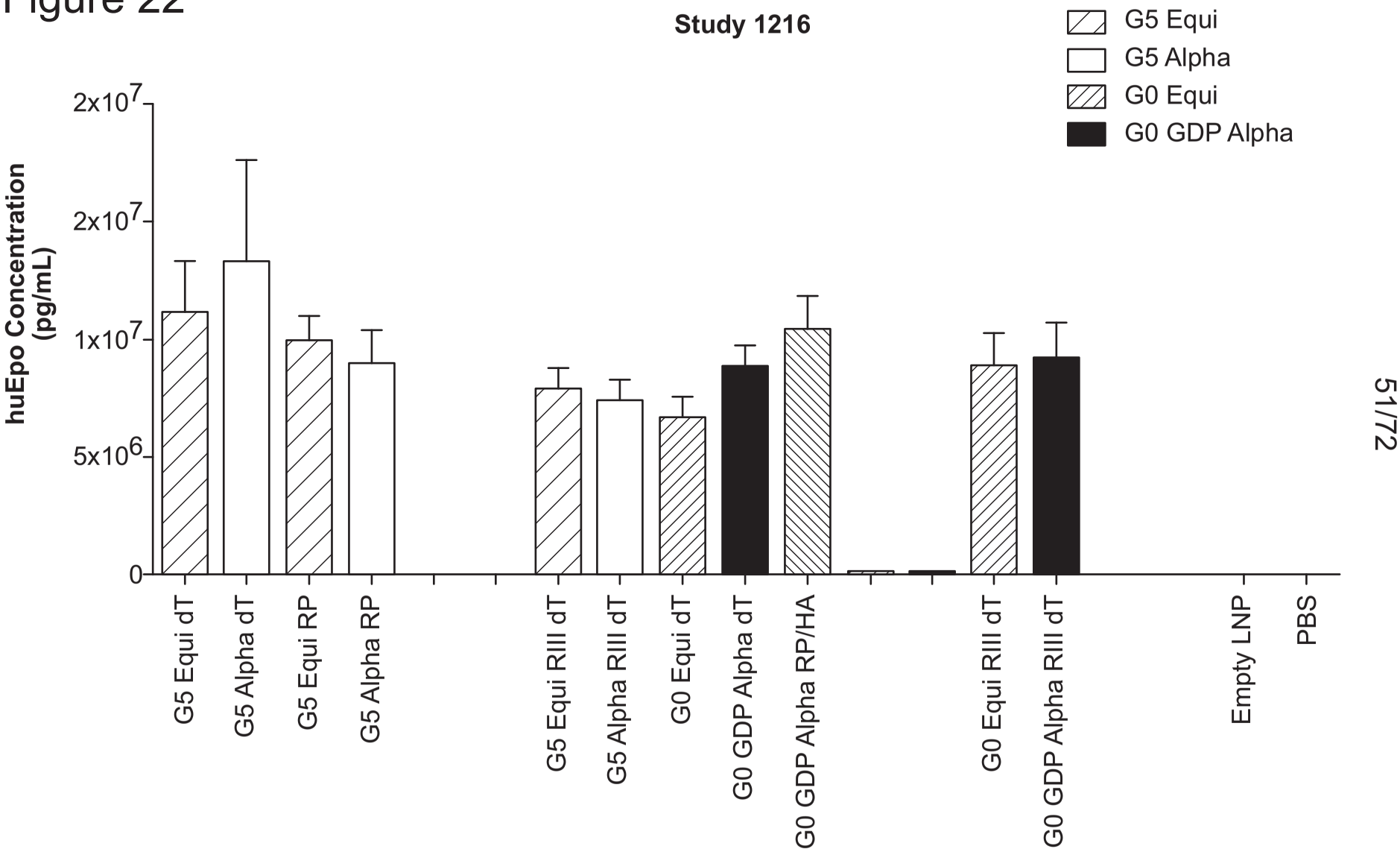

Study 1216
G5 Equi
G5 Alpha
G0 Equi
G0 GDP Alpha
huEpo Concentration (pg/mL)
2x10^7
2x10^7
1x10^7
5x10^6
0
G5 Equi dT
G5 Alpha dT
G5 Equi RP
G5 Alpha RP
G5 Equi RIII dT
G5 Alpha RIII dT
G0 Equi dT
G0 GDP Alpha dT
G0 GDP Alpha RP/HA
G0 Equi RIII dT
G0 GDP Alpha RIII dT
Empty LNP
PBS

Figure 23A

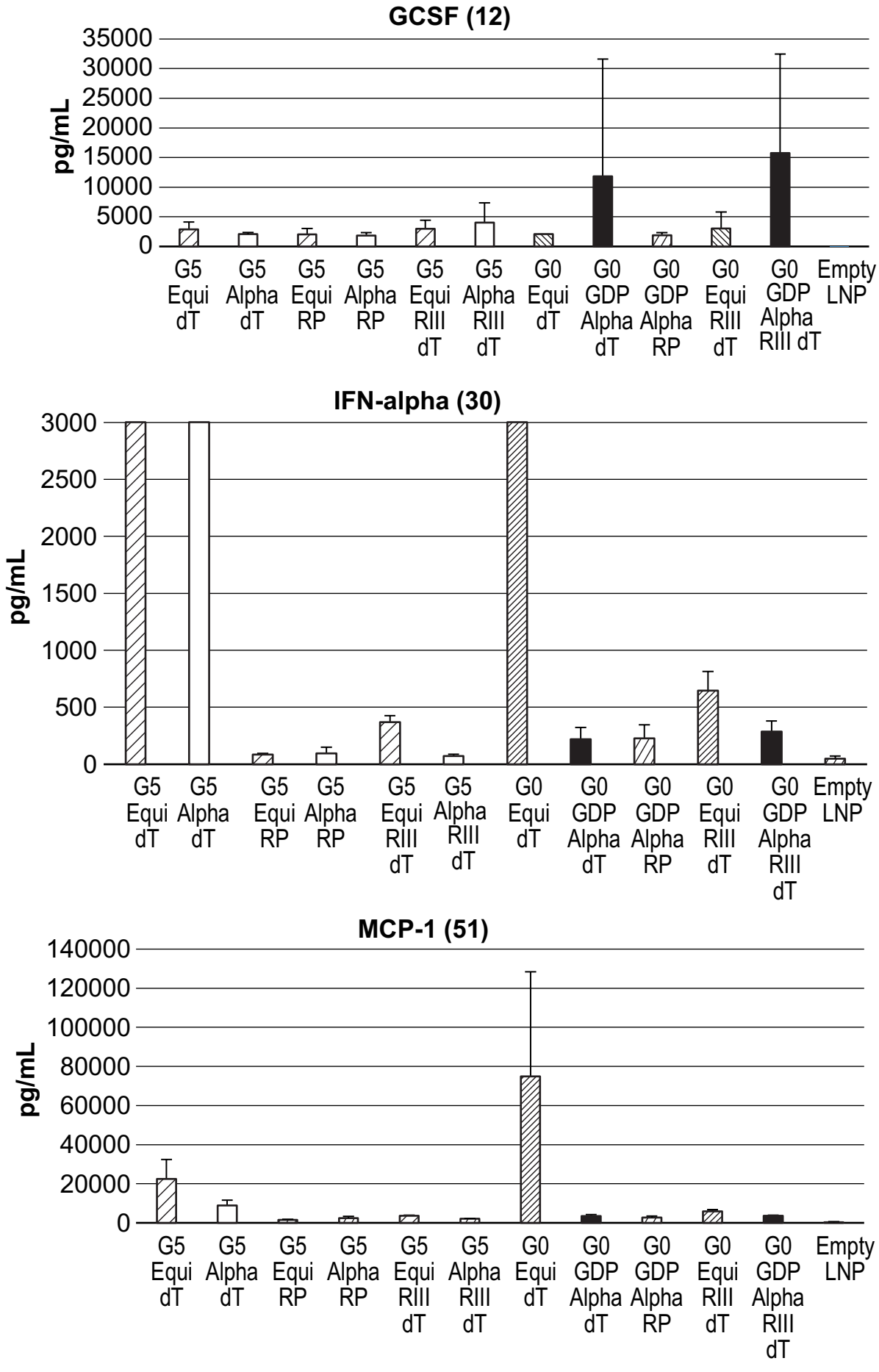

GCSF (12)
pg/mL
35000
30000
25000
20000
15000
10000
5000
0
G5 Equi dT
G5 Alpha dT
G5 Equi RP
G5 Alpha RP
G5 Equi RIII dT
G5 Alpha RIII dT
G0 Equi dT
G0 GDP Alpha dT
G0 GDP Alpha RP
G0 Equi RIII dT
G0 GDP Alpha RIII dT
Empty LNP
IFN-alpha (30)
pg/mL
3000
2500
2000
1500
1000
500
0
G5 Equi dT
G5 Alpha dT
G5 Equi RP
G5 Alpha RP
G5 Equi RIII dT
G5 Alpha RIII dT
G0 Equi dT
G0 GDP Alpha dT
G0 GDP Alpha RP
G0 Equi RIII dT
G0 GDP Alpha RIII dT
Empty LNP
MCP-1 (51)
pg/mL
140000
120000
100000
80000
60000
40000
20000
0
G5 Equi dT
G5 Alpha dT
G5 Equi RP
G5 Alpha RP
G5 Equi RIII dT
G5 Alpha RIII dT
G0 Equi dT
G0 GDP Alpha dT
G0 GDP Alpha RP
G0 Equi RIII dT
G0 GDP Alpha RIII dT
Empty LNP

Figure 23B

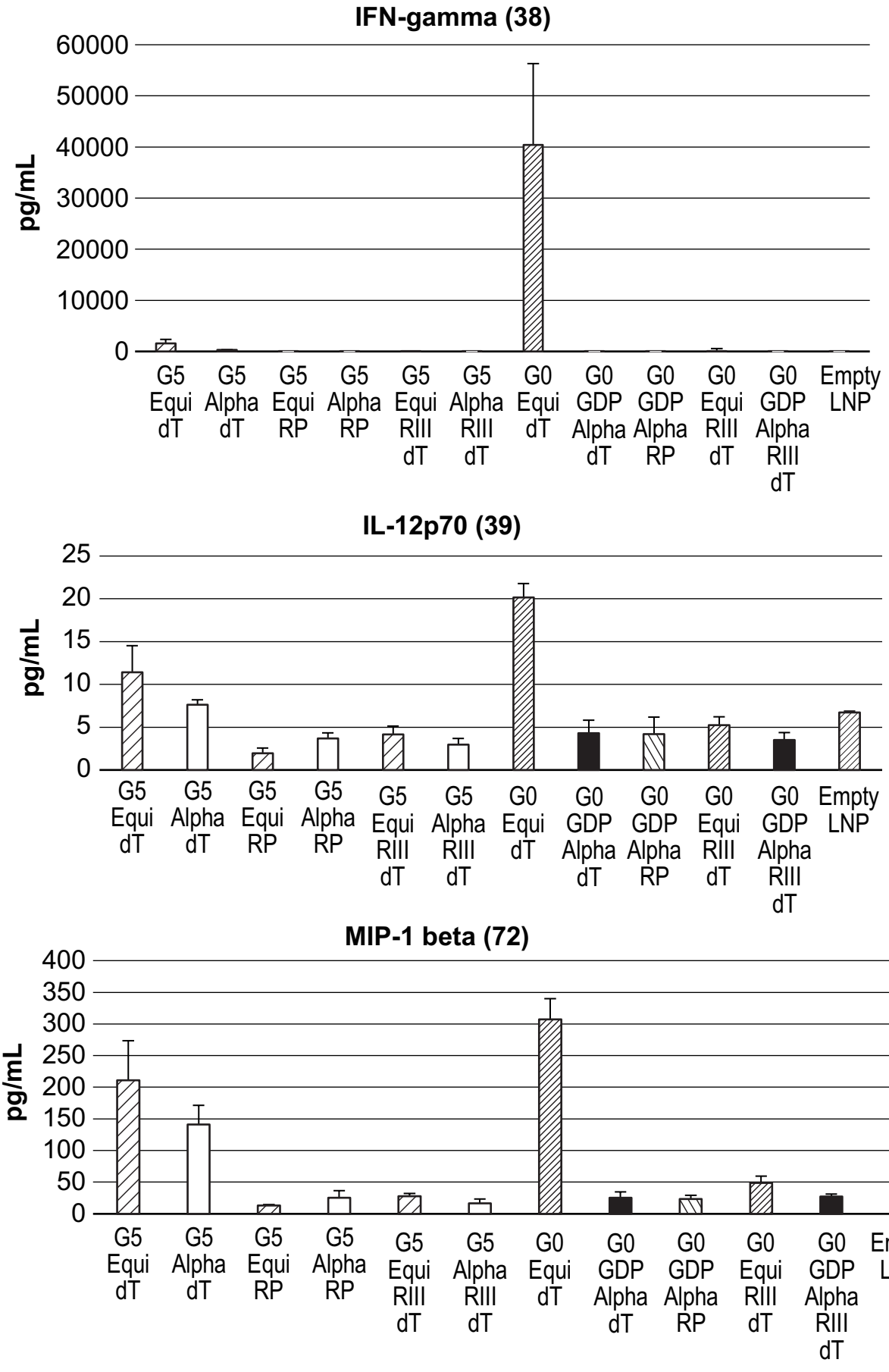

IFN-gamma (38)
pg/mL
60000
50000
40000
30000
20000
10000
0
G5 Equi dT
G5 Alpha dT
G5 Equi RP
G5 Alpha RP
G5 Equi RIII dT
G5 Alpha RIII dT
G0 Equi dT
G0 GDP Alpha dT
G0 GDP Alpha RP
G0 Equi RIII dT
G0 GDP Alpha RIII dT
Empty LNP
IL-12p70 (39)
pg/mL
25
20
15
10
5
0
G5 Equi dT
G5 Alpha dT
G5 Equi RP
G5 Alpha RP
G5 Equi RIII dT
G5 Alpha RIII dT
G0 Equi dT
G0 GDP Alpha dT
G0 GDP Alpha RP
G0 Equi RIII dT
G0 GDP Alpha RIII dT
Empty LNP
MIP-1 beta (72)
pg/mL
400
350
300
250
200
150
100
50
0
G5 Equi dT
G5 Alpha dT
G5 Equi RP
G5 Alpha RP
G5 Equi RIII dT
G5 Alpha RIII dT
G0 Equi dT
G0 GDP Alpha dT
G0 GDP Alpha RP
G0 Equi RIII dT
G0 GDP Alpha RIII dT
Empty LNP

Figure 23C

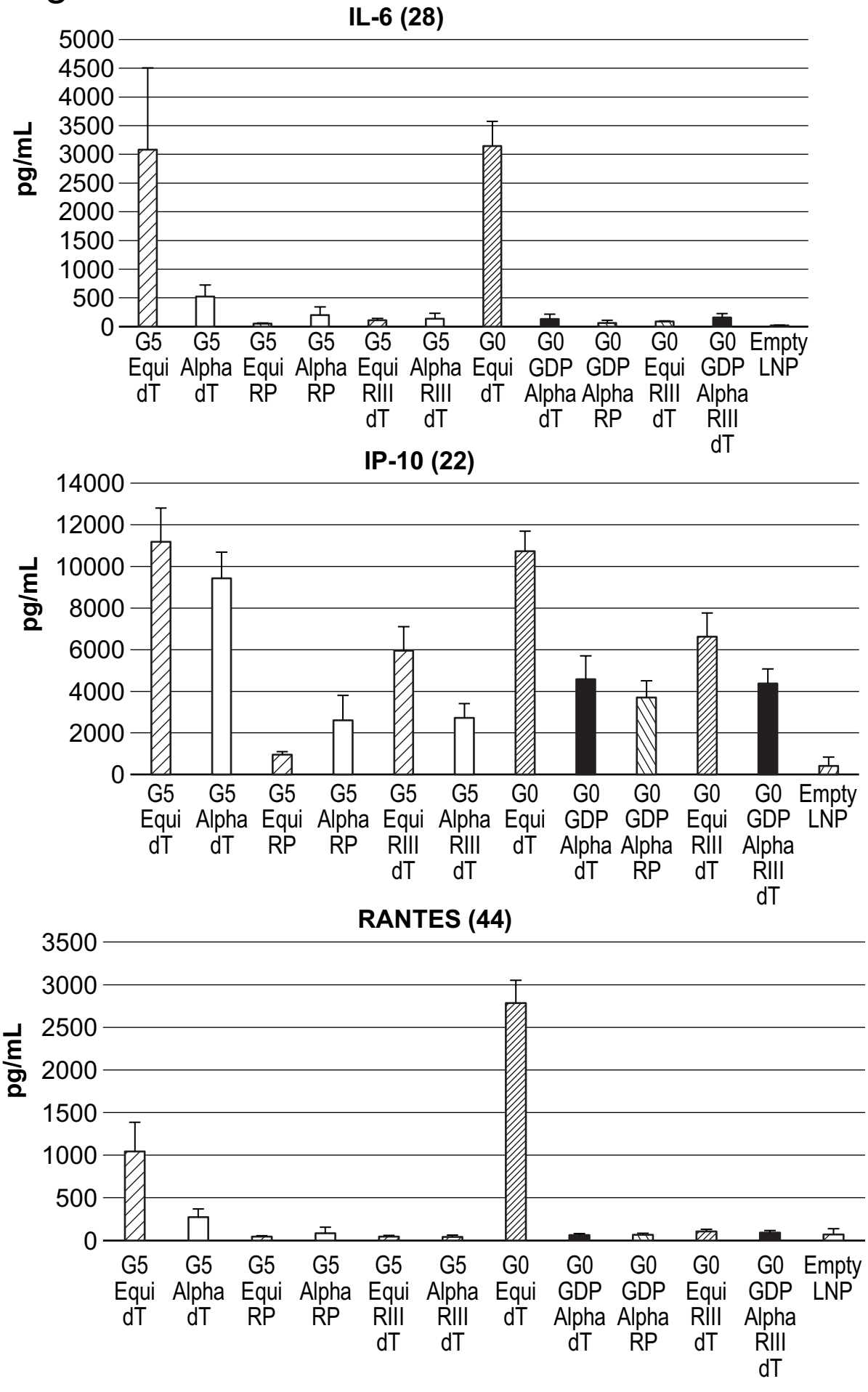

IL-6 (28)
pg/mL
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
G5 Equi dT
G5 Alpha dT
G5 Equi RP
G5 Alpha RP
G5 Equi RIII dT
G5 Alpha RIII dT
G0 Equi dT
G0 GDP Alpha dT
G0 GDP Alpha RP
G0 Equi RIII dT
G0 GDP Alpha RIII dT
Empty LNP
IP-10 (22)
pg/mL
14000
12000
10000
8000
6000
4000
2000
0
G5 Equi dT
G5 Alpha dT
G5 Equi RP
G5 Alpha RP
G5 Equi RIII dT
G5 Alpha RIII dT
G0 Equi dT
G0 GDP Alpha dT
G0 GDP Alpha RP
G0 Equi RIII dT
G0 GDP Alpha RIII dT
Empty LNP
RANTES (44)
pg/mL
3500
3000
2500
2000
1500
1000
500
0
G5 Equi dT
G5 Alpha dT
G5 Equi RP
G5 Alpha RP
G5 Equi RIII dT
G5 Alpha RIII dT
G0 Equi dT
G0 GDP Alpha dT
G0 GDP Alpha RP
G0 Equi RIII dT
G0 GDP Alpha RIII dT
Empty LNP

Figure 23D

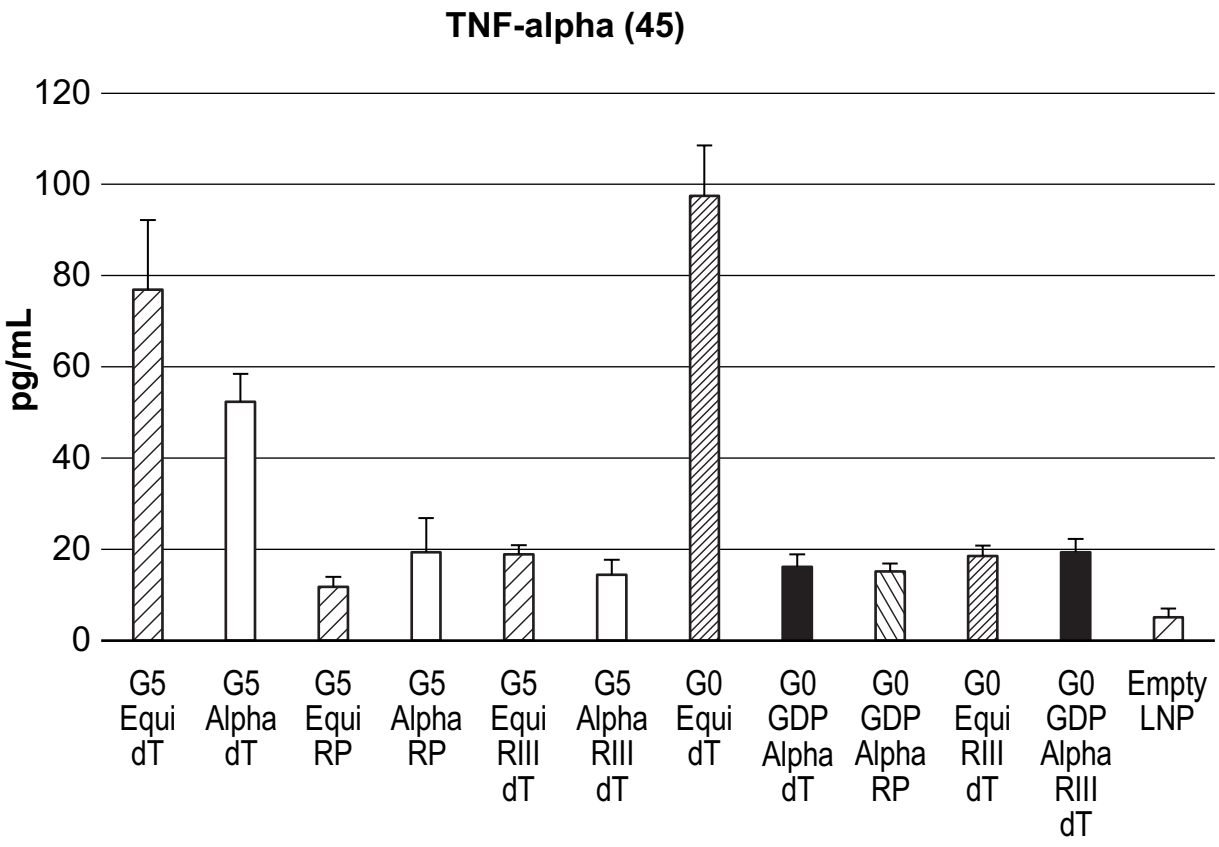

TNF-alpha (45)
pg/mL
120
100
80
60
40
20
0
G5 Equi dT
G5 Alpha dT
G5 Equi RP
G5 Alpha RP
G5 Equi RIII dT
G5 Alpha RIII dT
G0 Equi dT
G0 GDP Alpha dT
G0 GDP Alpha RP
G0 Equi RIII dT
G0 GDP Alpha RIII dT
Empty LNP

Figure 24

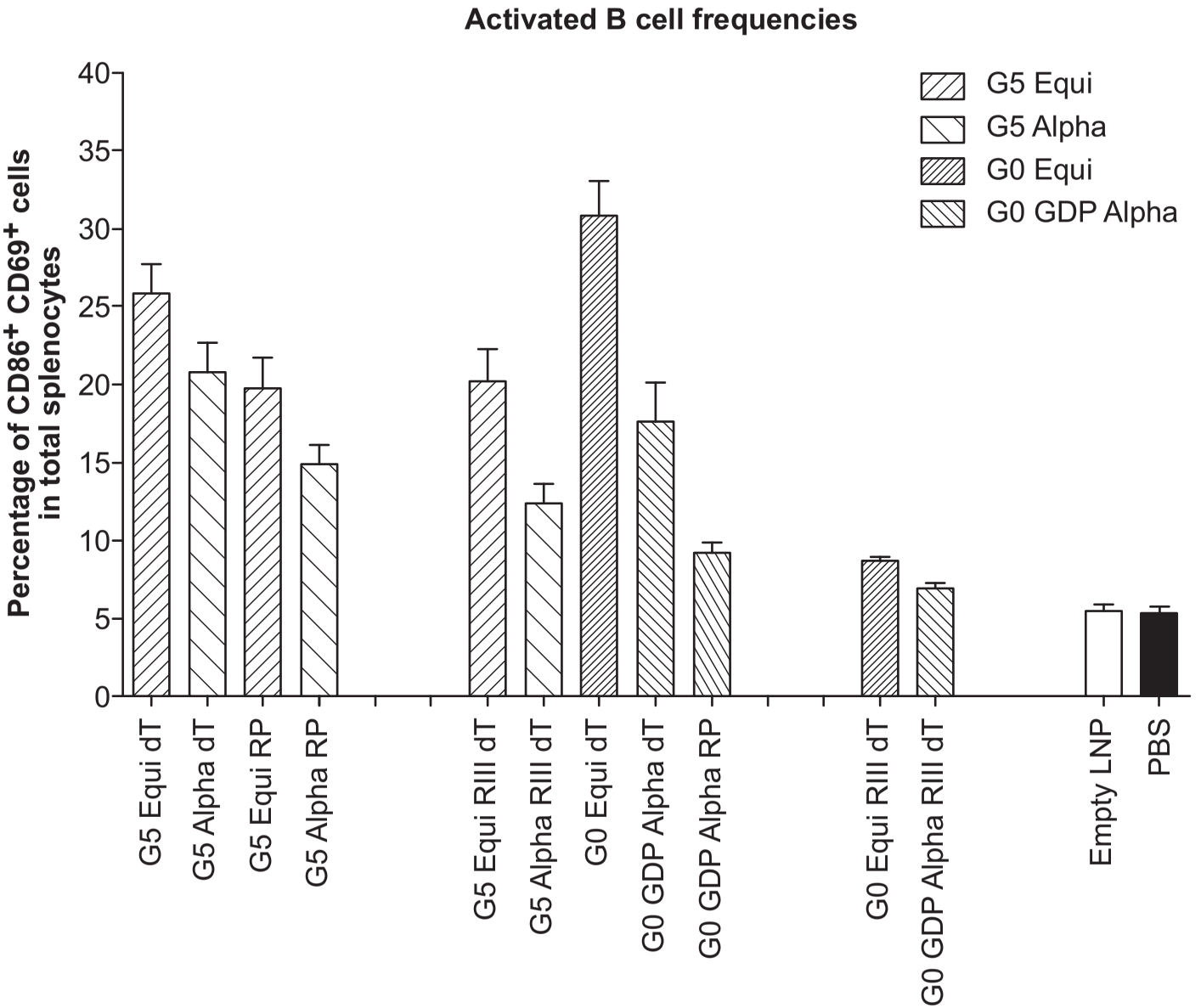

Activated B cell frequencies
Percentage of CD86+ CD69+ cells in total splenocytes
0
5
10
15
20
25
30
35
40
G5 Equi
G5 Alpha
G0 Equi
G0 GDP Alpha
G5 Equi dT
G5 Alpha dT
G5 Equi RP
G5 Alpha RP
G5 Equi RIII dT
G5 Alpha RIII dT
G0 Equi dT
G0 GDP Alpha dT
G0 GDP Alpha RP
G0 Equi RIII dT
G0 GDP Alpha RIII dT
Empty LNP
PBS

Figure 25

ppp-F8	ppp-GGGAAAUA	
ppp-F12	ppp-GGGAAAUAAGAG	SEQ ID NO: 7
ppp-RC8	ppp-UAUUUCCC	
ppp-RC12	ppp-CUCUUAUUUCCC	SEQ ID NO: 8
ppp-F8 + pppRC8	ppp-GGGAAAUA CCCUUUAU-ppp	SEQ ID NO: 9
ppp-F12 + pppRC12	ppp-GGGAAAUAAGAG CCCUUUAUUCUC-ppp	SEQ ID NO: 10
ppp-F8 + pppRC12	ppp-GGGAAAUA CCCUUUAUUCUC-ppp	SEQ ID NO: 11
ppp-F12 + pppRC8	ppp-GGGAAAUAAGAG CCCUUUAU-ppp	SEQ ID NO: 12
ppp-RC8 + hEPO	ppp-GGGAAAUAAGAGAAAAGAAGAGU...hEPO(850mer) CCCUUUAU-ppp	SEQ ID NO: 13
ppp-RC12 + hEPO	ppp-GGGAAAUAAGAGAAAAGAAGAGU...hEPO(850mer) CCCUUUAUUCUC-ppp	SEQ ID NO: 14

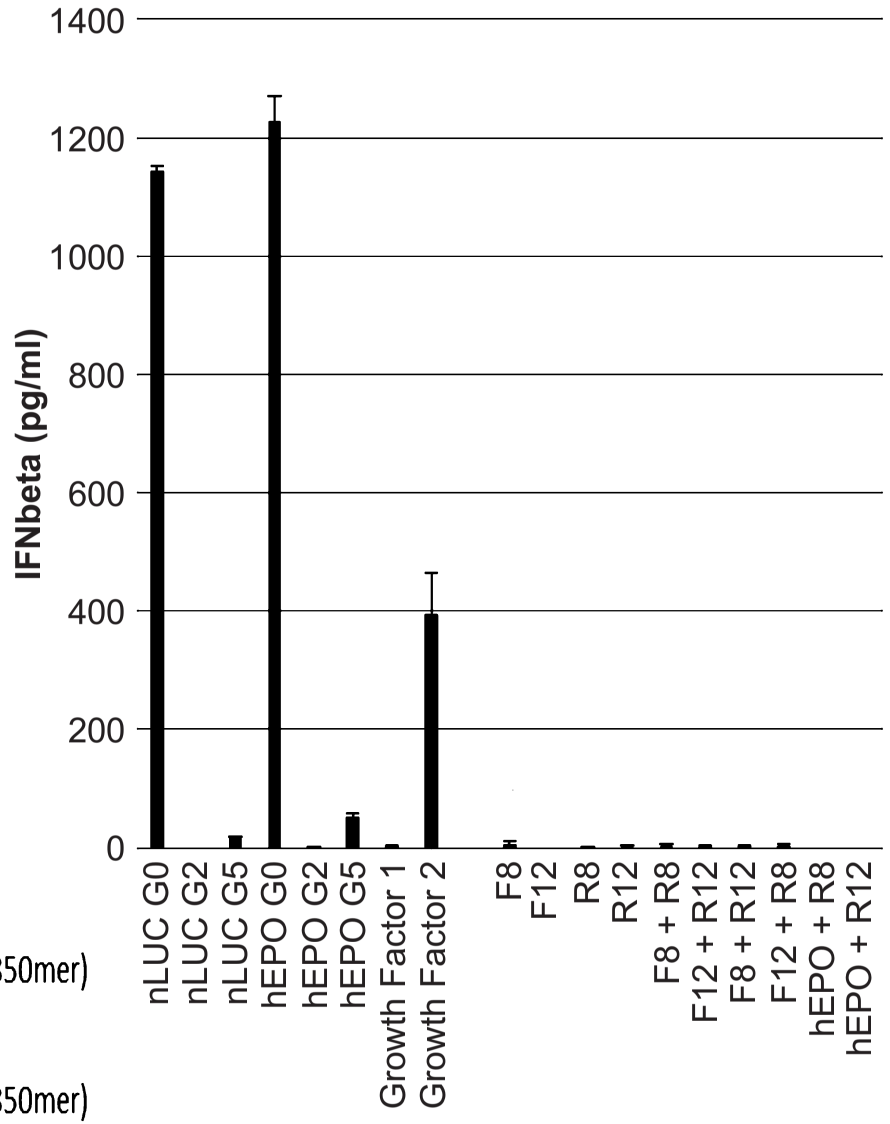


Figure 26

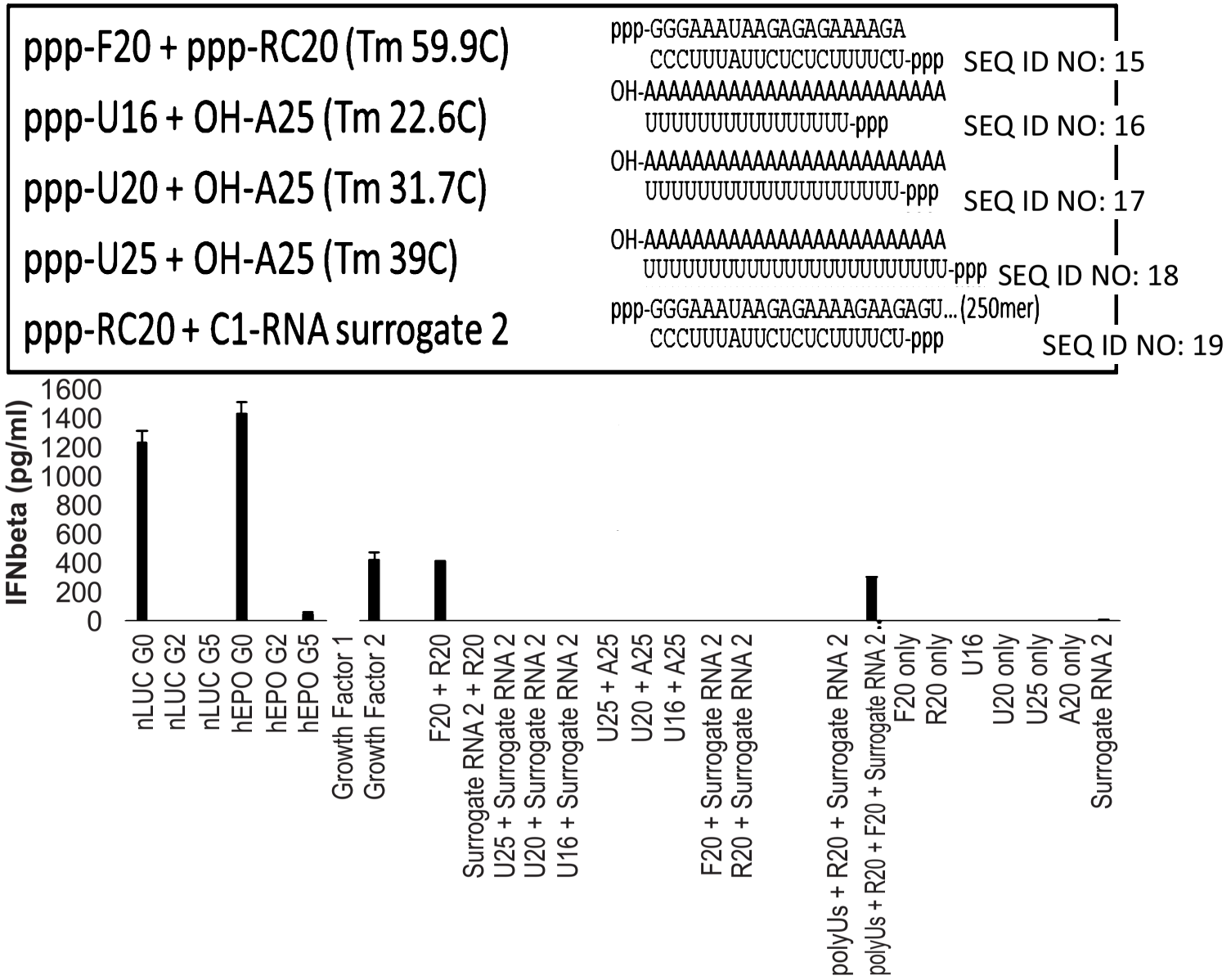

ppp-F20 + ppp-RC20 (Tm 59.9C)
ppp-U16 + OH-A25 (Tm 22.6C)
ppp-U20 + OH-A25 (Tm 31.7C)
ppp-U25 + OH-A25 (Tm 39C)
ppp-RC20 + C1-RNA surrogate 2
ppp-GGGAAAUAAGAGAGAAAAGA
CCCUUUAUUCUCUCUUUUCU-ppp
SEQ ID NO: 15
OH-AAAAAAAAAAAAAAAAAAAAAAAA
UUUUUUUUUUUUUUUU-ppp
SEQ ID NO: 16
OH-AAAAAAAAAAAAAAAAAAAAAAAA
UUUUUUUUUUUUUUUUUUUU-ppp
SEQ ID NO: 17
OH-AAAAAAAAAAAAAAAAAAAAAAAA
UUUUUUUUUUUUUUUUUUUUUUUU-ppp
SEQ ID NO: 18
ppp-GGGAAAUAAGAGAAAAGAAGAGU...(250mer)
CCCUUUAUUCUCUCUUUUCU-ppp
SEQ ID NO: 19
IFNbeta (pg/ml)
1600
1400
1200
1000
800
600
400
200
0
nLUC G0
nLUC G2
nLUC G5
hEPO G0
hEPO G2
hEPO G5
Growth Factor 1
Growth Factor 2
F20 + R20
Surrogate RNA 2 + R20
U25 + Surrogate RNA 2
U20 + Surrogate RNA 2
U16 + Surrogate RNA 2
U25 + A25
U20 + A25
U16 + A25
F20 + Surrogate RNA 2
R20 + Surrogate RNA 2
polyUs + R20 + Surrogate RNA 2
polyUs + R20 + F20 + Surrogate RNA 2
F20 only
R20 only
U16
U20 only
U25 only
A20 only
Surrogate RNA 2

Figure 27

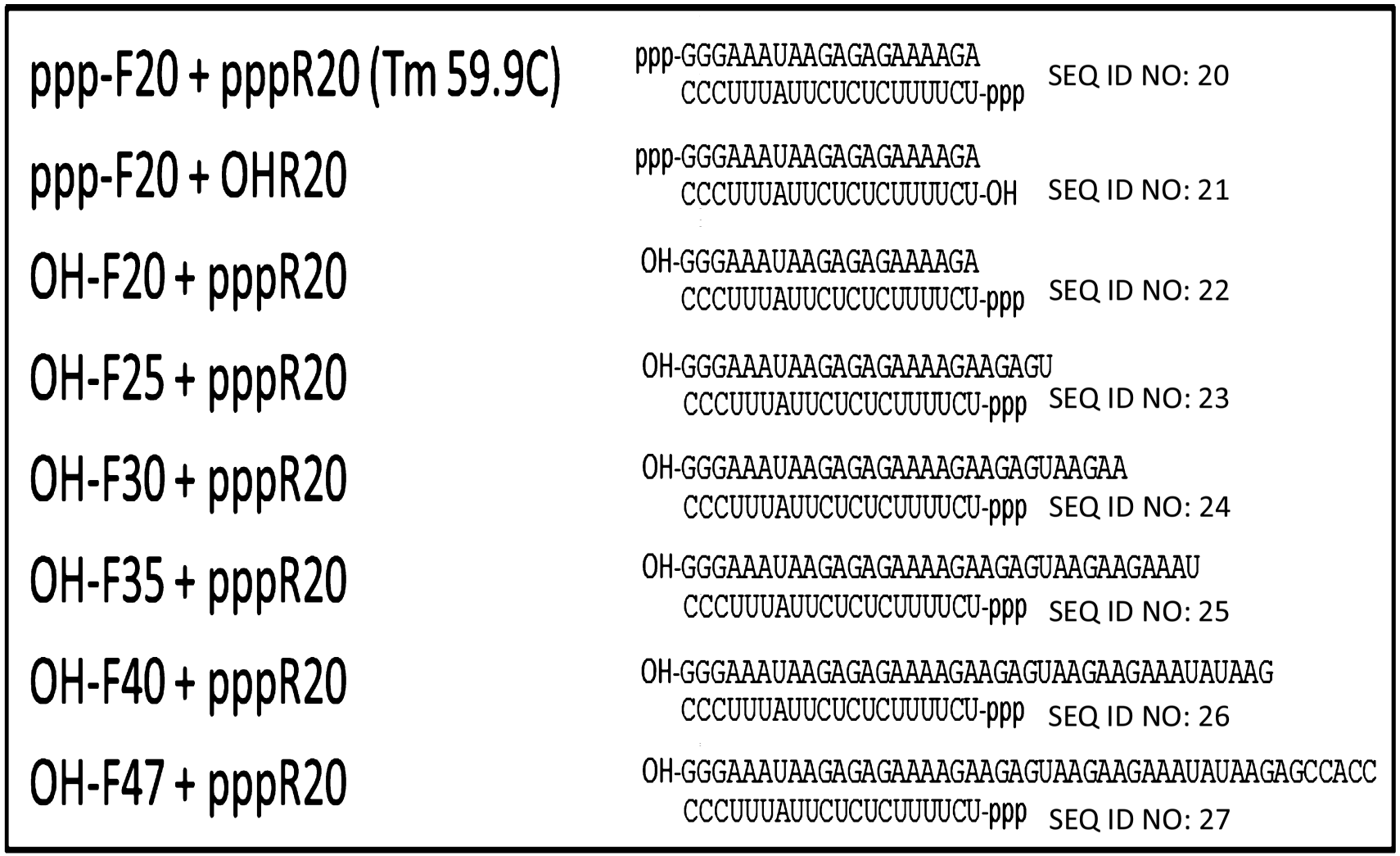

ppp-F20 + pppR20 (Tm 59.9C)
ppp-GGGAAAUAAGAGAGAAAAGA
CCCUUUAUUCUCUCUUUUCU-ppp
SEQ ID NO: 20
ppp-F20 + OHR20
ppp-GGGAAAUAAGAGAGAAAAGA
CCCUUUAUUCUCUCUUUUCU-OH
SEQ ID NO: 21
OH-F20 + pppR20
OH-GGGAAAUAAGAGAGAAAAGA
CCCUUUAUUCUCUCUUUUCU-ppp
SEQ ID NO: 22
OH-F25 + pppR20
OH-GGGAAAUAAGAGAGAAAAGAAGAGU
CCCUUUAUUCUCUCUUUUCU-ppp
SEQ ID NO: 23
OH-F30 + pppR20
OH-GGGAAAUAAGAGAGAAAAGAAGAGUAAGAA
CCCUUUAUUCUCUCUUUUCU-ppp
SEQ ID NO: 24
OH-F35 + pppR20
OH-GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAU
CCCUUUAUUCUCUCUUUUCU-ppp
SEQ ID NO: 25
OH-F40 + pppR20
OH-GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAG
CCCUUUAUUCUCUCUUUUCU-ppp
SEQ ID NO: 26
OH-F47 + pppR20
OH-GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCCACC
CCCUUUAUUCUCUCUUUUCU-ppp
SEQ ID NO: 27

Figure 27 (continued)

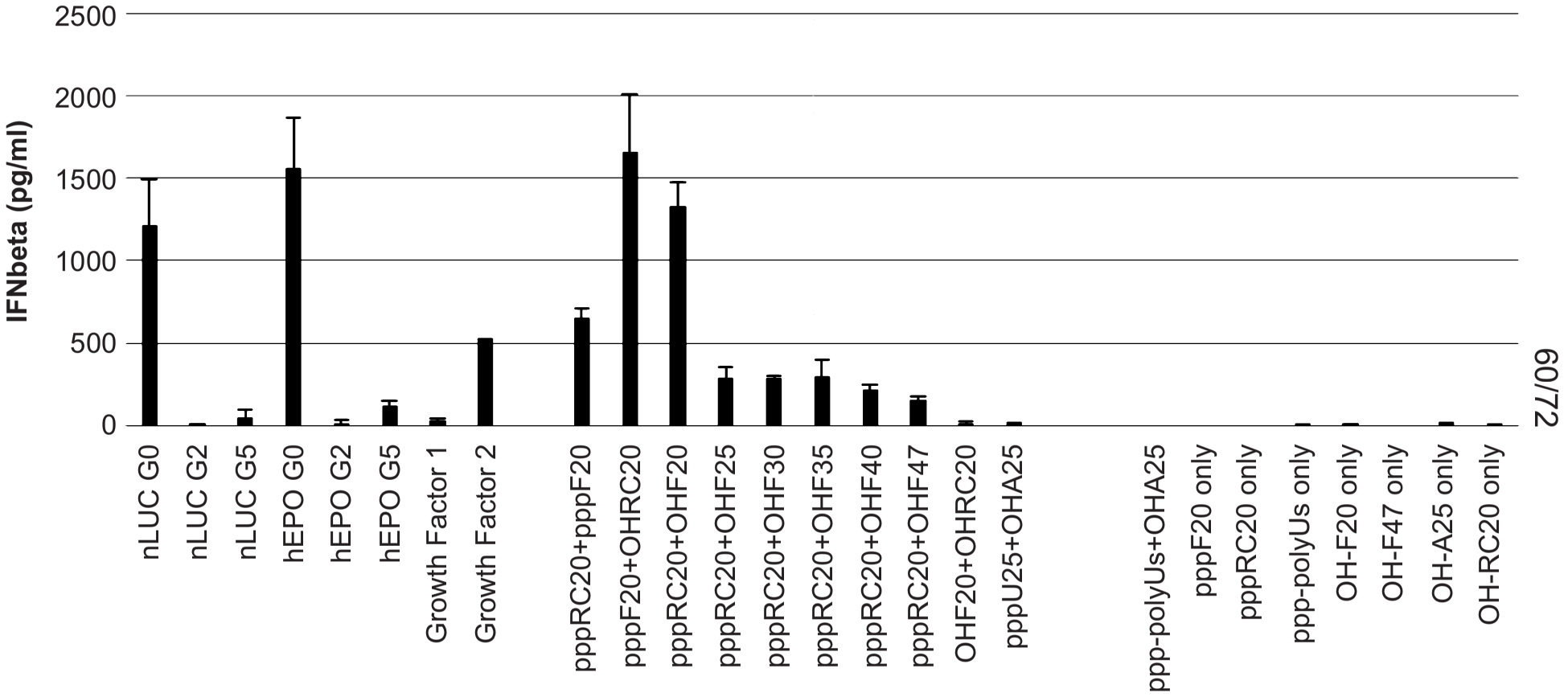


Figure 28

pg/ml
0
500
1000
1500
2000
2500
3000
nLUC G0
nLUC G2
nLUC G5
hEPO G0
hEPO G2
hEPO G5
Growth Factor 1
Growth Factor 2
pppR25+OHF20
pppR25+OHF25
pppR25+OHF30
pppR25+OHF35
pppR25+OHF40
pppR30+OHF20
pppR30+OHF25
pppR30+OHF30
pppR30+OHF35
pppR30+OHF40
pppR35+OHF20
pppR35+OHF25
pppR35+OHF30
pppR35+OHF35
pppR35+OHF40
pppR40+OHF20
pppR40+OHF25
pppR40+OHF30
pppR40+OHF35
pppR40+OHF40
pppR20+pppF20
pppR25 only
pppR30 only
pppR35 only
pppR40 only
OHF40 only
pppU30+OHA30
pppU40+OHA30
pppU50+OHA30
pppU50 only

Figure 29

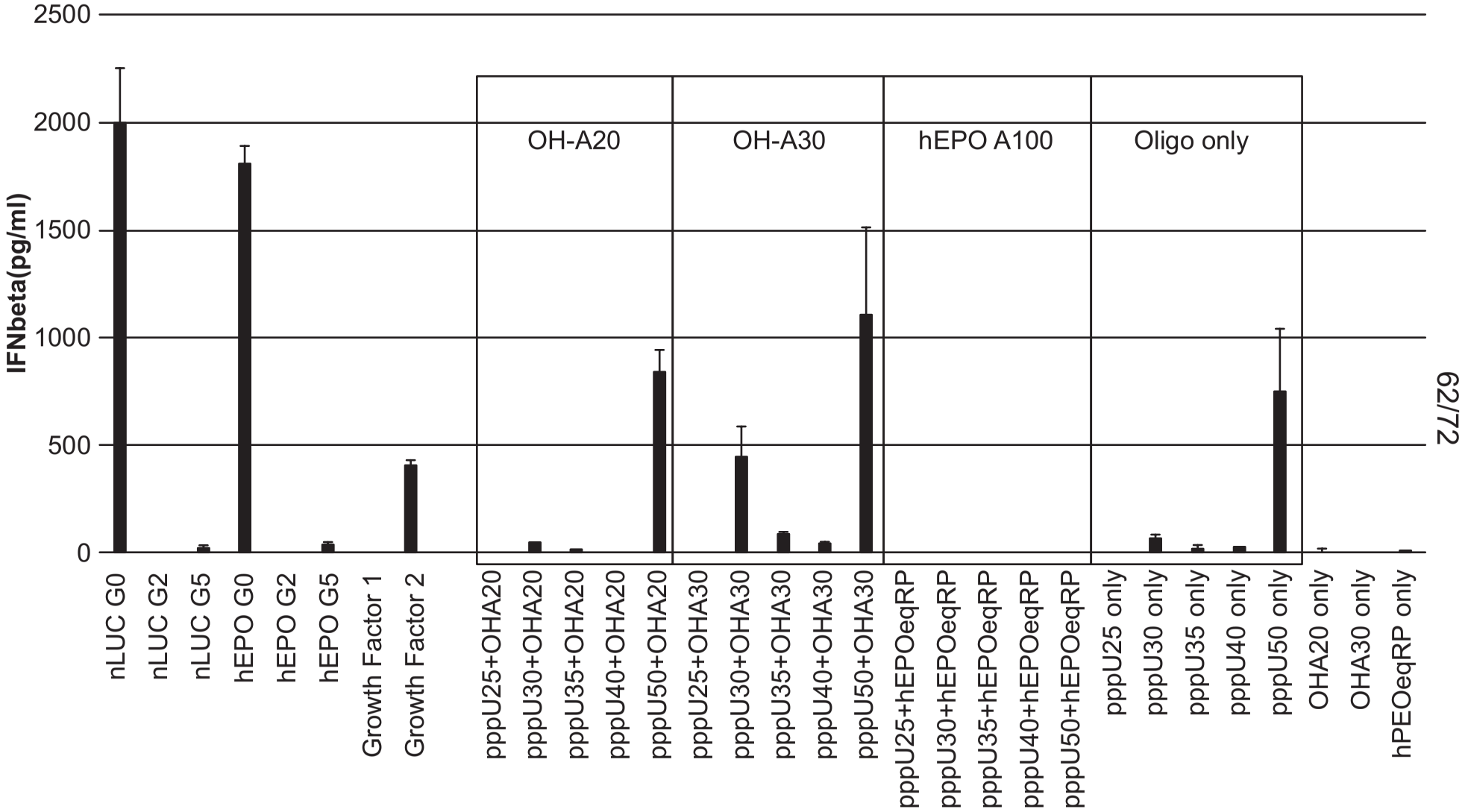
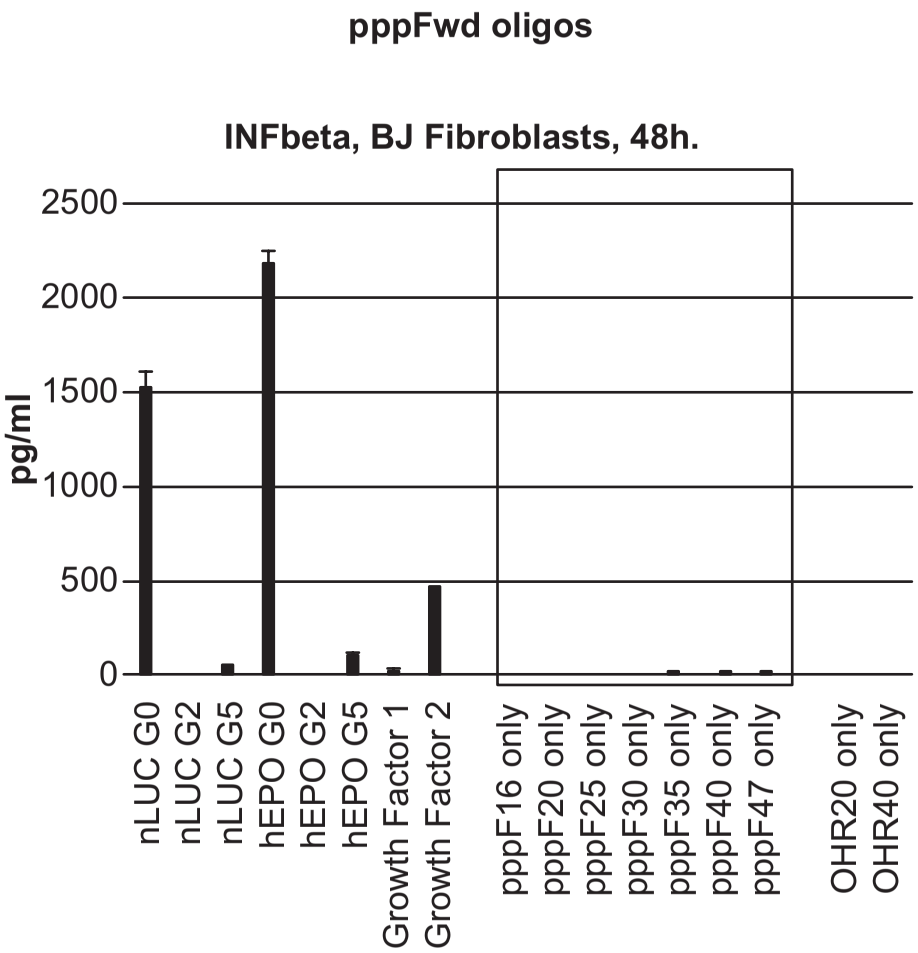

2500
2000
1500
1000
500
0
IFNbeta(pg/ml)
OH-A20
OH-A30
hEPO A100
Oligo only
nLUC G0
nLUC G2
nLUC G5
hEPO G0
hEPO G2
hEPO G5
Growth Factor 1
Growth Factor 2
pppU25+OHA20
pppU30+OHA20
pppU35+OHA20
pppU40+OHA20
pppU50+OHA20
pppU25+OHA30
pppU30+OHA30
pppU35+OHA30
pppU40+OHA30
pppU50+OHA30
pppU25+hEPOeqRP
pppU30+hEPOeqRP
pppU35+hEPOeqRP
pppU40+hEPOeqRP
pppU50+hEPOeqRP
pppU25 only
pppU30 only
pppU35 only
pppU40 only
pppU50 only
OHA20 only
OHA30 only
hPEOeqRP only

Figure 30


pppFwd oligos
INFbeta, BJ Fibroblasts, 48h.
pg/ml
2500
2000
1500
1000
500
0
nLUC G0
nLUC G2
nLUC G5
hEPO G0
hEPO G2
hEPO G5
Growth Factor 1
Growth Factor 2
pppF16 only
pppF20 only
pppF25 only
pppF30 only
pppF35 only
pppF40 only
pppF47 only
OHR20 only
OHR40 only

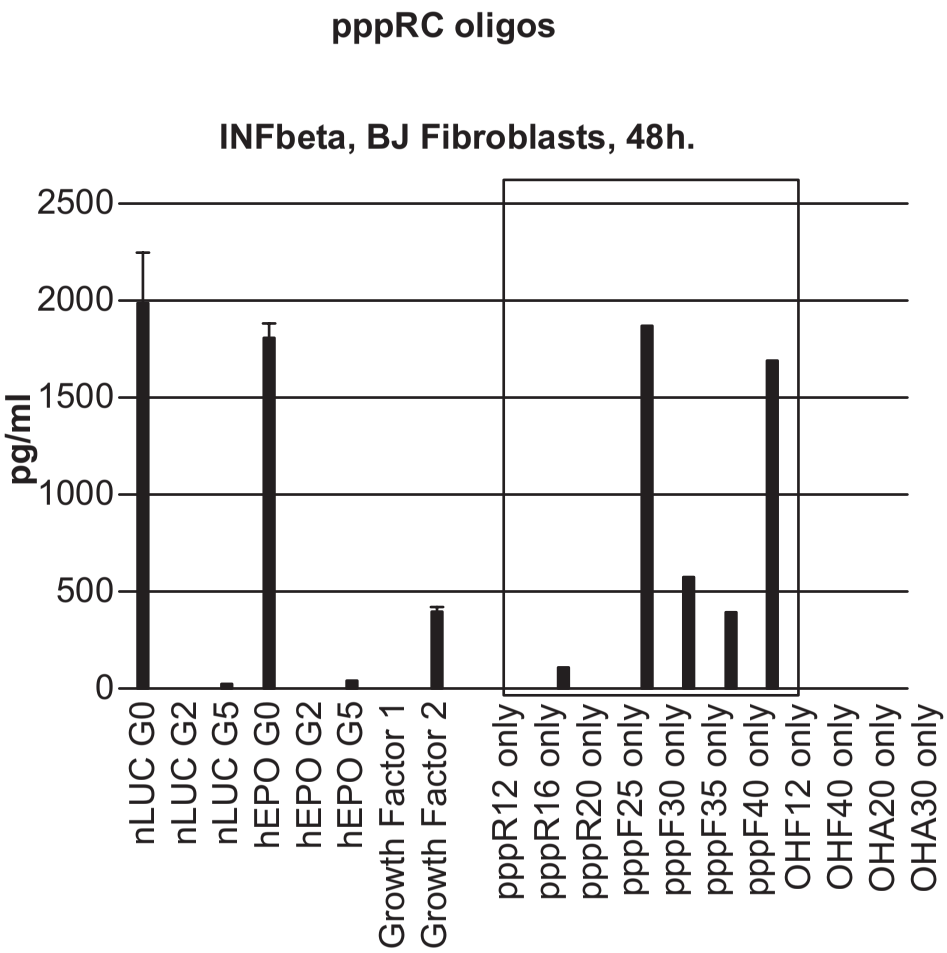

pppRC oligos
INFbeta, BJ Fibroblasts, 48h.
pg/ml
2500
2000
1500
1000
500
0
nLUC G0
nLUC G2
nLUC G5
hEPO G0
hEPO G2
hEPO G5
Growth Factor 1
Growth Factor 2
pppR12 only
pppR16 only
pppR20 only
pppF25 only
pppF30 only
pppF35 only
pppF40 only
OHF12 only
OHF40 only
OHA20 only
OHA30 only

Figure 31

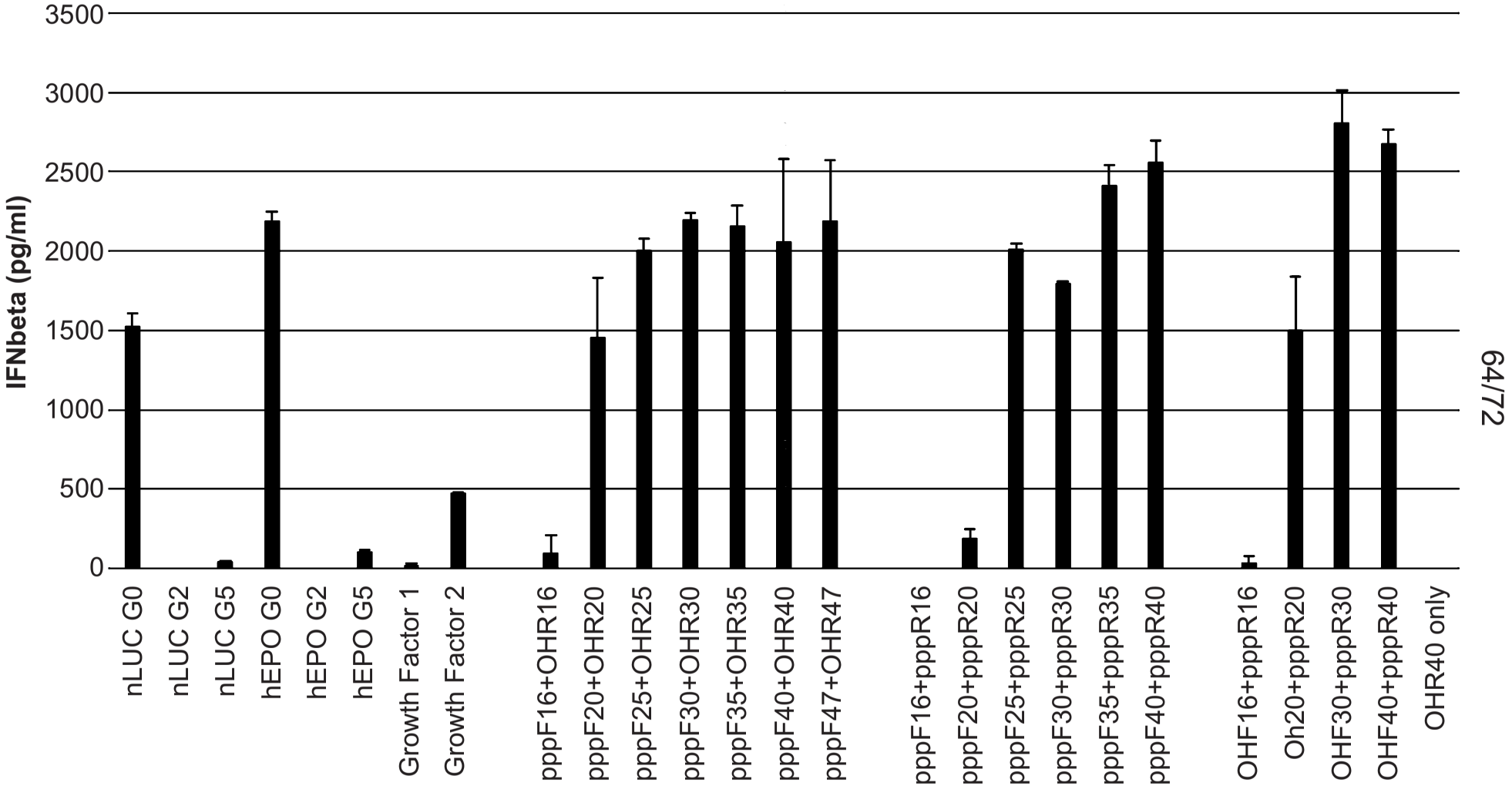

IFNbeta (pg/ml)
3500
3000
2500
2000
1500
1000
500
0
nLUC G0
nLUC G2
nLUC G5
hEPO G0
hEPO G2
hEPO G5
Growth Factor 1
Growth Factor 2
pppF16+OHR16
pppF20+OHR20
pppF25+OHR25
pppF30+OHR30
pppF35+OHR35
pppF40+OHR40
pppF47+OHR47
pppF16+pppR16
pppF20+pppR20
pppF25+pppR25
pppF30+pppR30
pppF35+pppR35
pppF40+pppR40
OHF16+pppR16
Oh20+pppR20
OHF30+pppR30
OHF40+pppR40
OHR40 only

Figure 32

No CIP
IFNbeta (pg/ml)
0
500
1000
1500
2000
2500
nLUC G0
nLUC G2
nLUC G5
hEPO G0
hEPO G2
hEPO G5
Growth Factor 1
Growth Factor 2
pppR30 only
pppR30+OHF20
pppR30+OHF25
pppR30+OHF30
pppR30+OHF35
pppR30+OHF40
+ CIP
IFNbeta (pg/ml)
0
500
1000
1500
2000
2500
3000
3500
4000
nLUC G0
nLUC G2
nLUC G5
hEPO G0
hEPO G2
hEPO G5
Growth Factor 1
Growth Factor 2
pppR30+OHF30
pppR30 only
pppR30+OHF20+CIP
pppR30+OHF25+CIP
pppR30+OHF30+CIP
pppR30+OHF35+CIP
pppR30+OHF40+CIP
OHF12 only

Figure 33

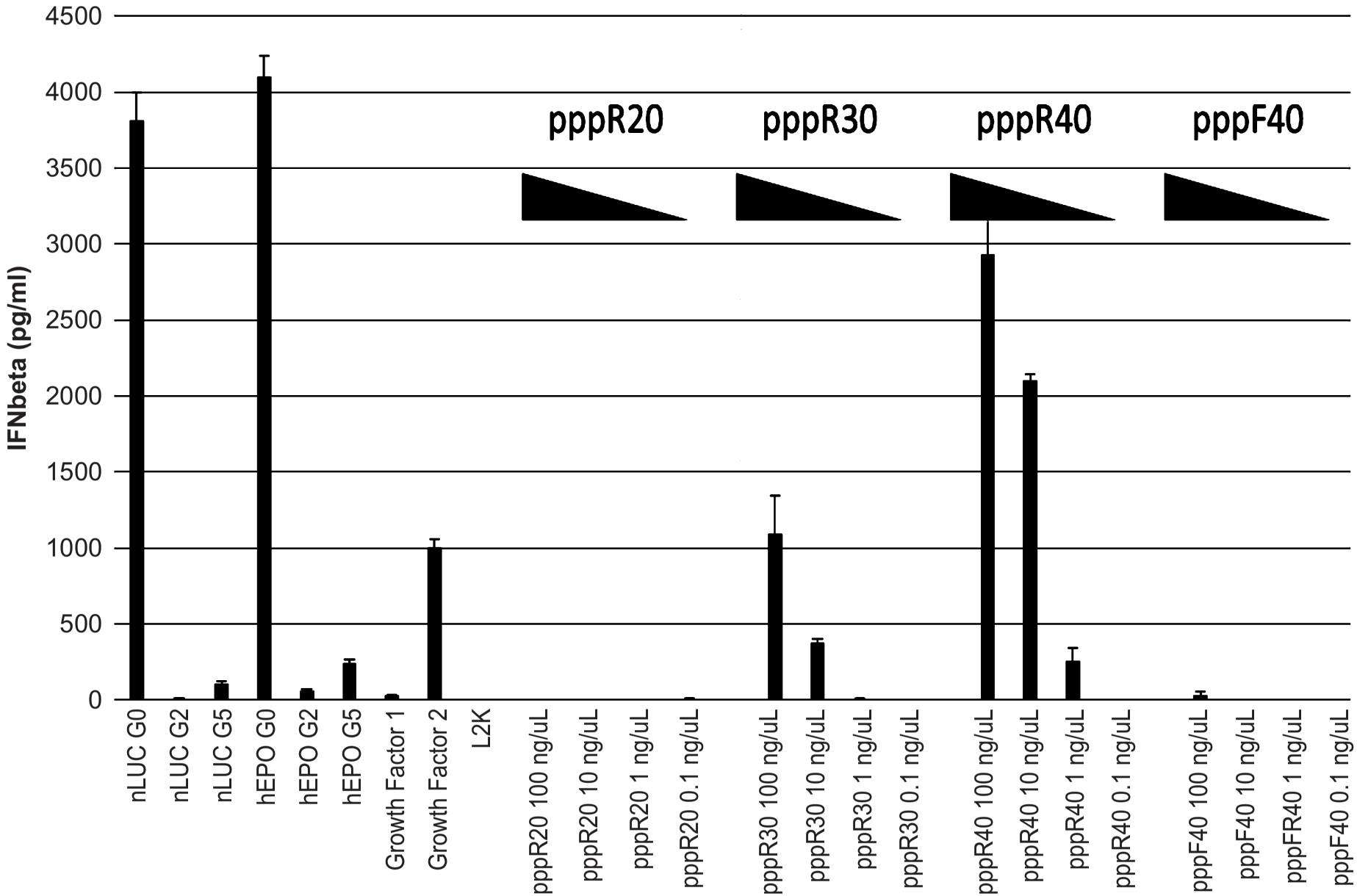

4500
4000
3500
3000
2500
2000
1500
1000
500
0
IFNbeta (pg/ml)
pppR20
pppR30
pppR40
pppF40
nLUC G0
nLUC G2
nLUC G5
hEPO G0
hEPO G2
hEPO G5
Growth Factor 1
Growth Factor 2
L2K
pppR20 100 ng/uL
pppR20 10 ng/uL
pppR20 1 ng/uL
pppR20 0.1 ng/uL
pppR30 100 ng/uL
pppR30 10 ng/uL
pppR30 1 ng/uL
pppR30 0.1 ng/uL
pppR40 100 ng/uL
pppR40 10 ng/uL
pppR40 1 ng/uL
pppR40 0.1 ng/uL
pppF40 100 ng/uL
pppF40 10 ng/uL
pppFR40 1 ng/uL
pppF40 0.1 ng/uL

Figure 34

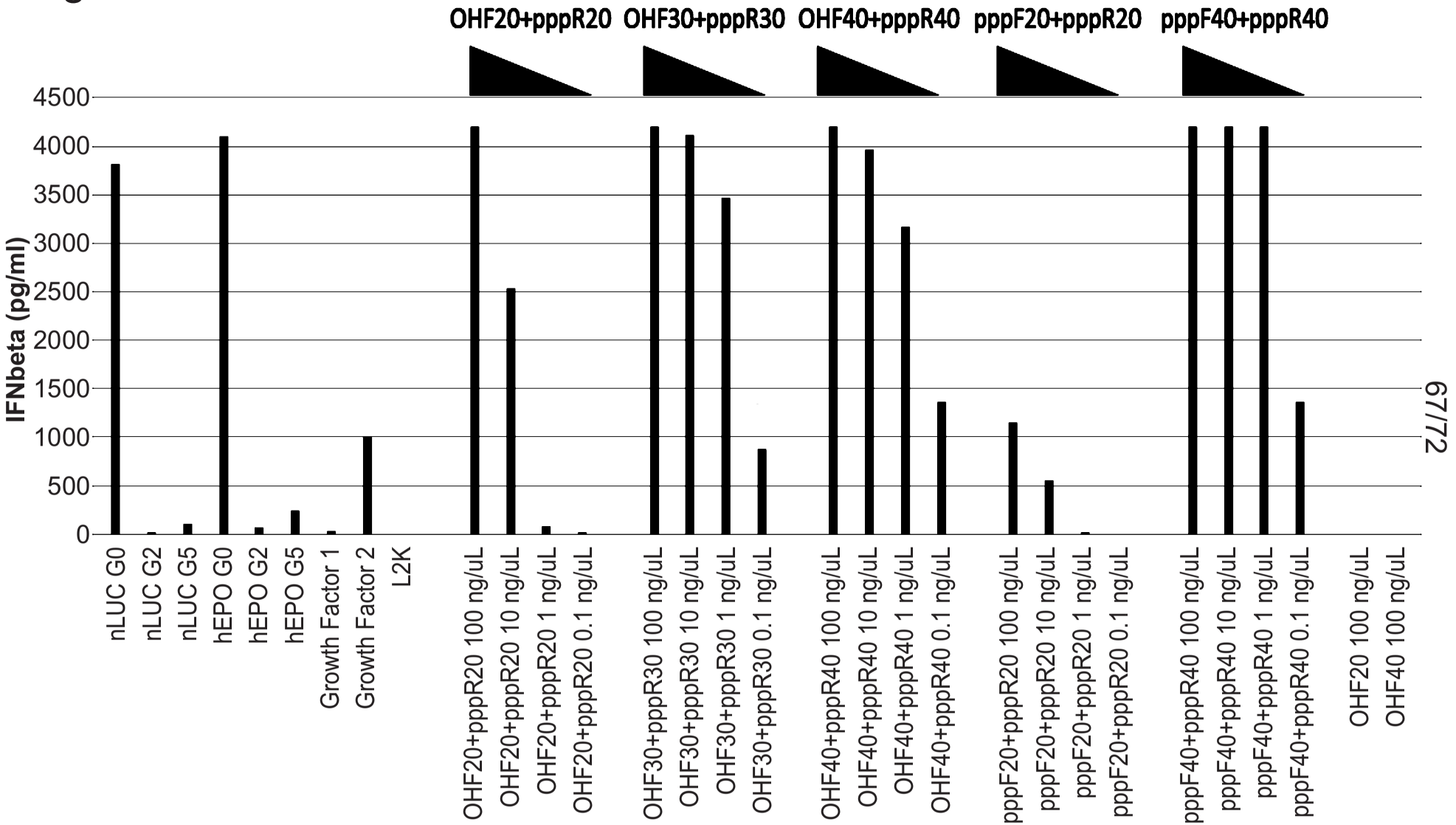
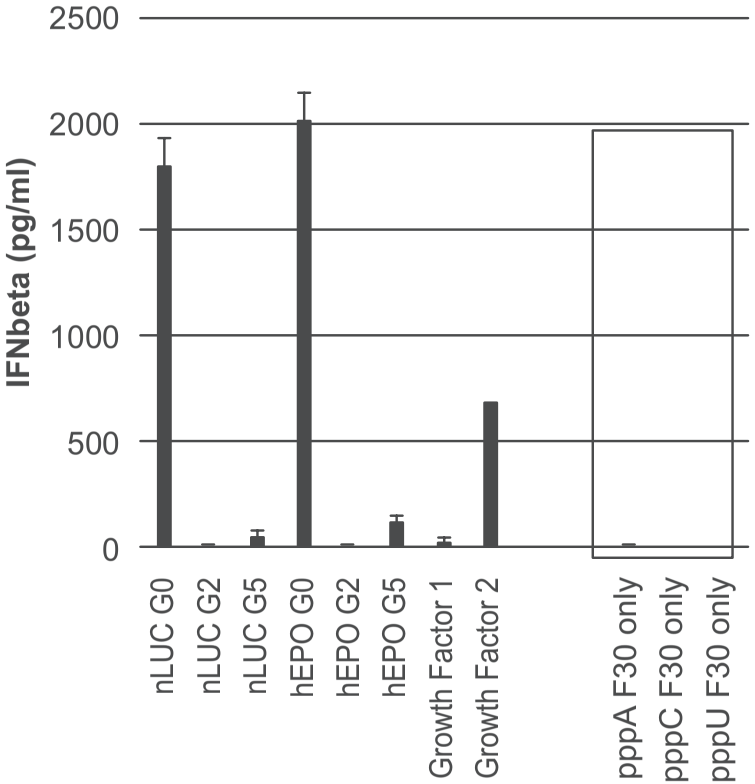
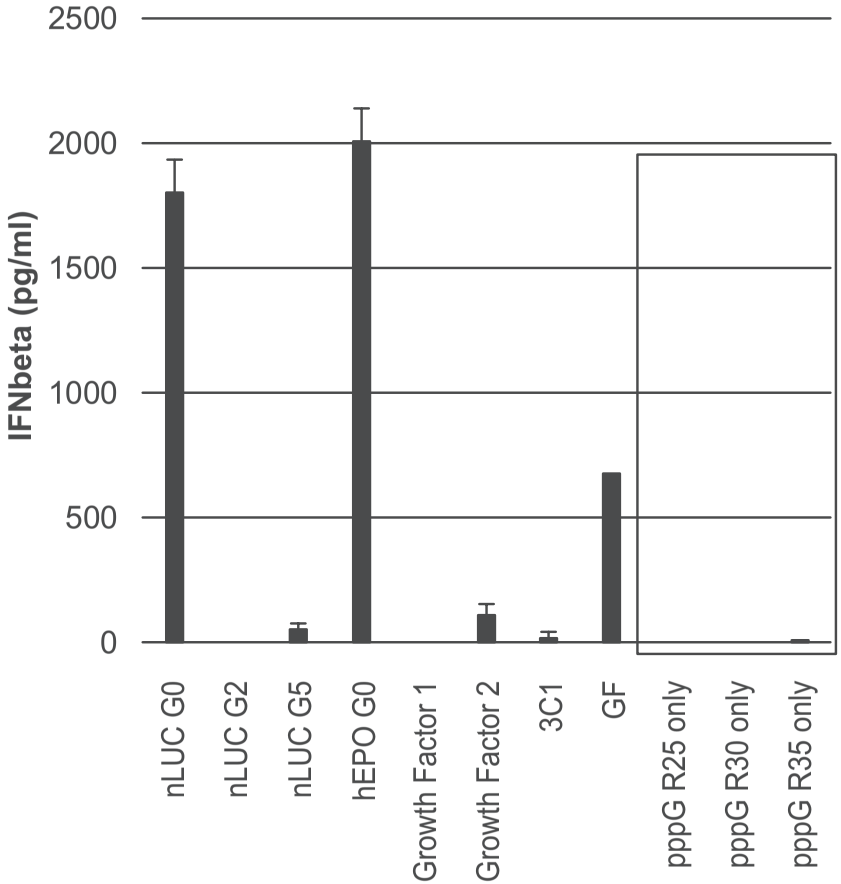

OHF20+pppR20
OHF30+pppR30
OHF40+pppR40
pppF20+pppR20
pppF40+pppR40
IFNbeta (pg/ml)
4500
4000
3500
3000
2500
2000
1500
1000
500
0
nLUC G0
nLUC G2
nLUC G5
hEPO G0
hEPO G2
hEPO G5
Growth Factor 1
Growth Factor 2
L2K
OHF20+pppR20 100 ng/uL
OHF20+pppR20 10 ng/uL
OHF20+pppR20 1 ng/uL
OHF20+pppR20 0.1 ng/uL
OHF30+pppR30 100 ng/uL
OHF30+pppR30 10 ng/uL
OHF30+pppR30 1 ng/uL
OHF30+pppR30 0.1 ng/uL
OHF40+pppR40 100 ng/uL
OHF40+pppR40 10 ng/uL
OHF40+pppR40 1 ng/uL
OHF40+pppR40 0.1 ng/uL
pppF20+pppR20 100 ng/uL
pppF20+pppR20 10 ng/uL
pppF20+pppR20 1 ng/uL
pppF20+pppR20 0.1 ng/uL
pppF40+pppR40 100 ng/uL
pppF40+pppR40 10 ng/uL
pppF40+pppR40 1 ng/uL
pppF40+pppR40 0.1 ng/uL
OHF20 100 ng/uL
OHF40 100 ng/uL

Figure 35



Oligo	Sequence (5' → 3')	
AF30	AGGGAAAUAAGAGAGAAAAGAAGAGUAAGAA	SEQ ID NO: 33
CF30	CGGGAAAUAAGAGAGAAAAGAAGAGUAAGAA	SEQ ID NO: 34
UF30	UGGGAAAUAAGAGAGAAAAGAAGAGUAAGAA	SEQ ID NO: 35

Figure 36



Oligo	Sequence (5' → 3')	
GR25	GACUCUUCUUUUCUCUCUUAUUUCCC	SEQ ID NO: 28
GR30	GUUCUUACUCUUCUUUUCUCUCUUAUUUCCC	SEQ ID NO: 29
GR35	GAUUUCUUCUUACUCUUCUUUUCUCUCUUAUUUCCC	SEQ ID NO: 30

Figure 37

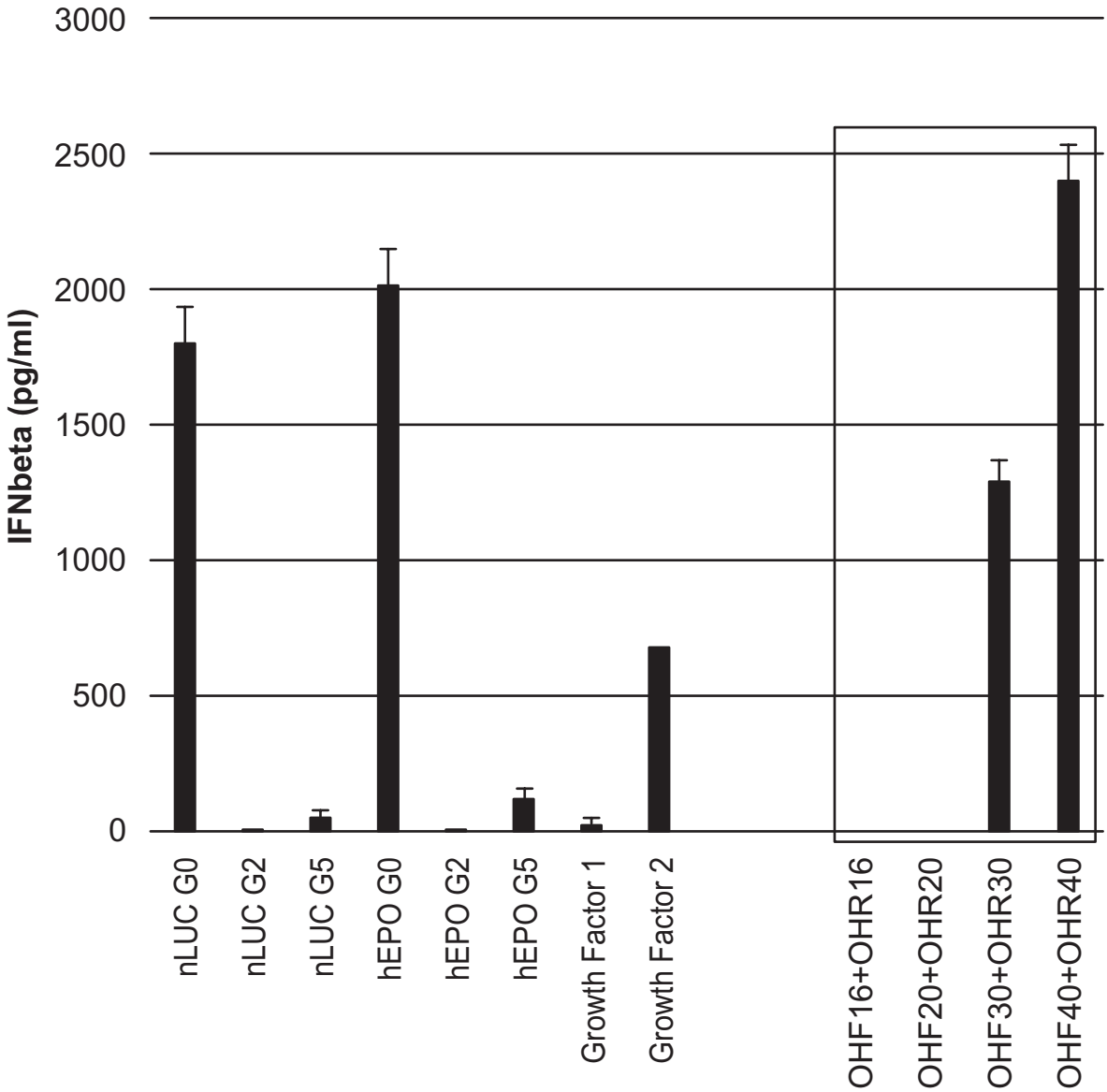

3000
2500
2000
1500
1000
500
0
IFNbeta (pg/ml)
nLUC G0
nLUC G2
nLUC G5
hEPO G0
hEPO G2
hEPO G5
Growth Factor 1
Growth Factor 2
OHF16+OHR16
OHF20+OHR20
OHF30+OHR30
OHF40+OHR40

Figure 38

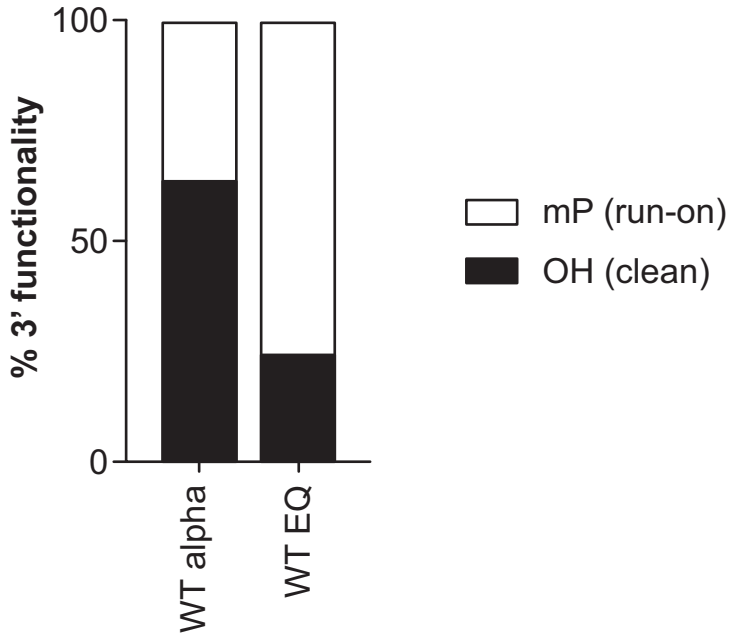


Figure 39

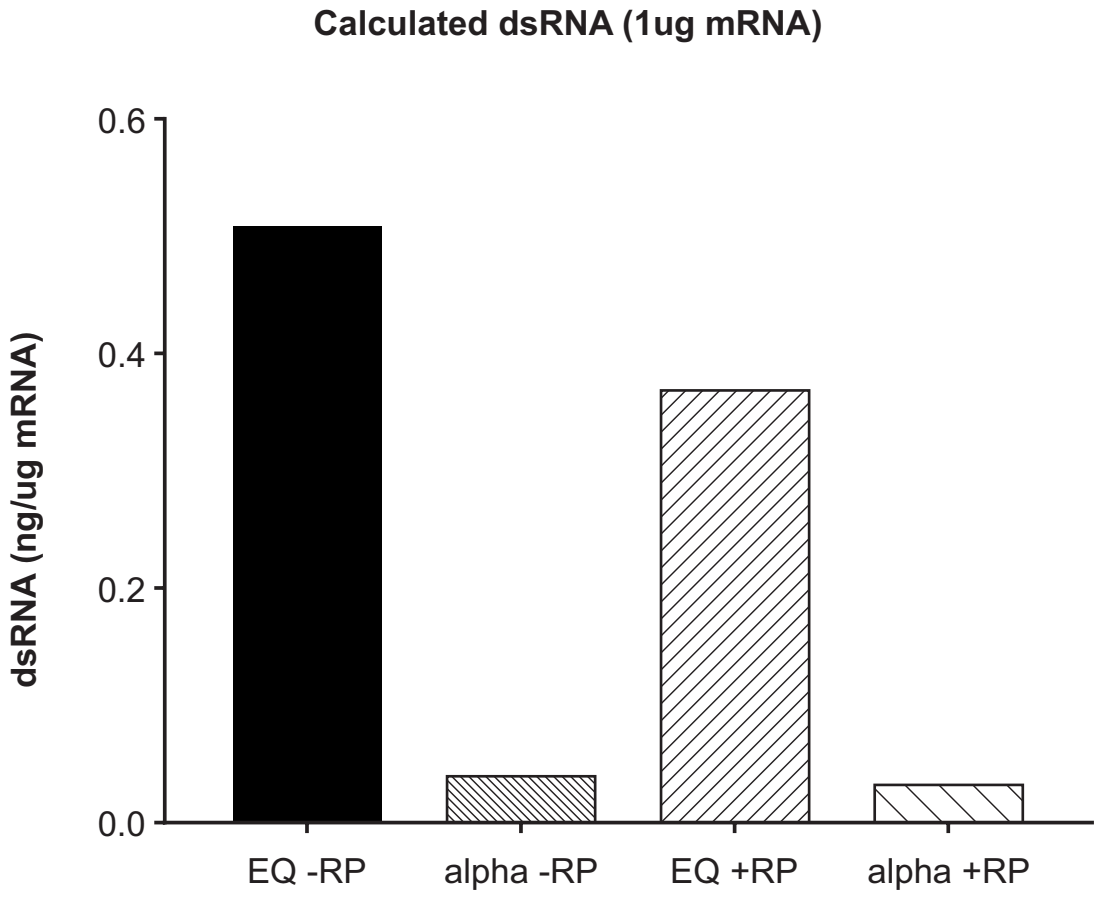
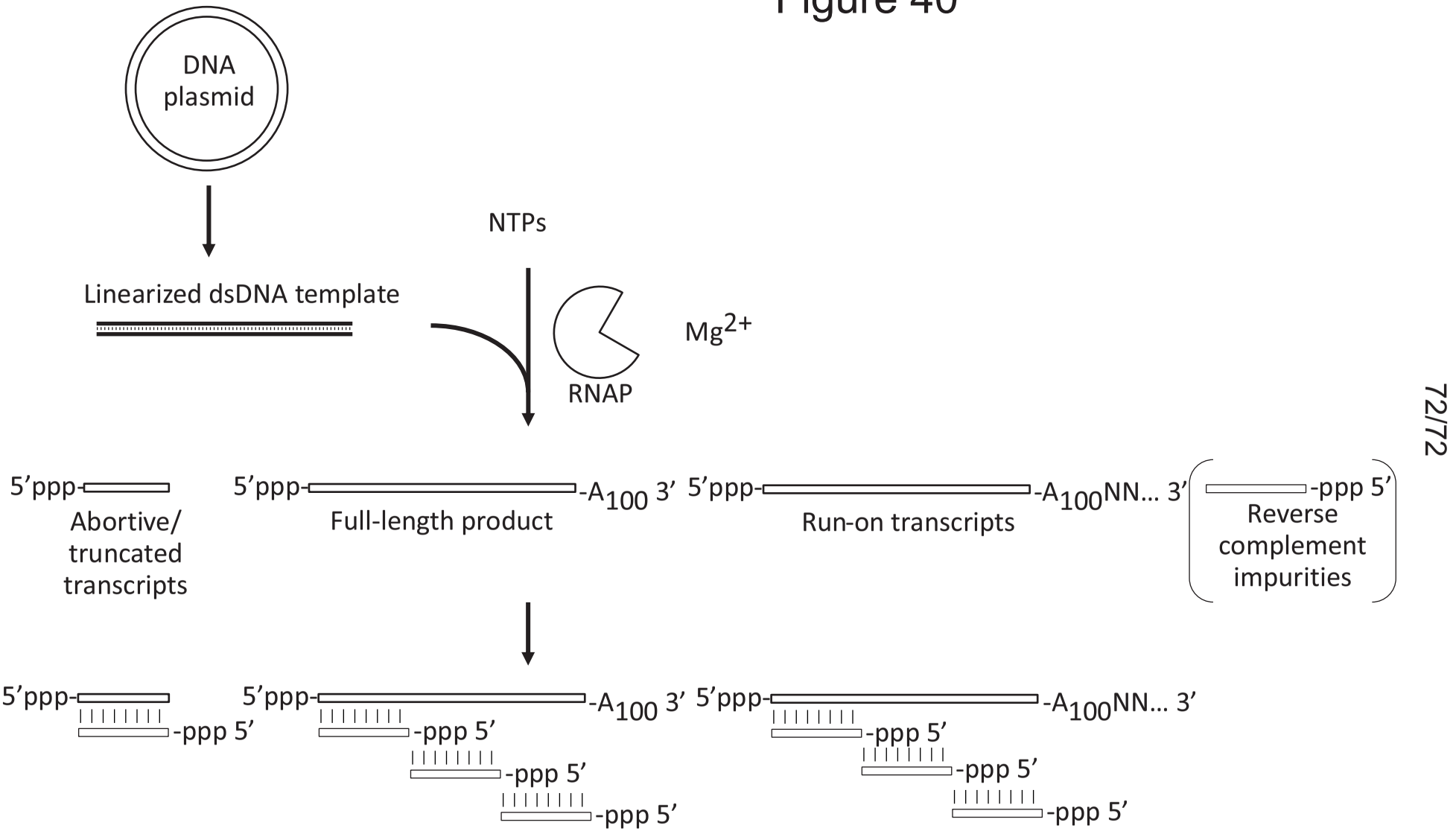


Figure 40


DNA plasmid
Linearized dsDNA template
NTPs
Mg2+
RNAP
5'ppp-
Abortive/ truncated transcripts
5'ppp-A100 3'
Full-length product
5'ppp-A100NN... 3'
Run-on transcripts
-ppp 5'
Reverse complement impurities