

FIG. 1A

Reconstituted in
PBS (pH 7.0)
2ng/ml NanoLuc enzyme

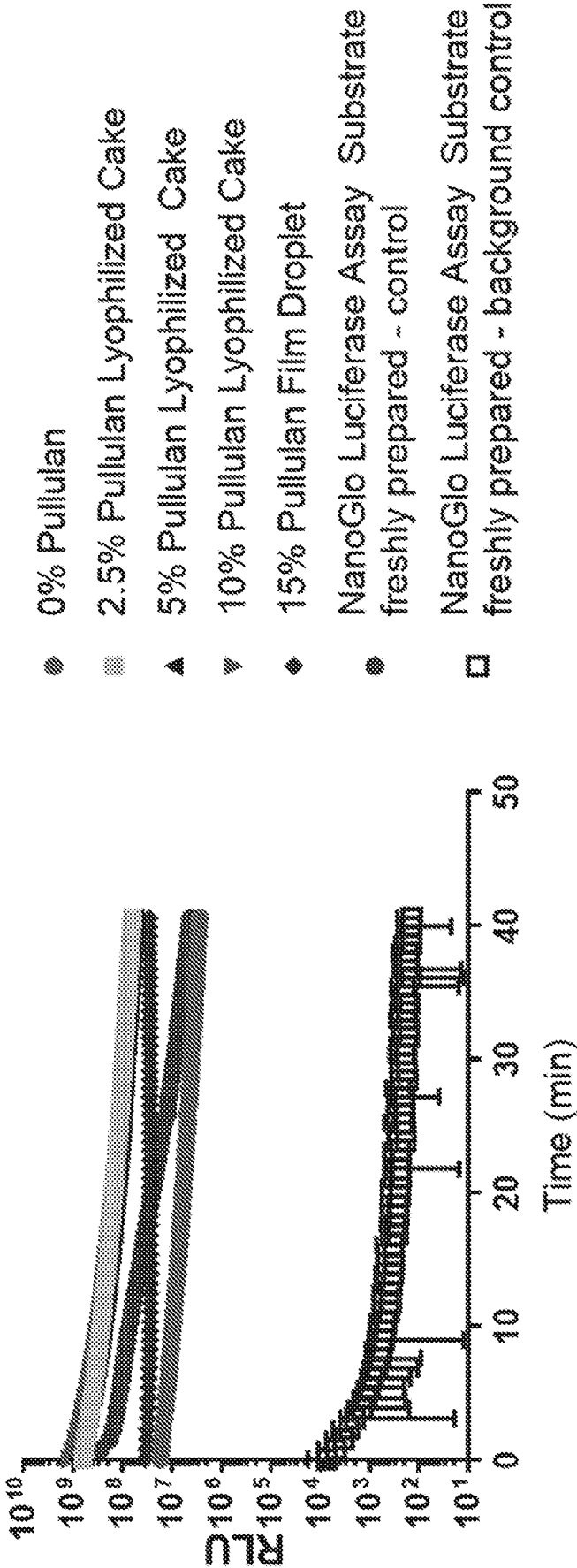


FIG. 1B

Reconstituted in
NanoGlo buffer
2ng/ml NanoLuc enzyme

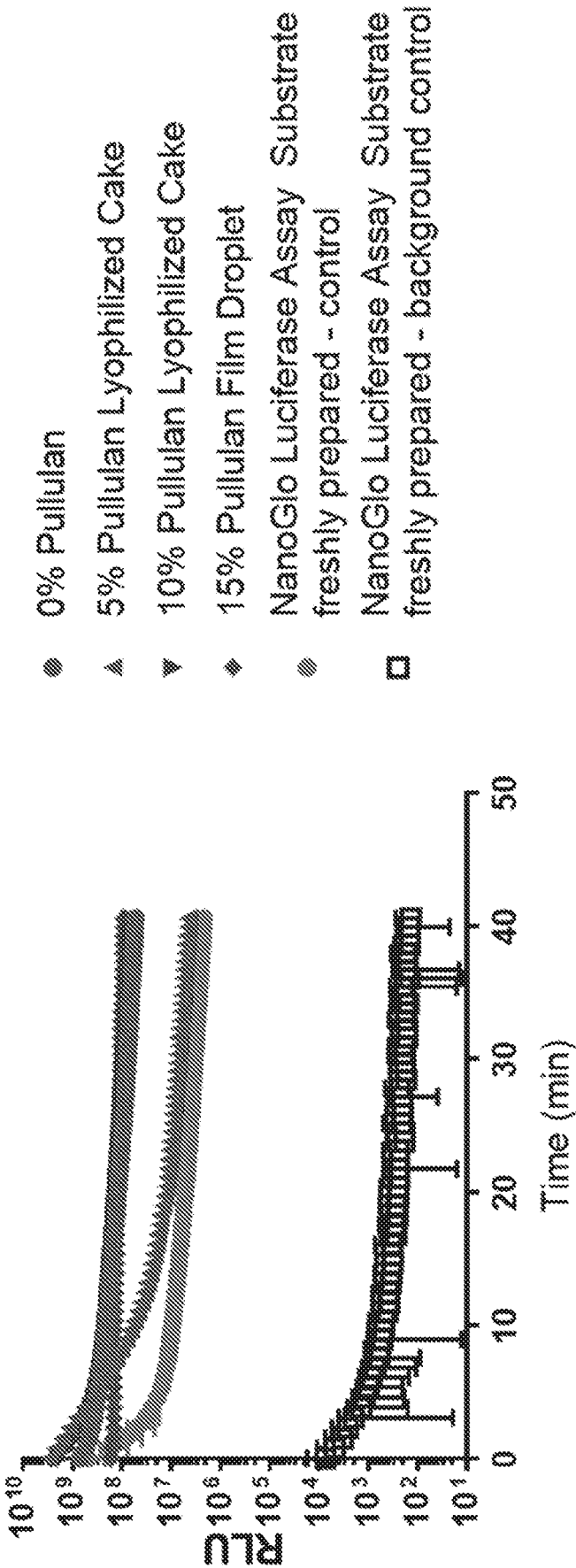
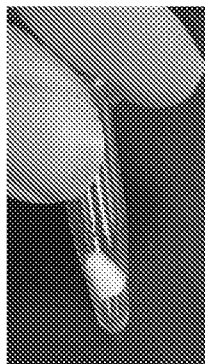


FIG. 1C

Lyophilized
Cake



Film
Droplet

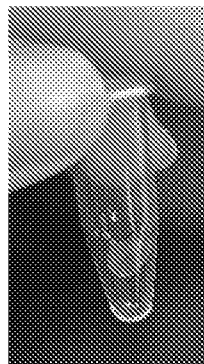


FIG. 2A

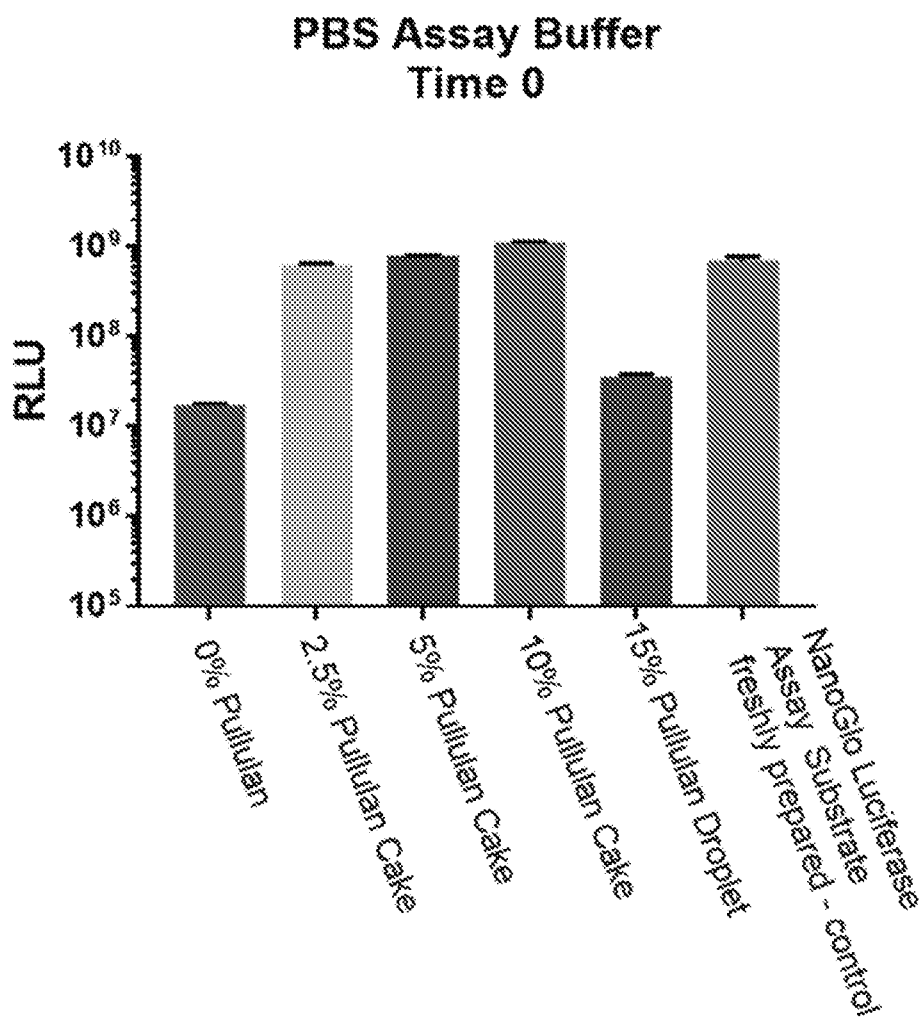


FIG. 2B

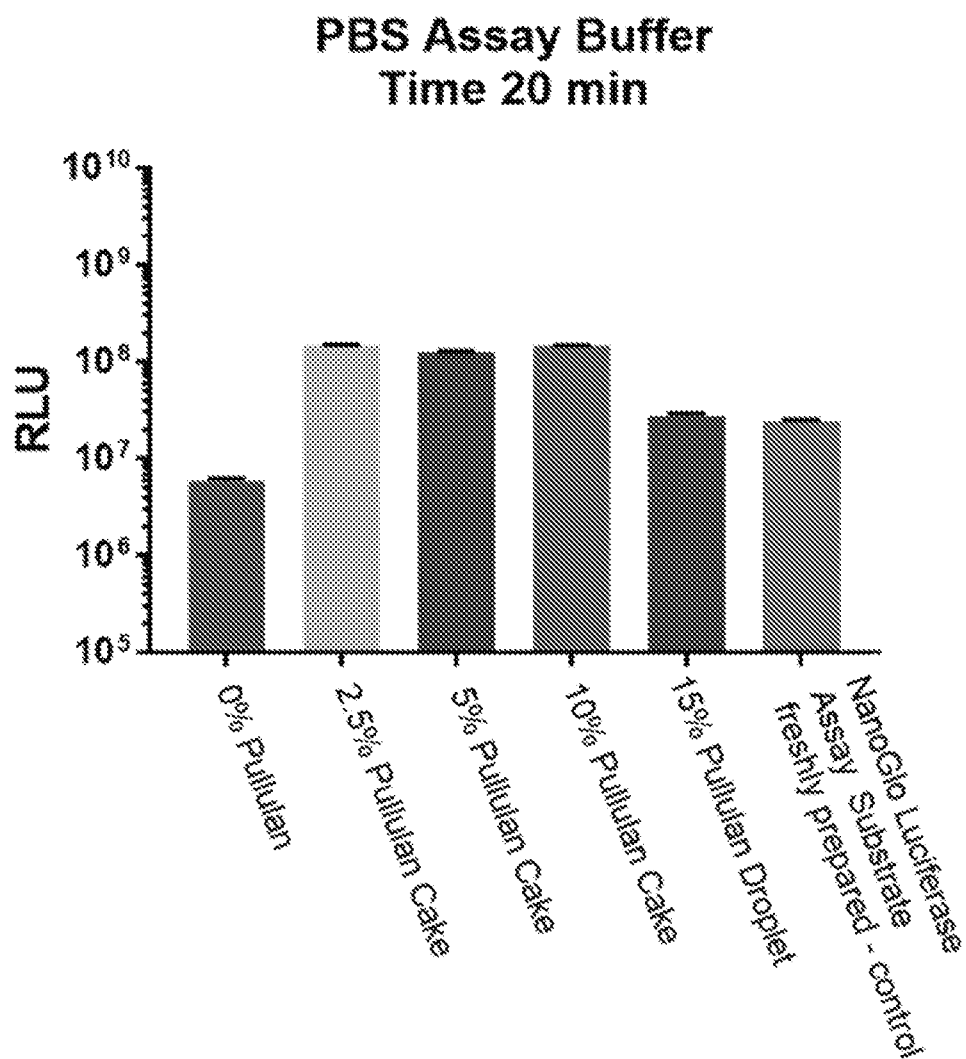


FIG. 2C

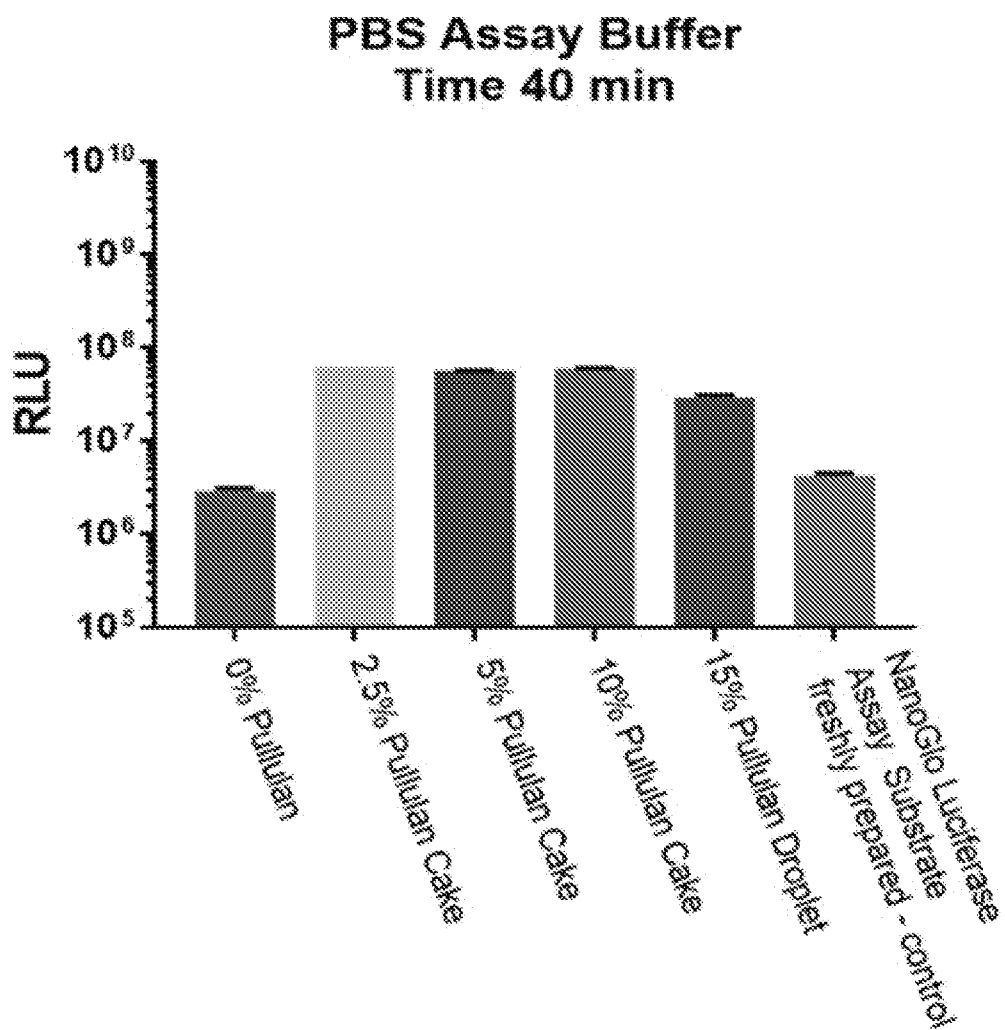


FIG. 3A

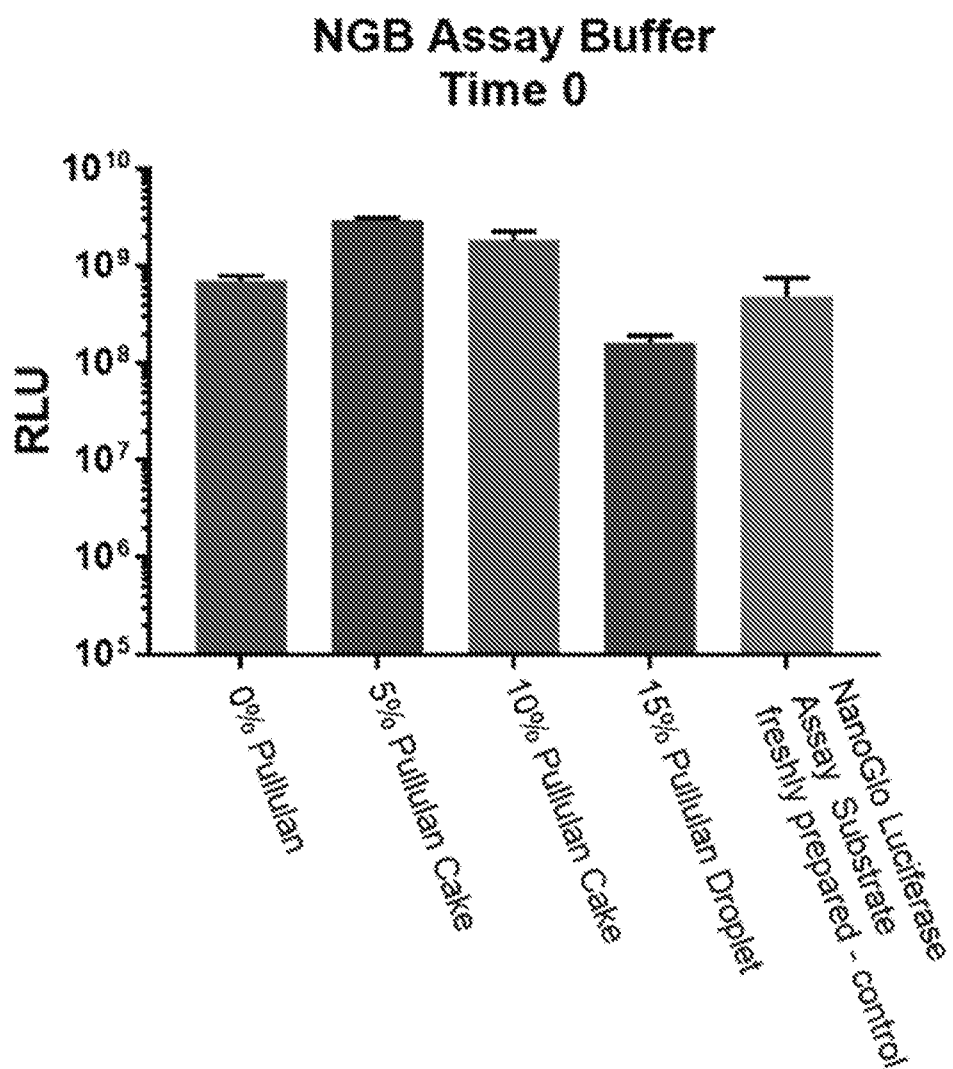


FIG. 3B

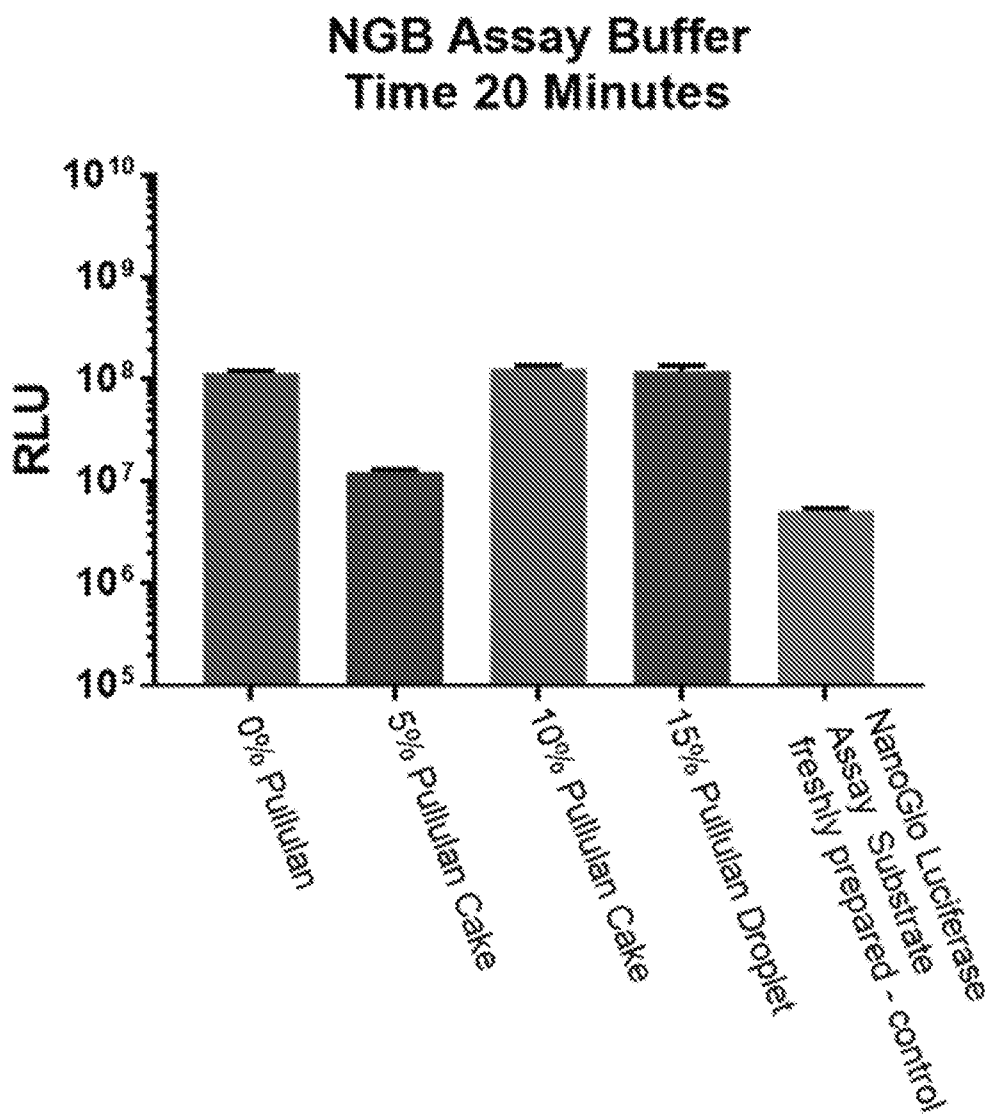


FIG. 3C

NGB Assay Buffer Time 40 Minutes

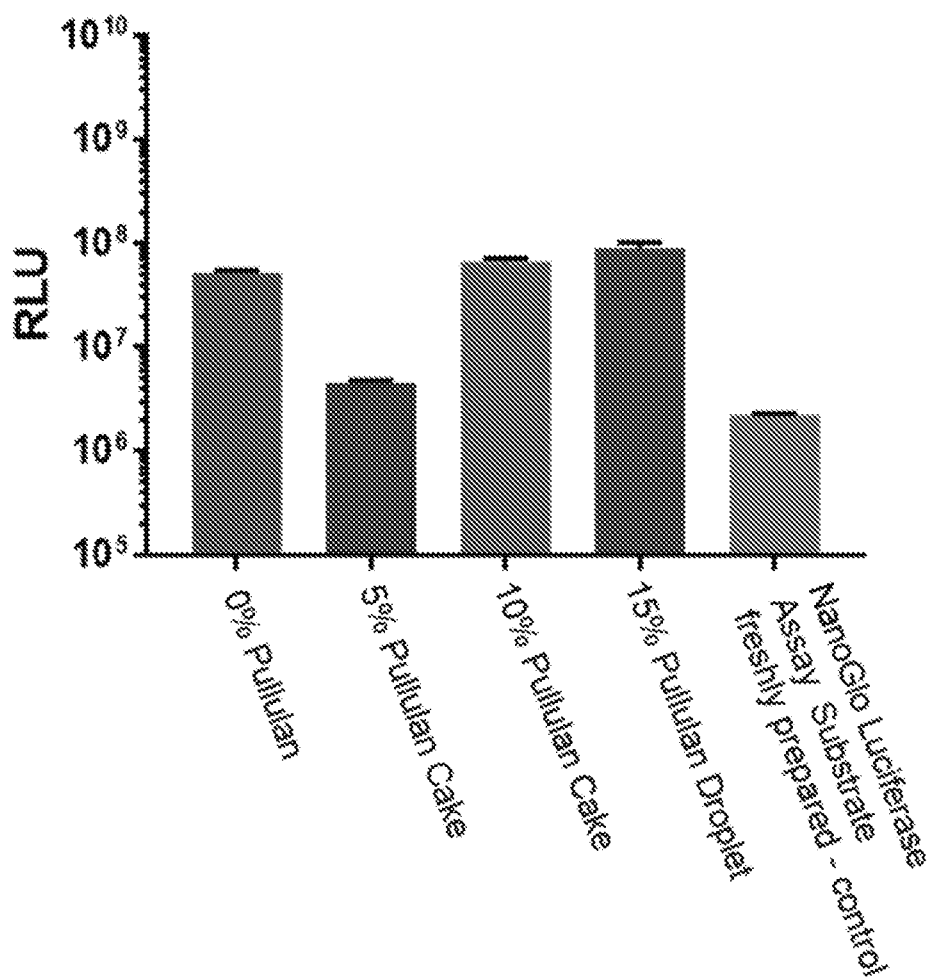


FIG. 4A

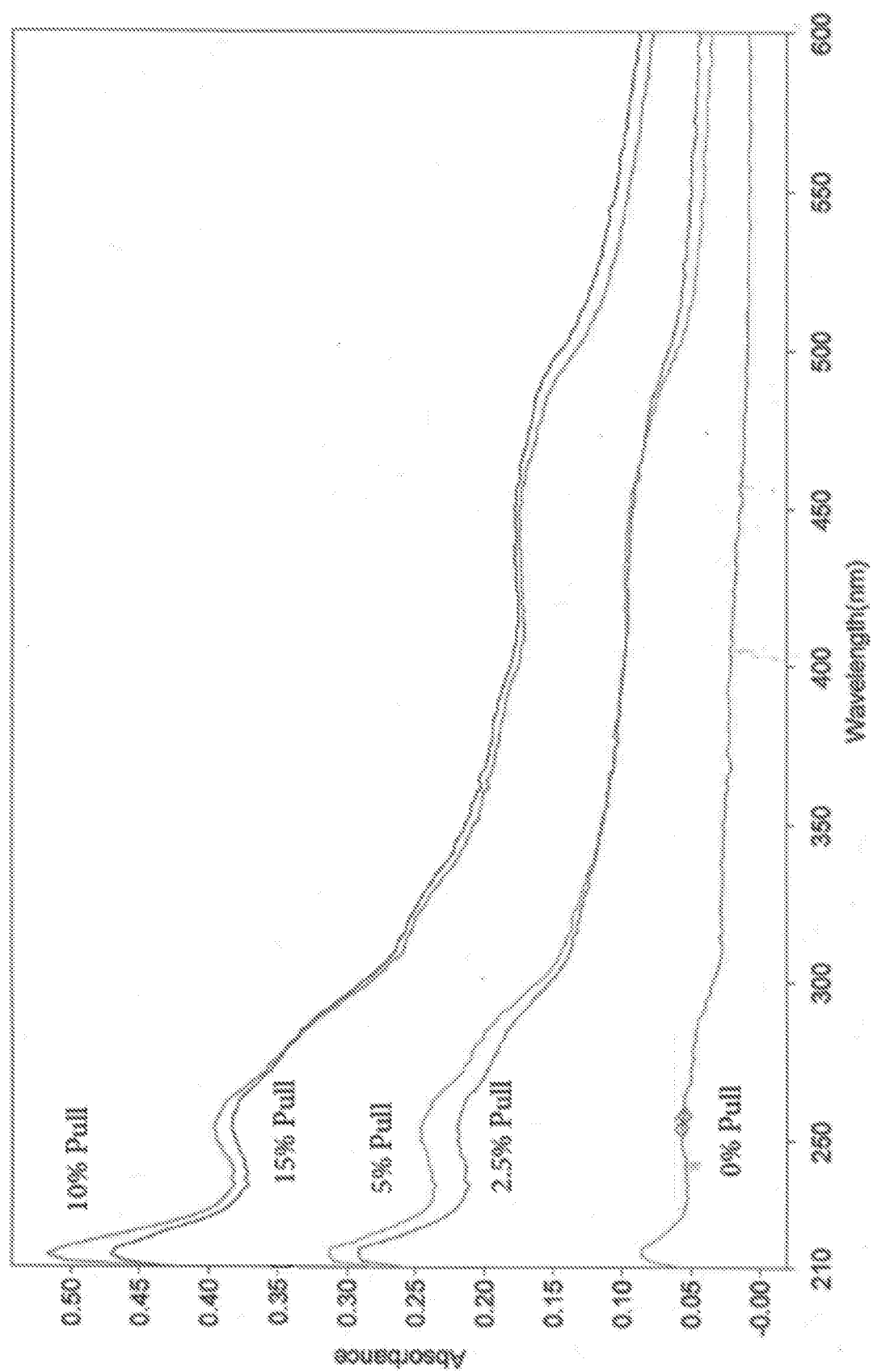


FIG. 4B

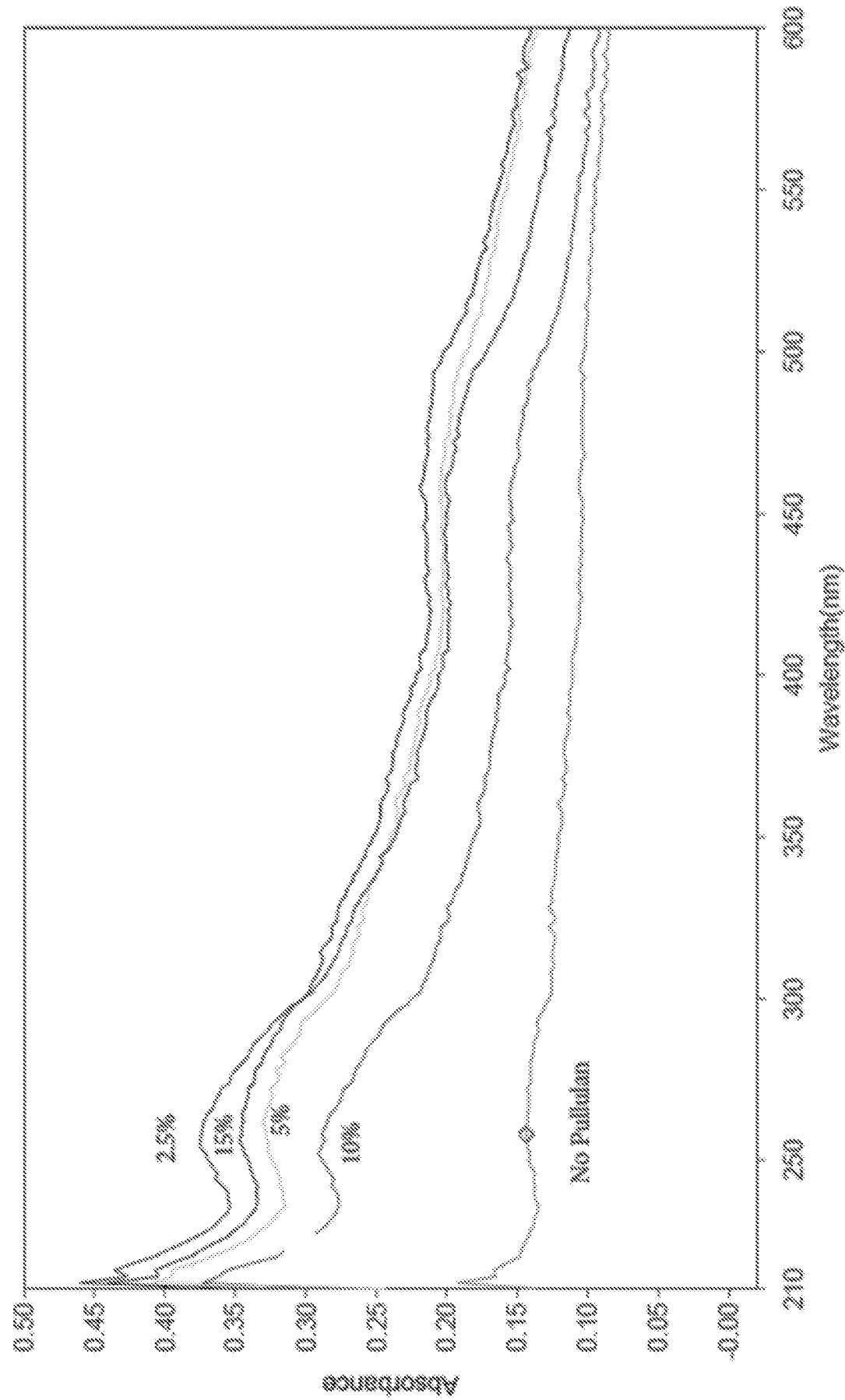


FIG. 4C

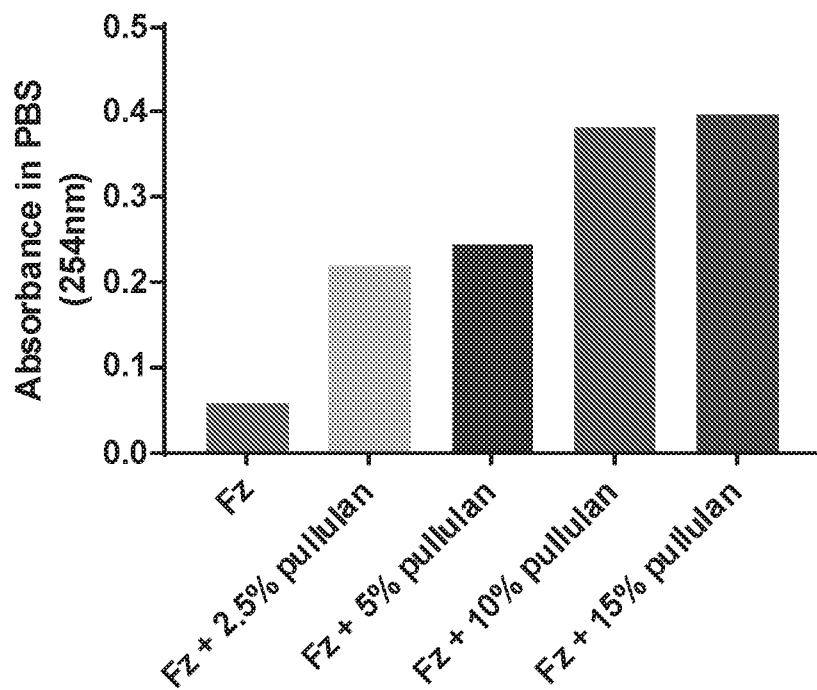


FIG. 5

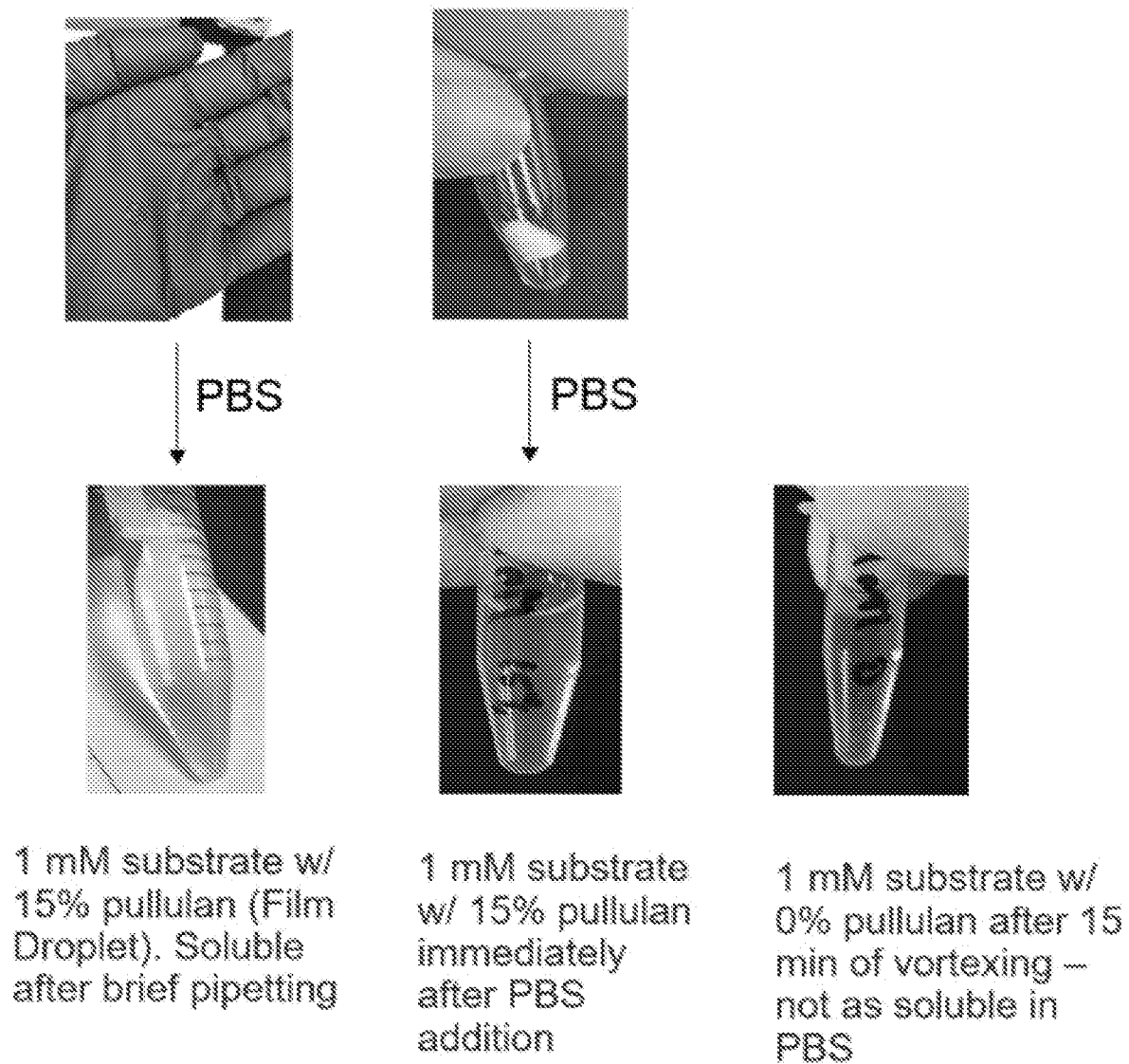


FIG. 6A

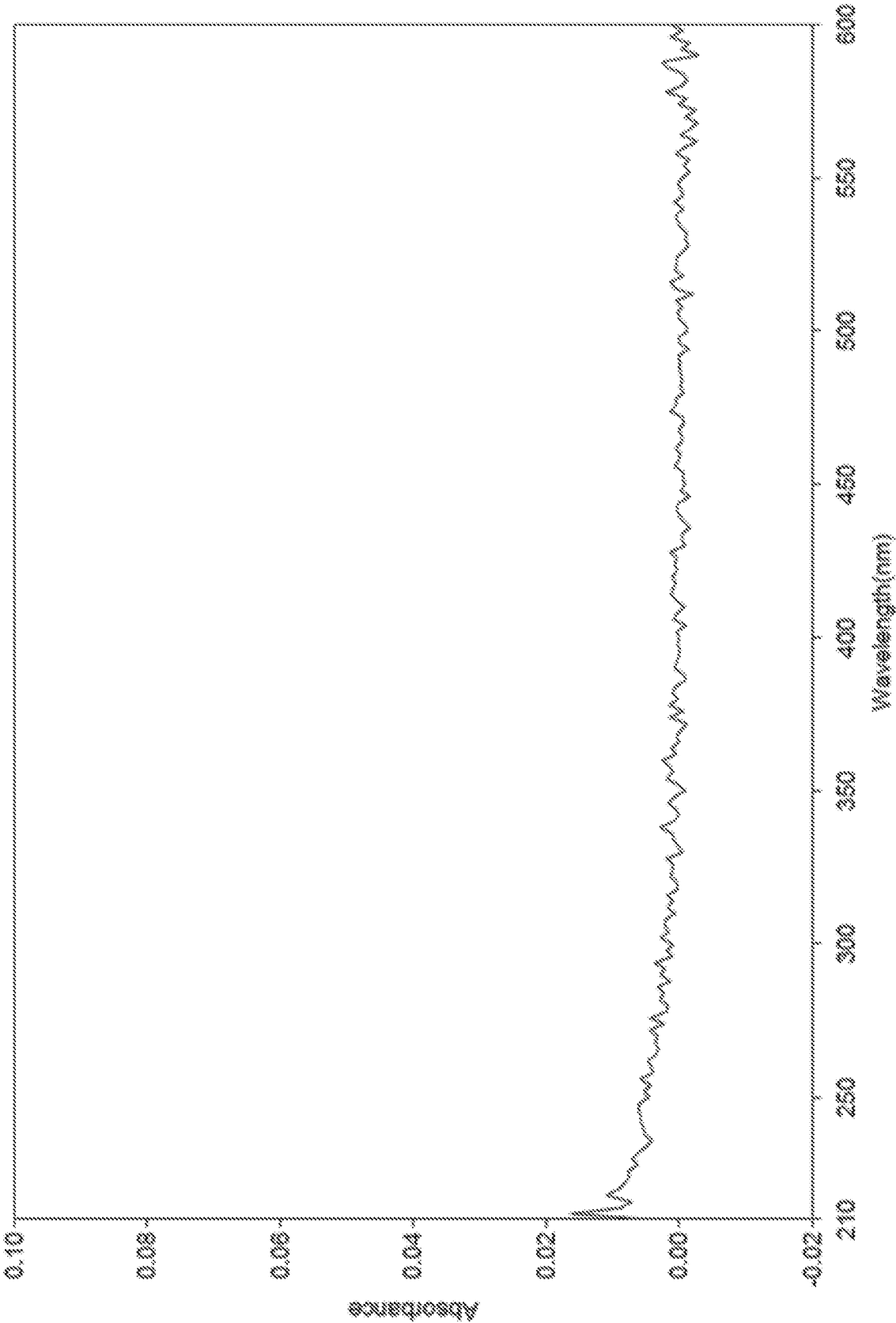


FIG. 6B

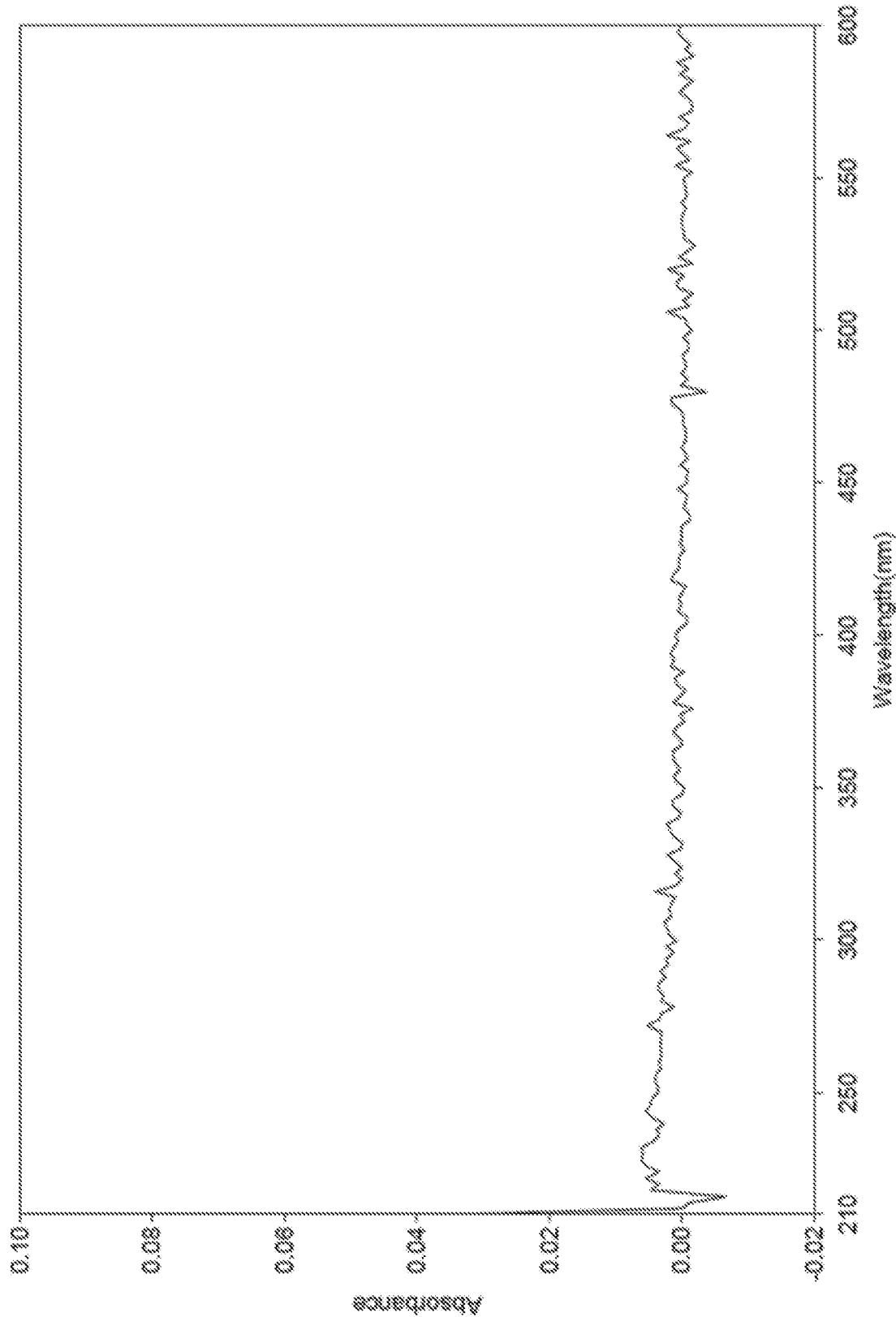
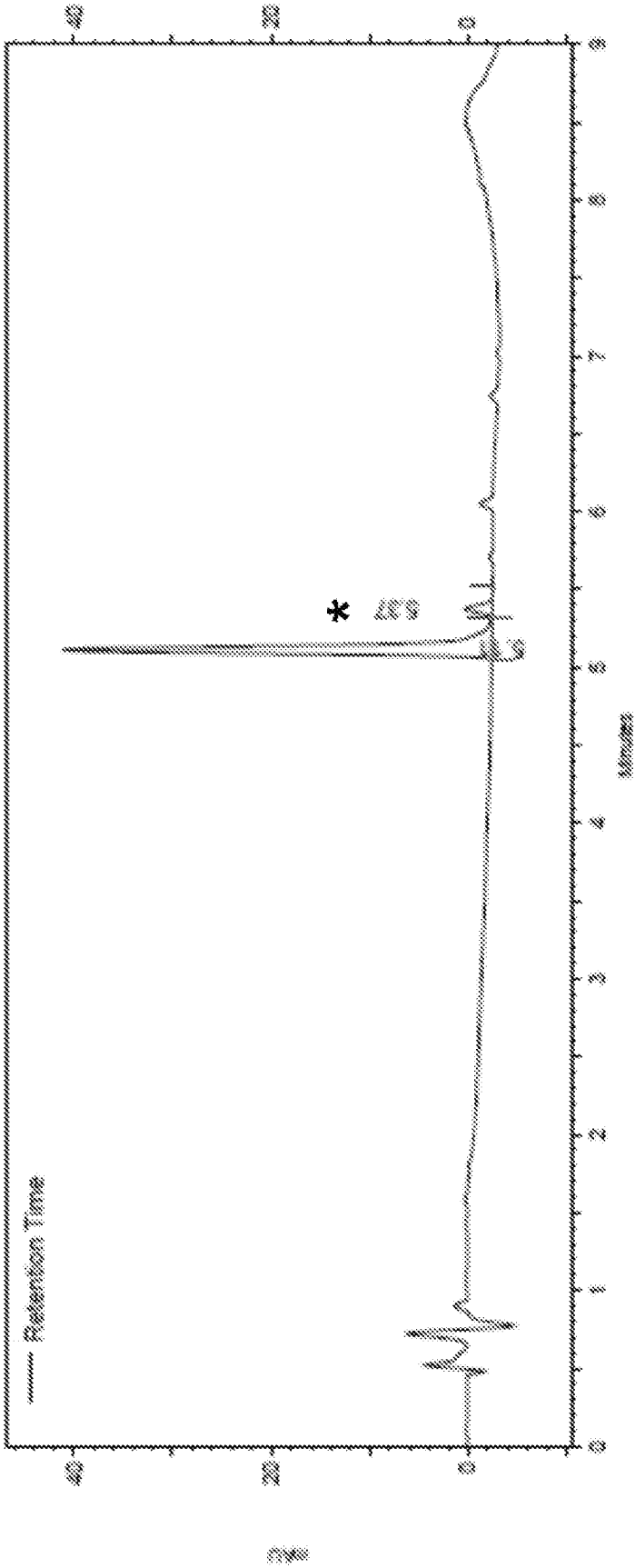


FIG. 7A



17/123

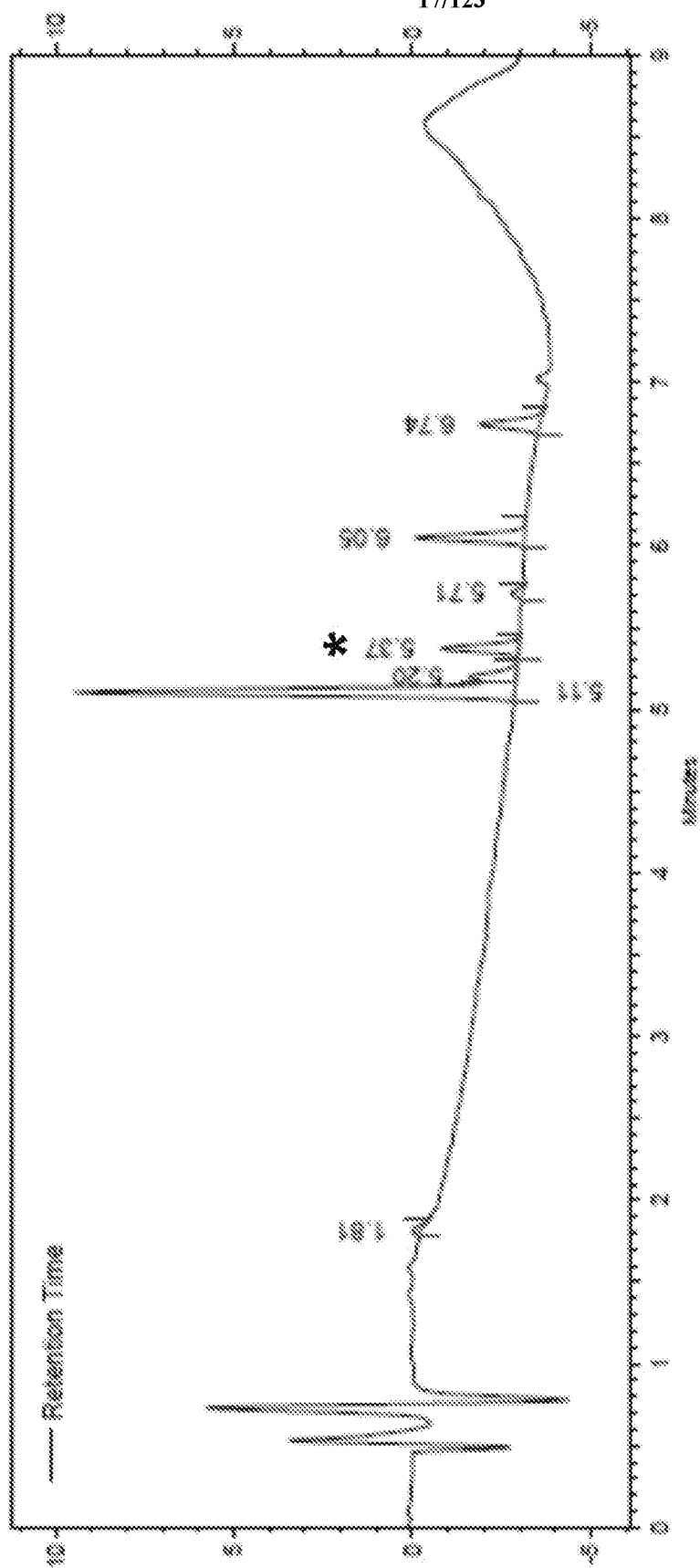


FIG. 7B

100

FIG. 8A

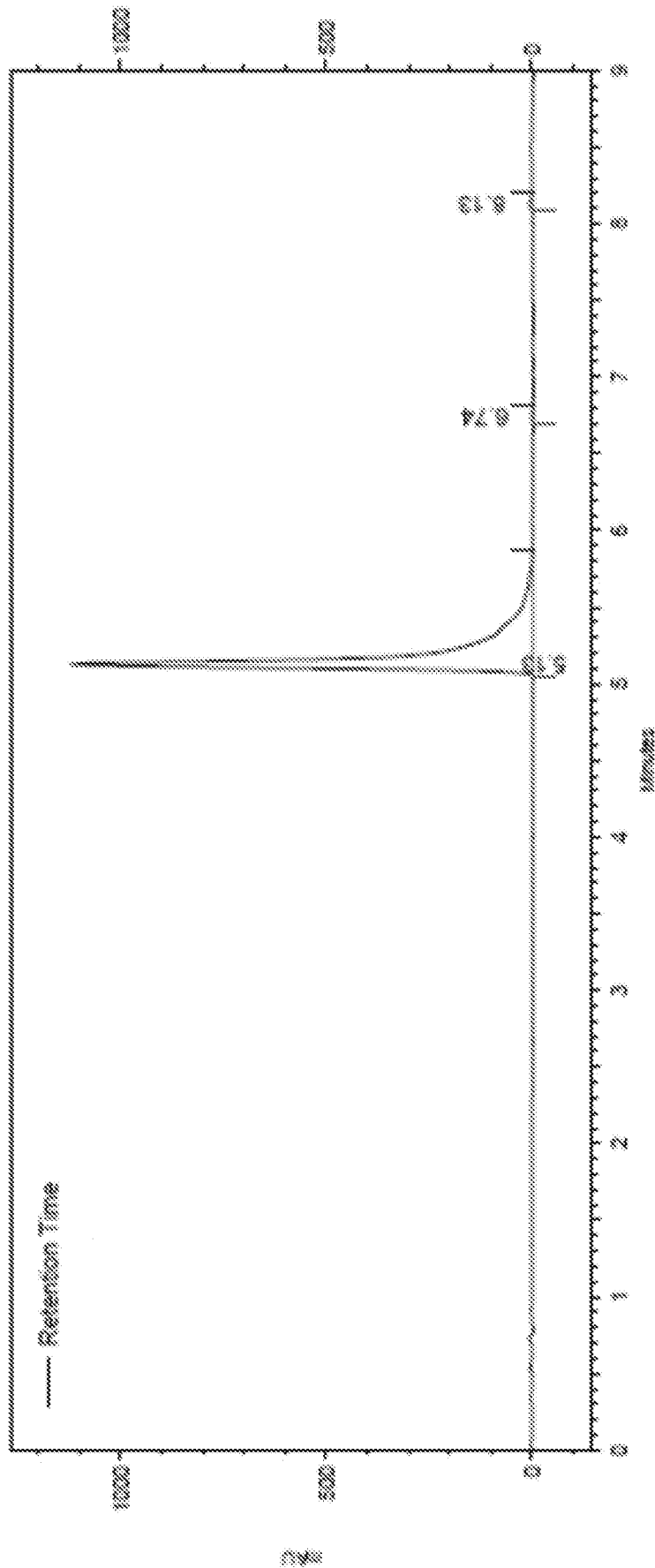


FIG. 8B

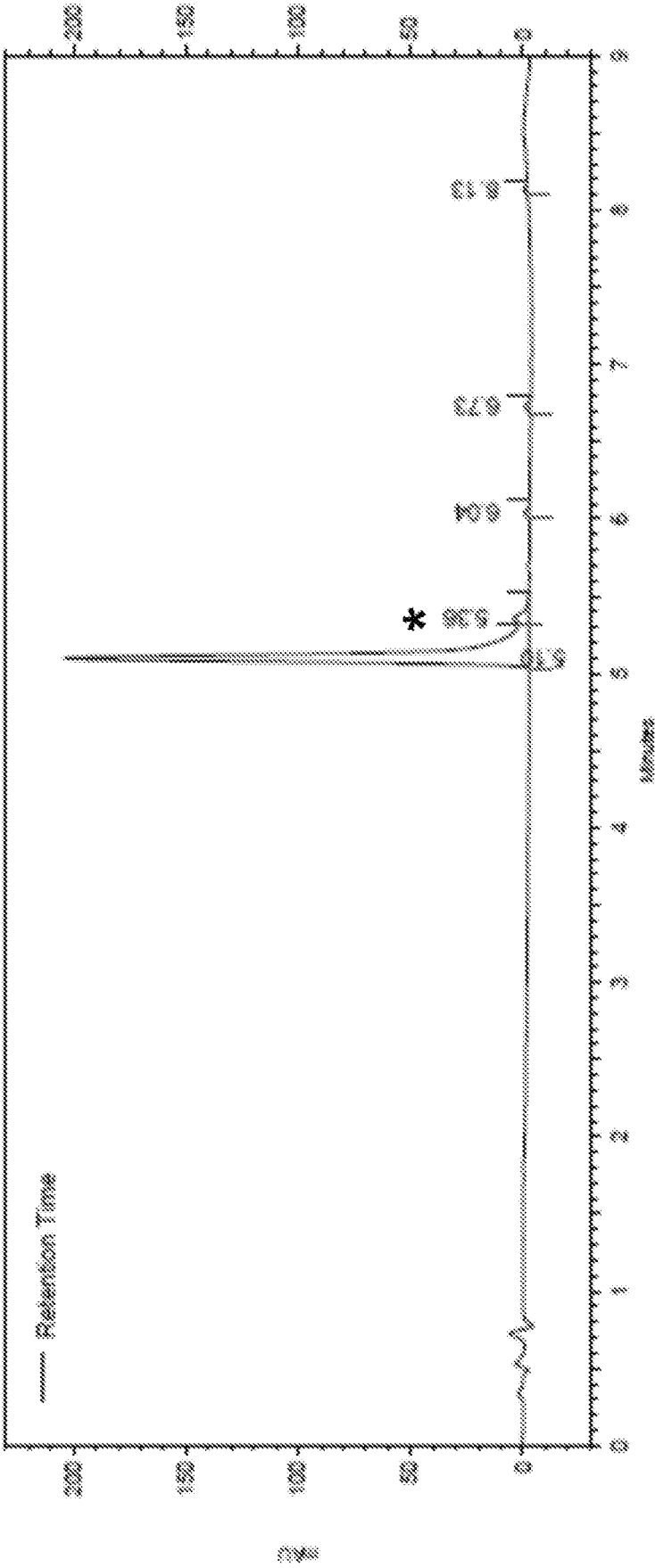


FIG. 9A

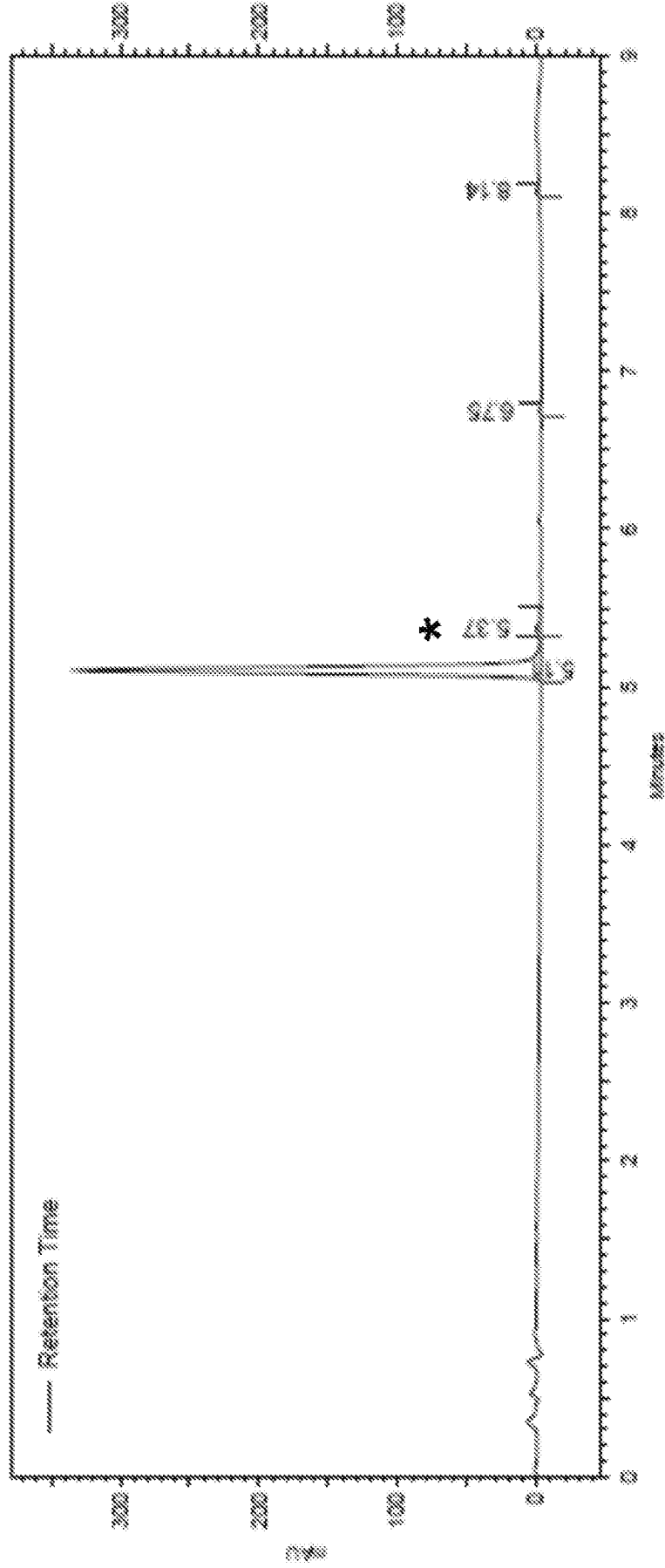


FIG. 9B

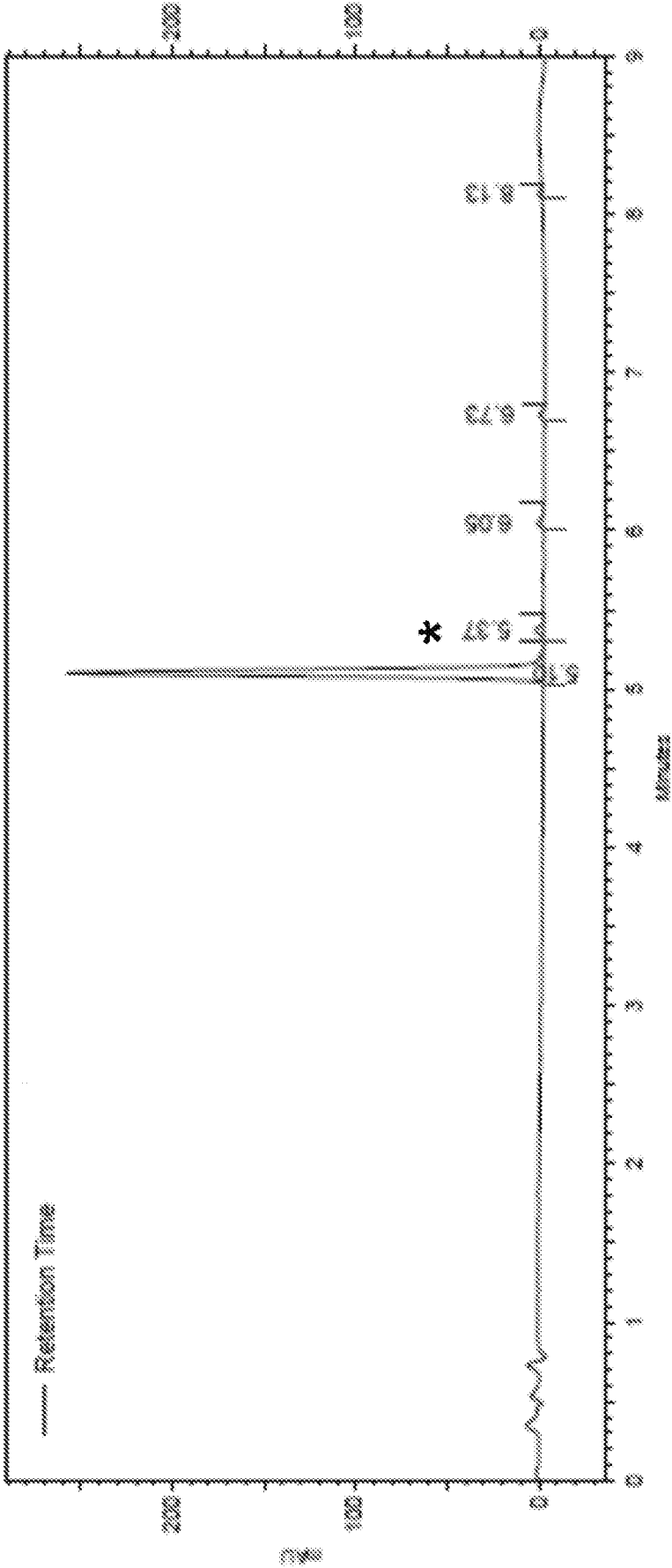


FIG. 10B

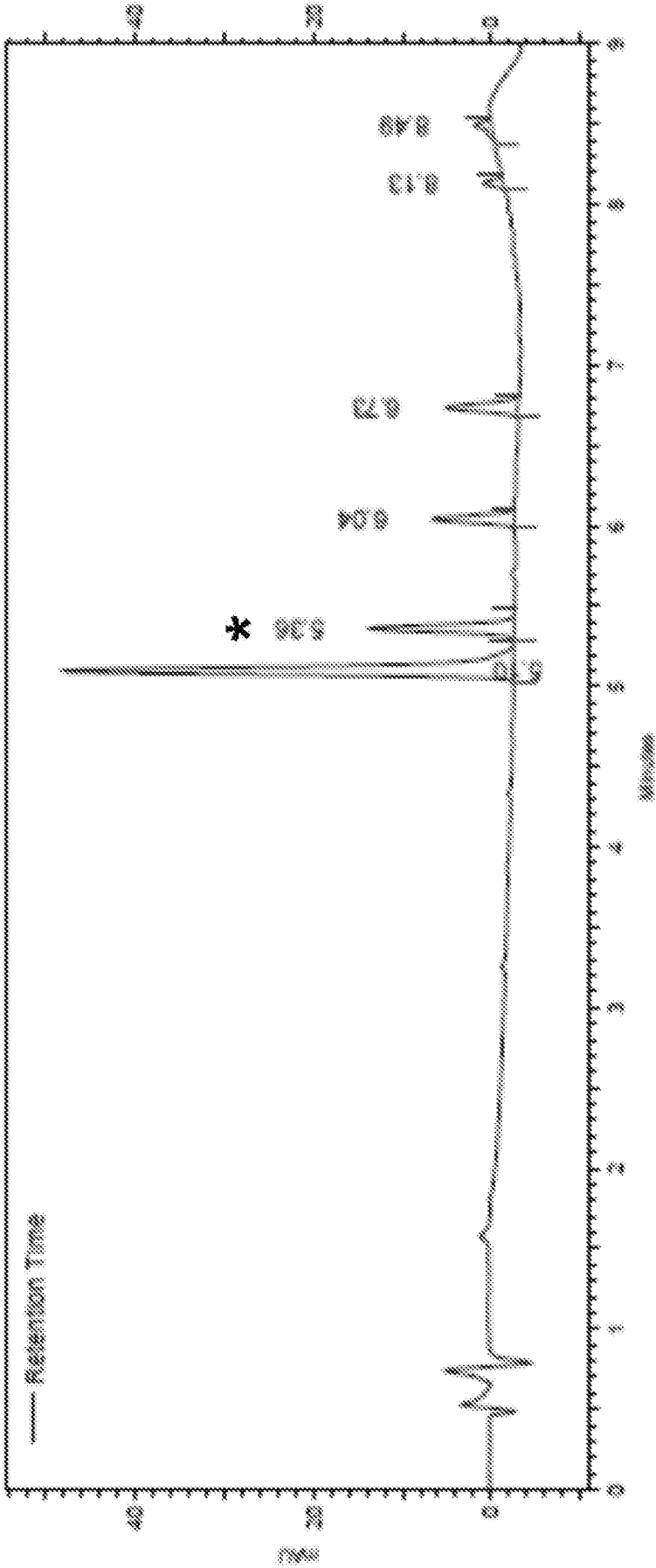


FIG. 11A

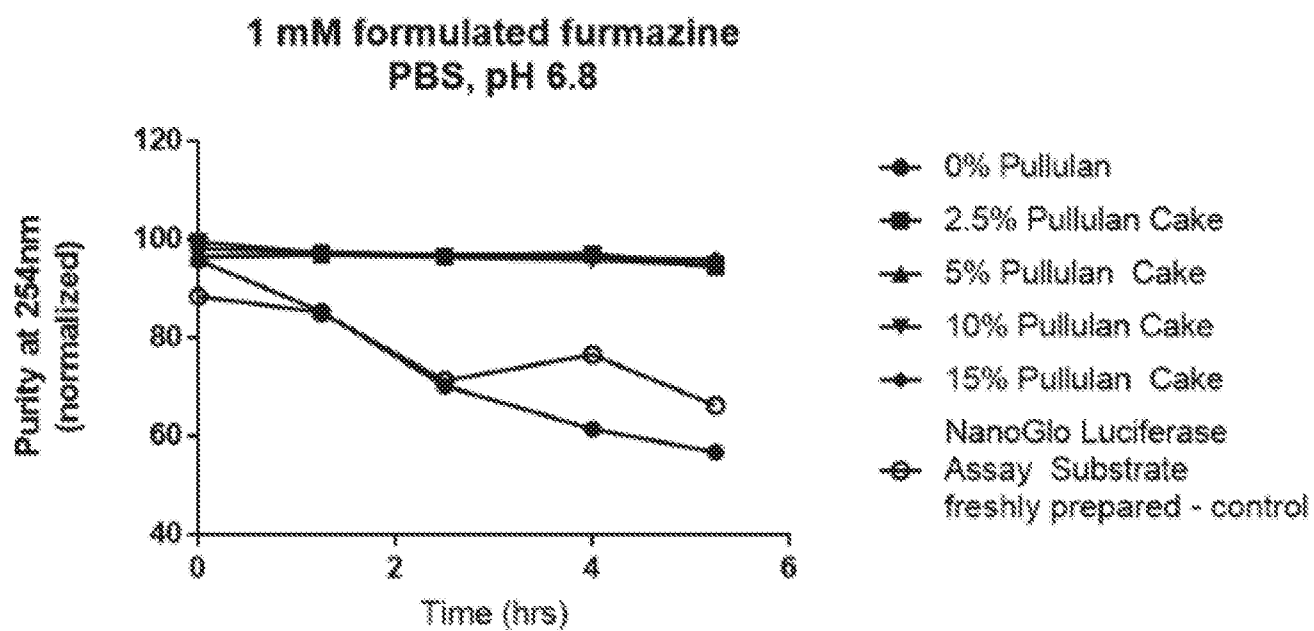


FIG. 11B

1 mM formulated furmazine
PBS, pH 6.8

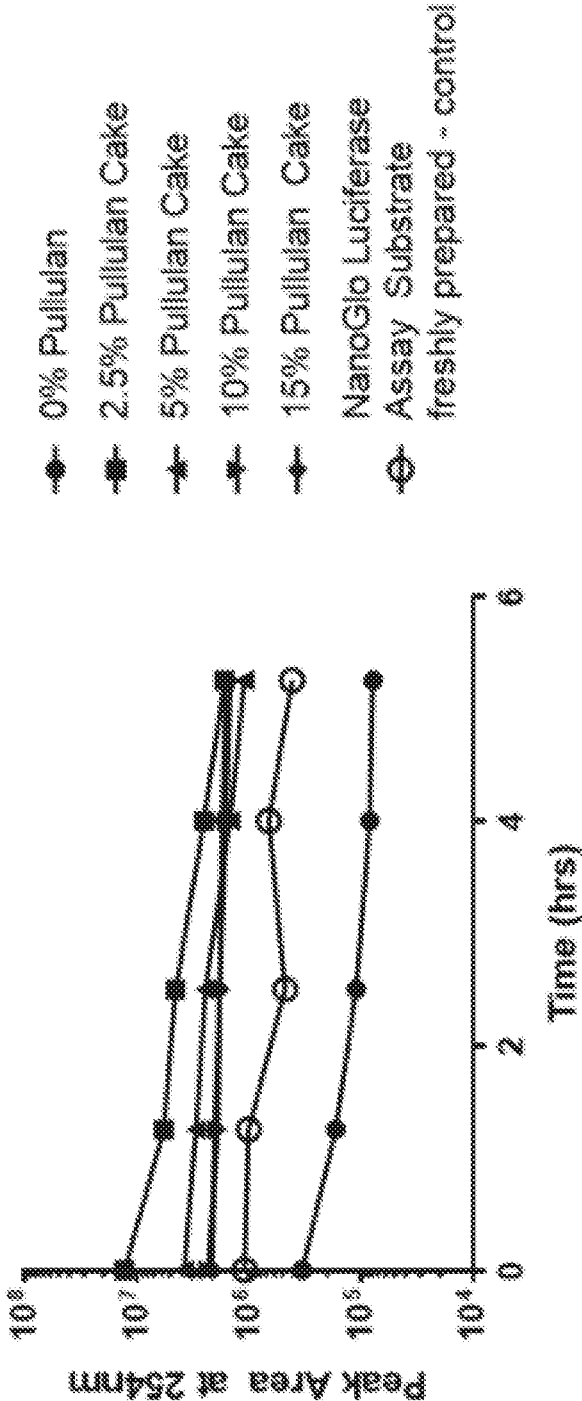


FIG. 12

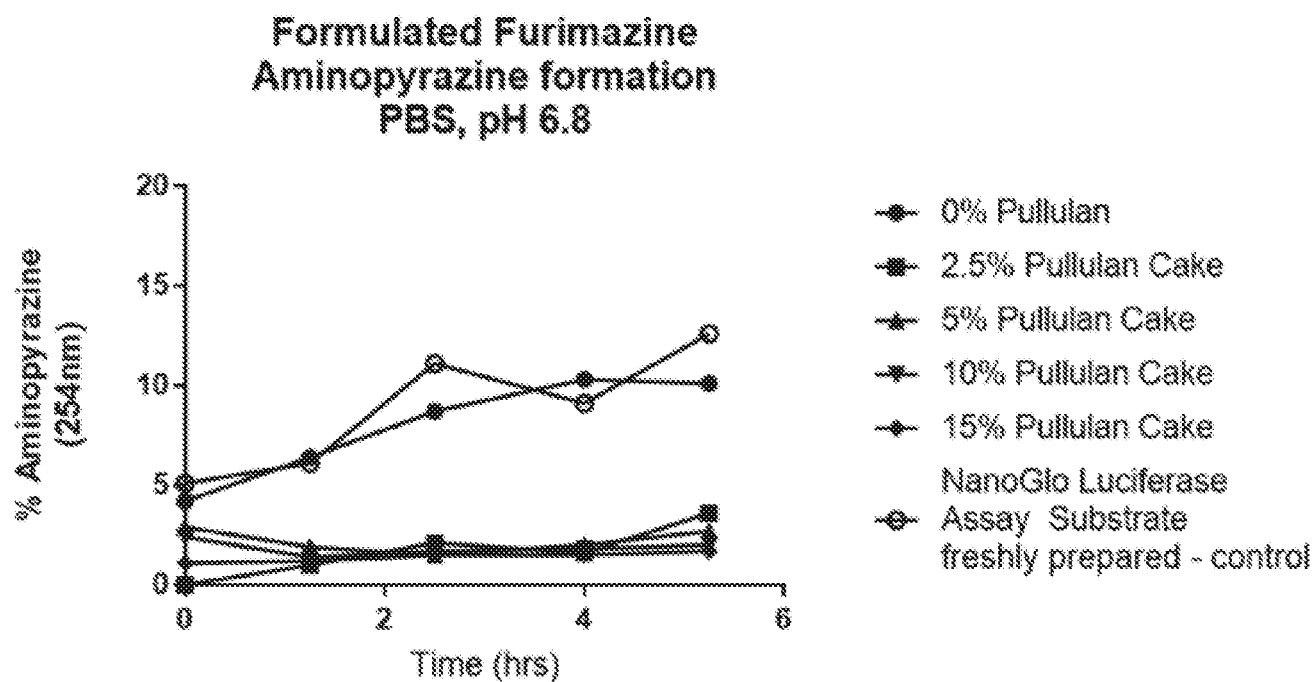
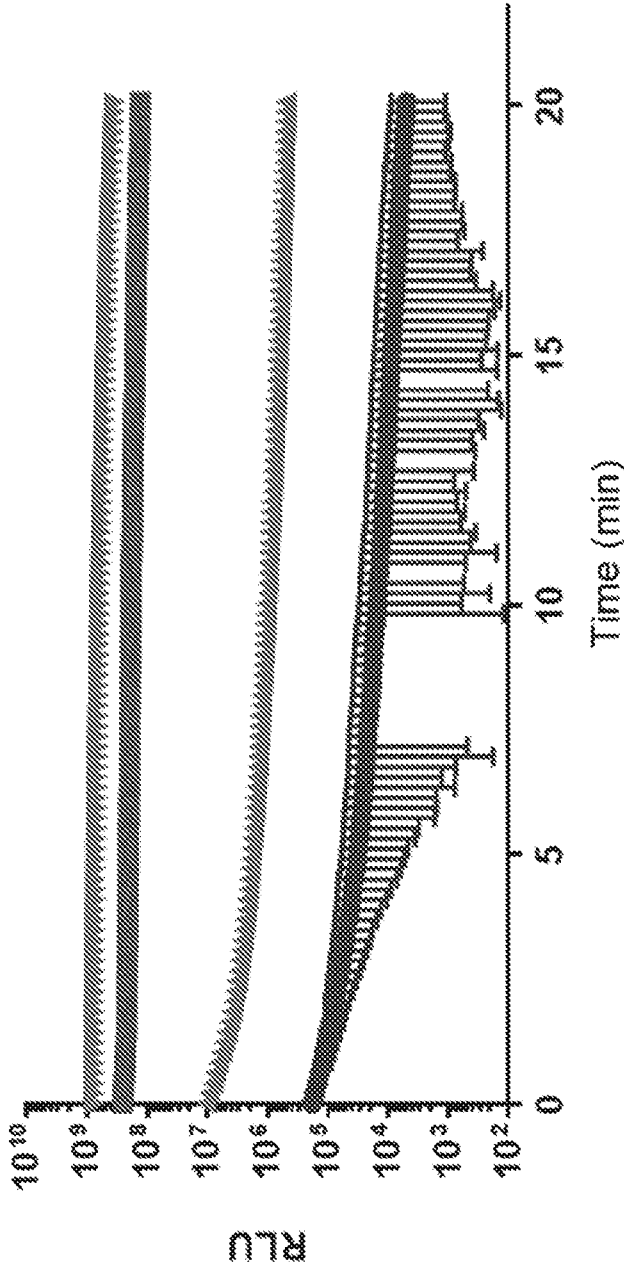


FIG. 13A

Substrate Formulations in
Solid Paper Matrix
PBS, pH 7.0
2 ng/ml NanoLuc enzyme



- ◆ 0.1 uM furimazine paper spot punched from Whatman 903 cards
- 10 uM furimazine paper spot punched from Whatman 903 cards
- ▲ 0.1 uM NanoGlo Luciferase Assay Substrate freshly prepared
- ▼ 10 uM NanoGlo Luciferase Assay Substrate freshly prepared

FIG. 13B

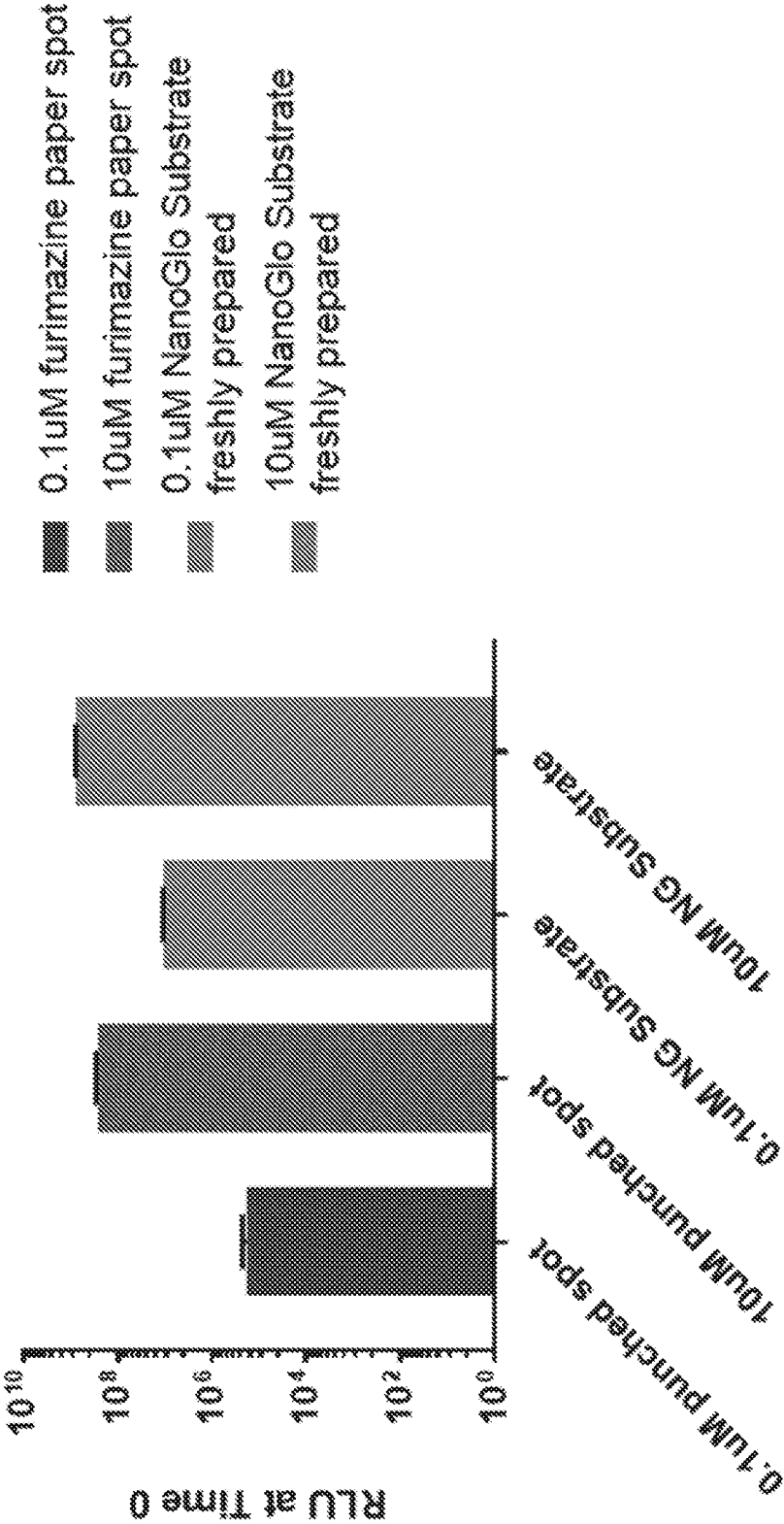


FIG. 13C



FIG. 14

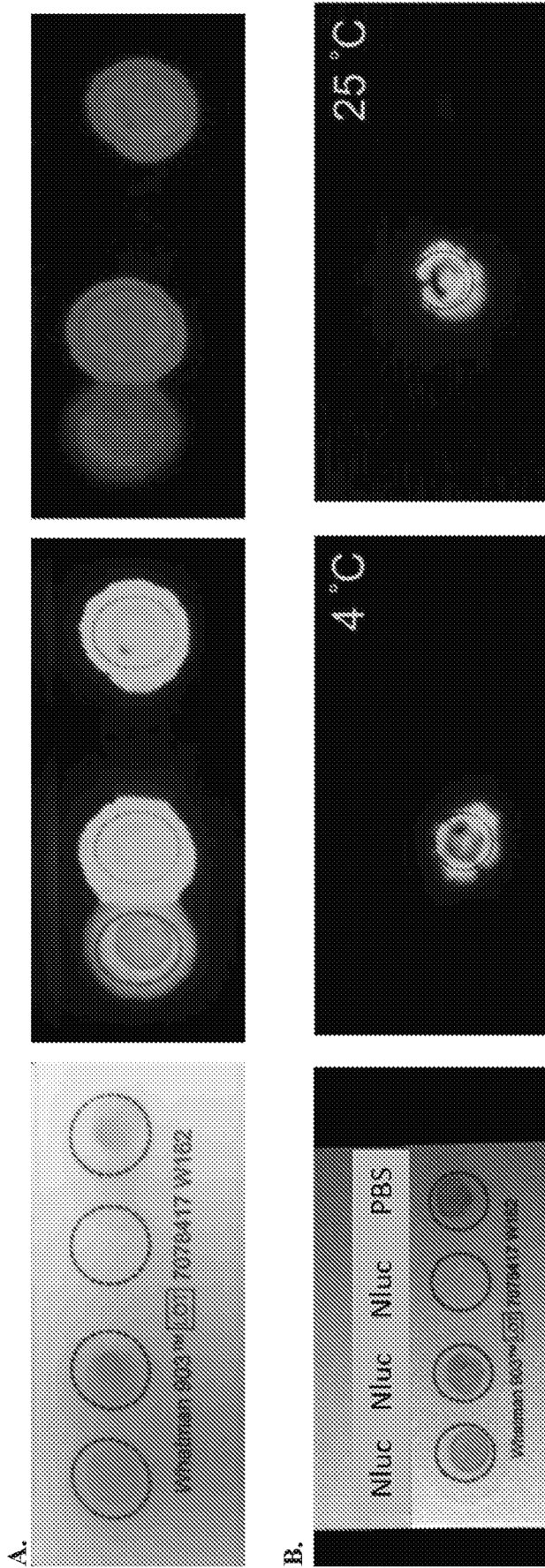
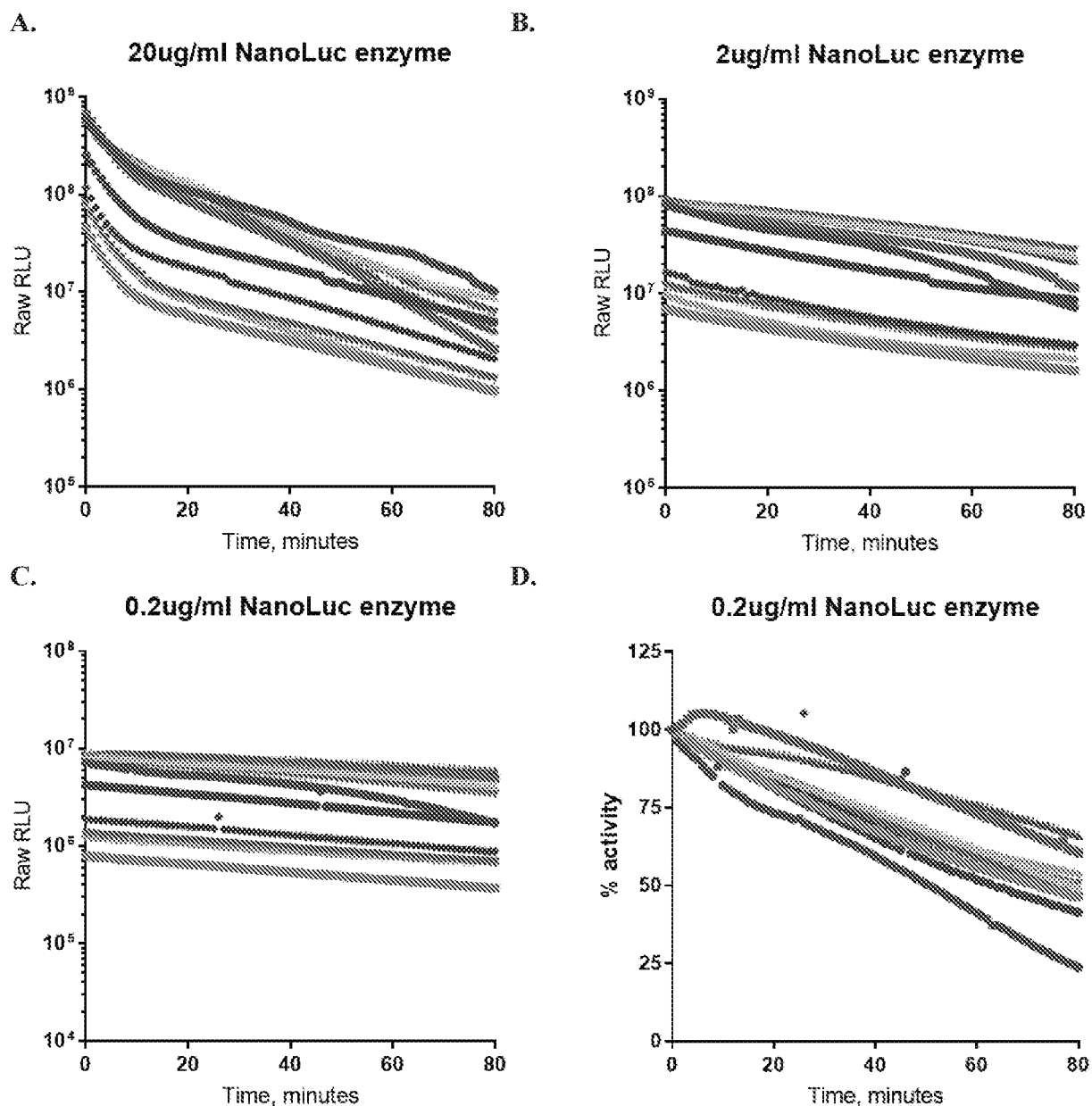


FIG. 15



1. Furimazine in water
2. Furimazine + Sucrose protein buffer
3. Furimazine + ATT (20mM)
4. Furimazine + ATT (50mM)
5. Furimazine + ATT (20mM) + Sucrose protein buffer
6. Furimazine + ATT (50mM) + Sucrose protein buffer
7. Furimazine + Thiourea (20mM)
8. Furimazine + Thiourea (100mM)
9. Furimazine + Thiourea (20mM) + Sucrose protein buffer
10. Furimazine + Thiourea (100mM) + in Sucrose protein buffer

FIG. 16

Formulated Furimazine in
Solid Paper Matrices - Whatman 903 card punches
PBS, pH 7.0
2 ng/ml NanoLuc enzyme

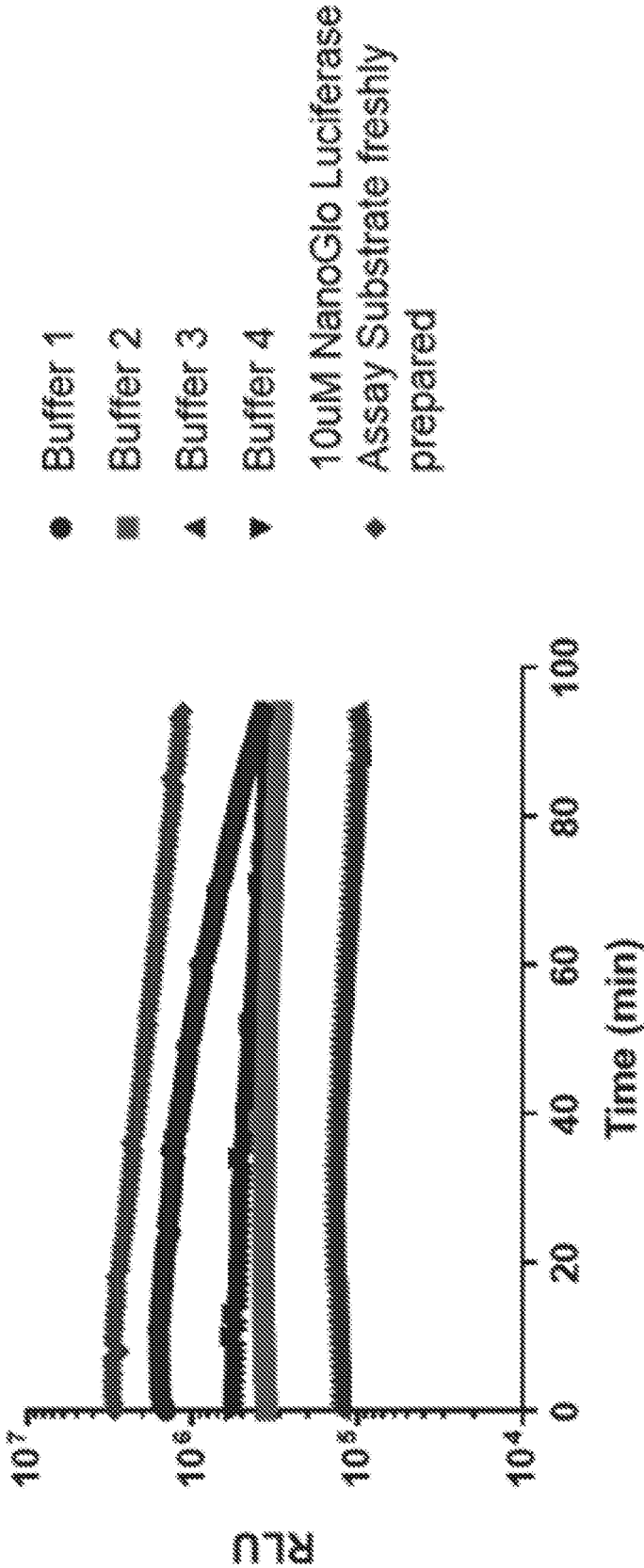


FIG. 17A

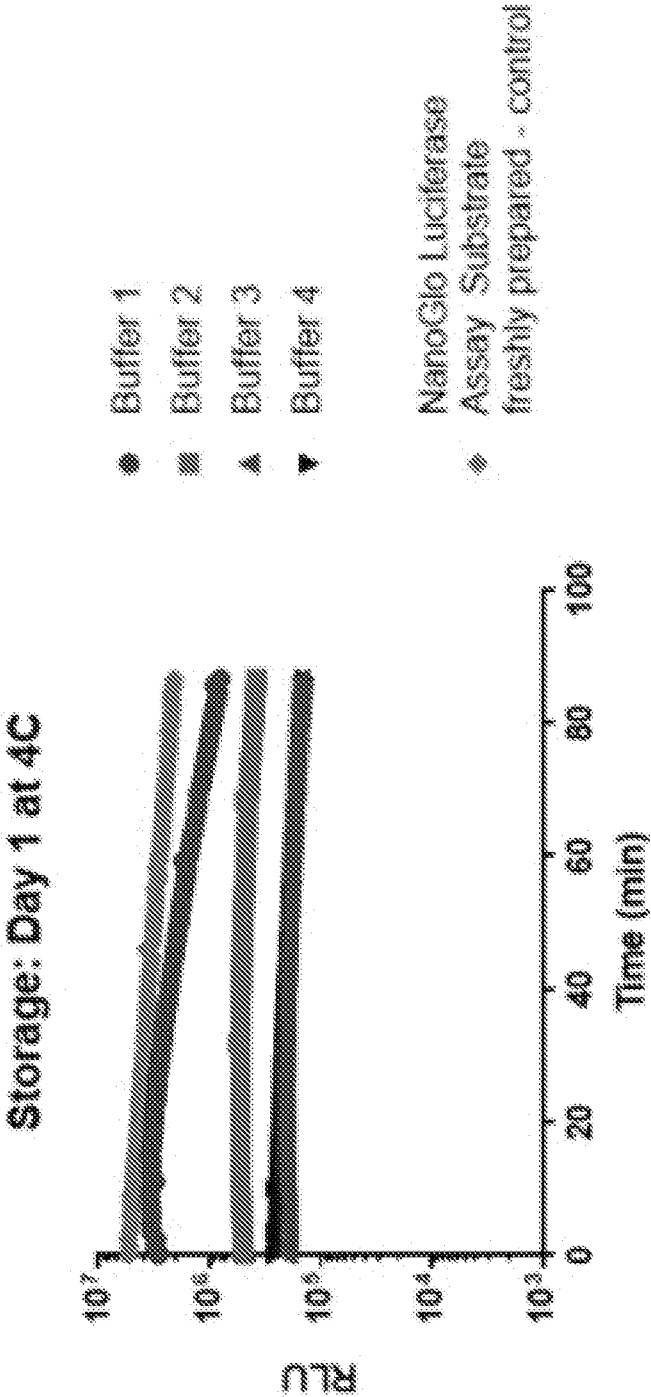


FIG. 17B

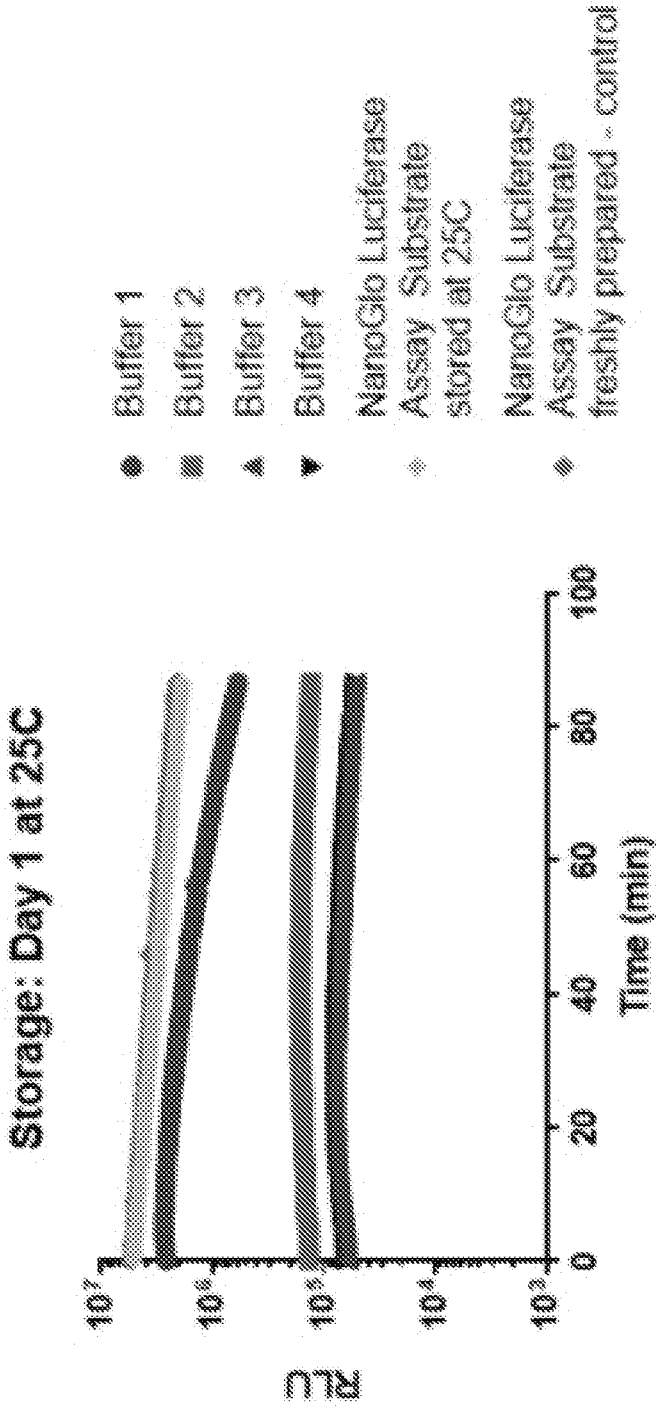


FIG. 17C

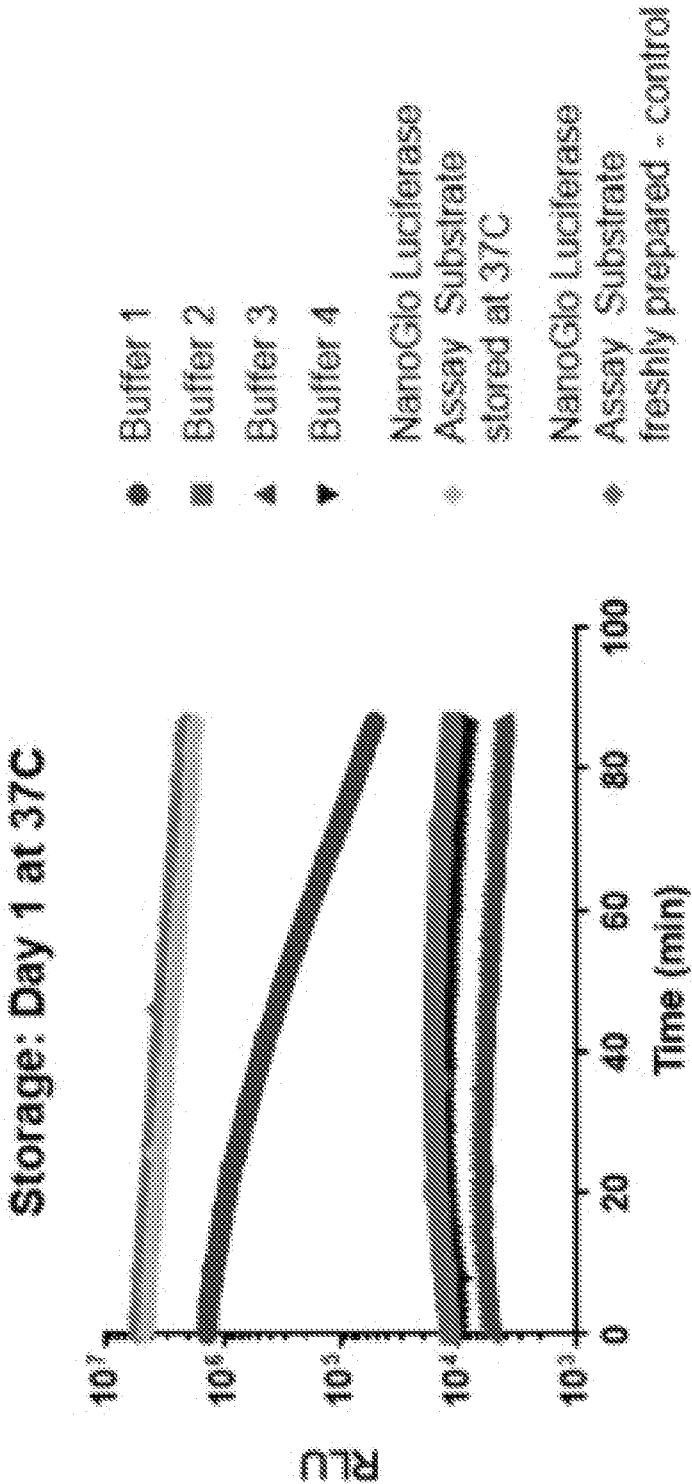


FIG. 18A

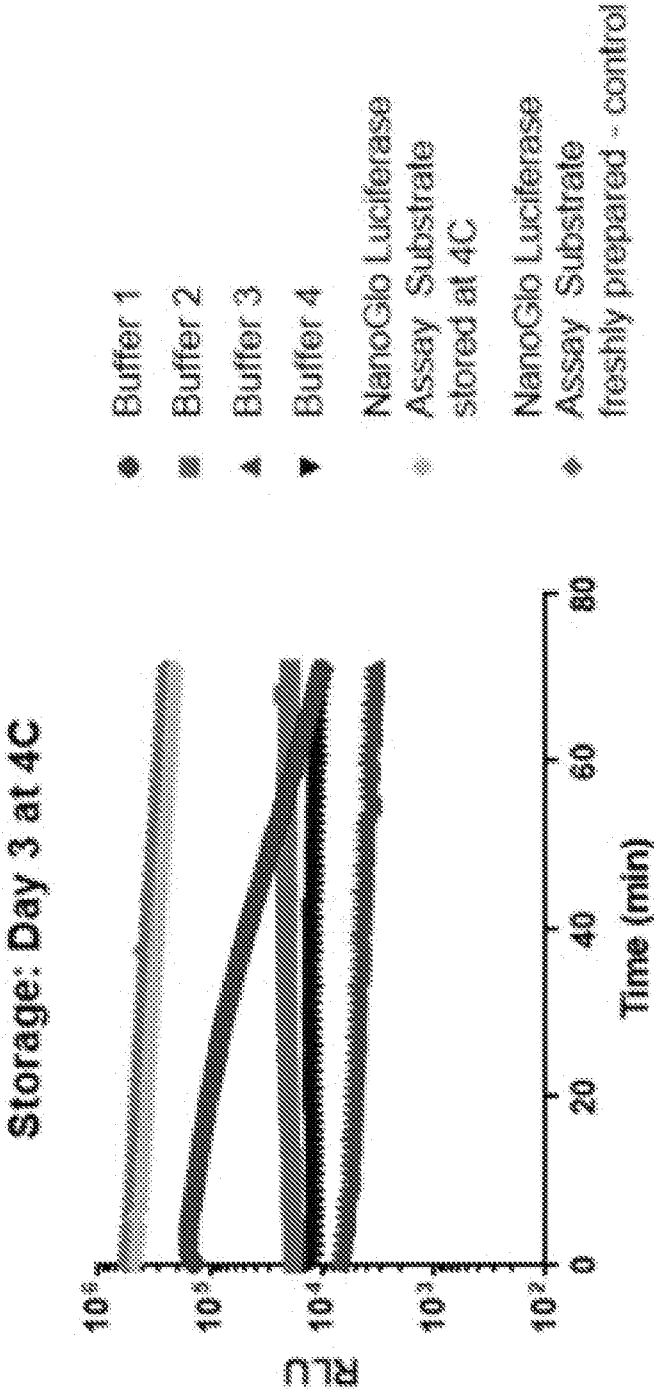


FIG. 18B

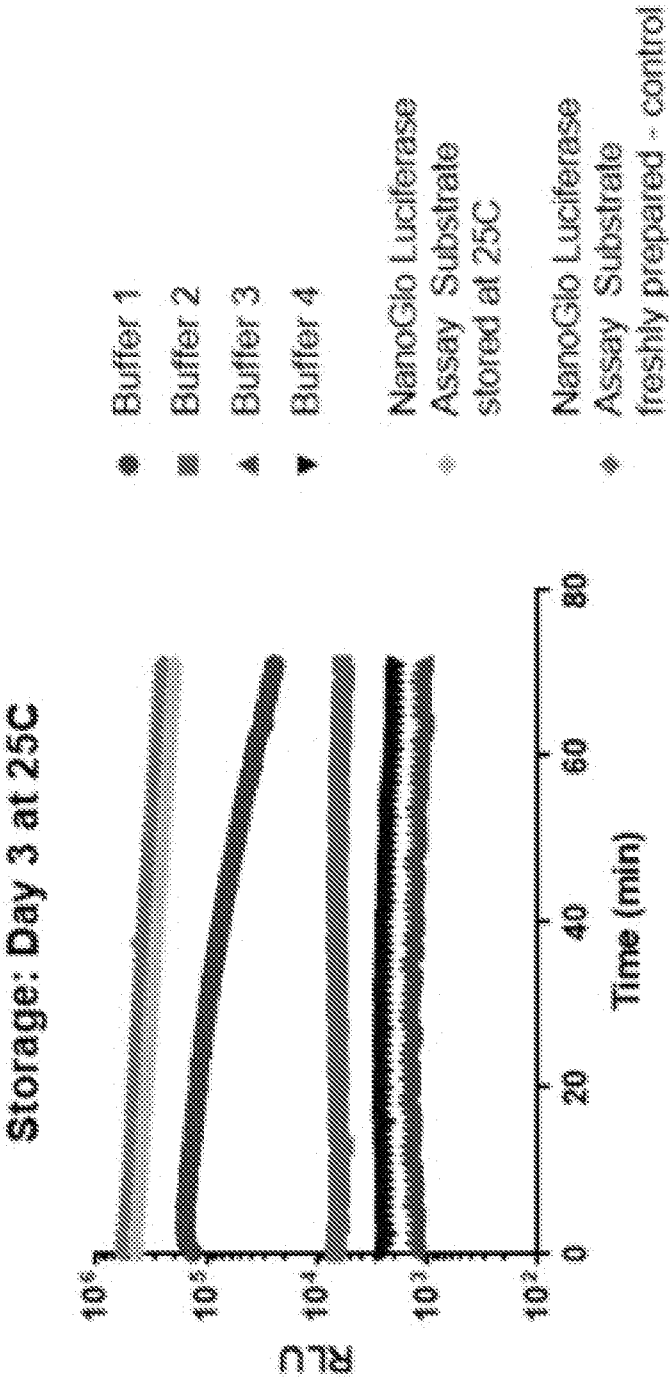


FIG. 18C

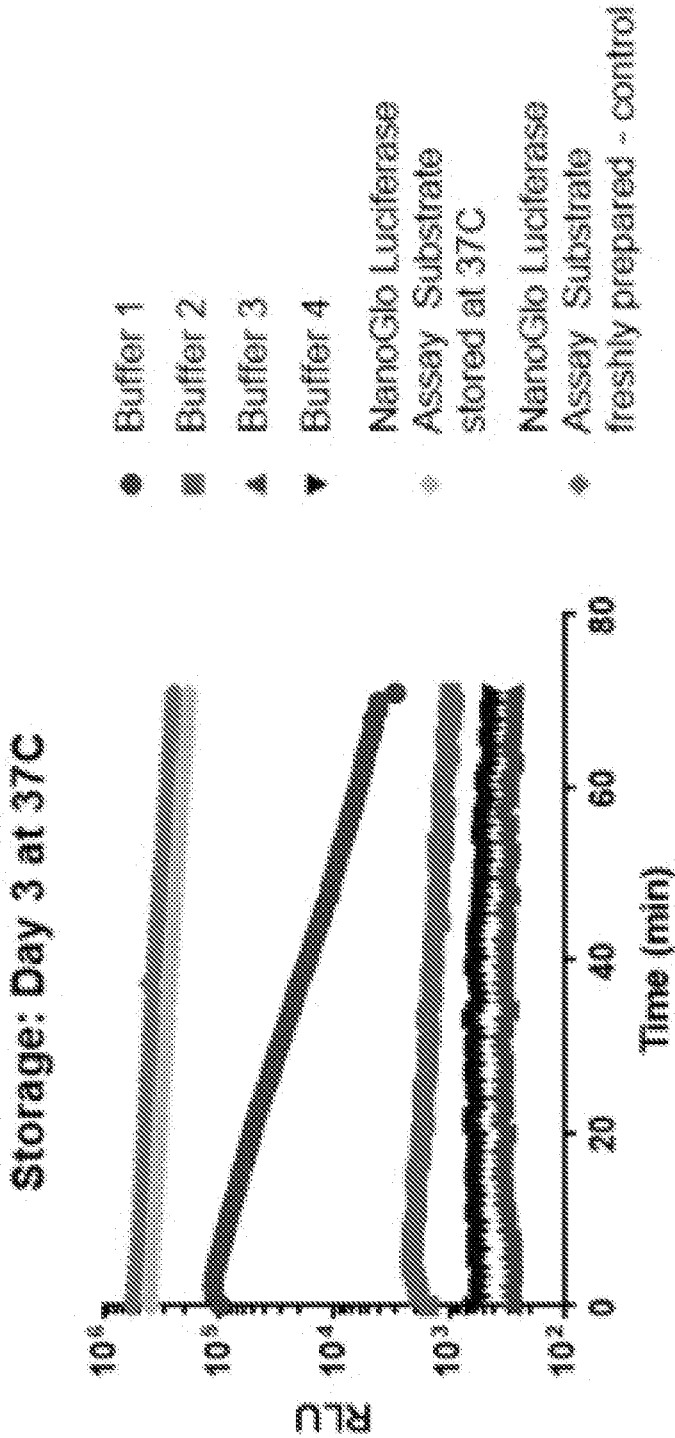


FIG. 19A

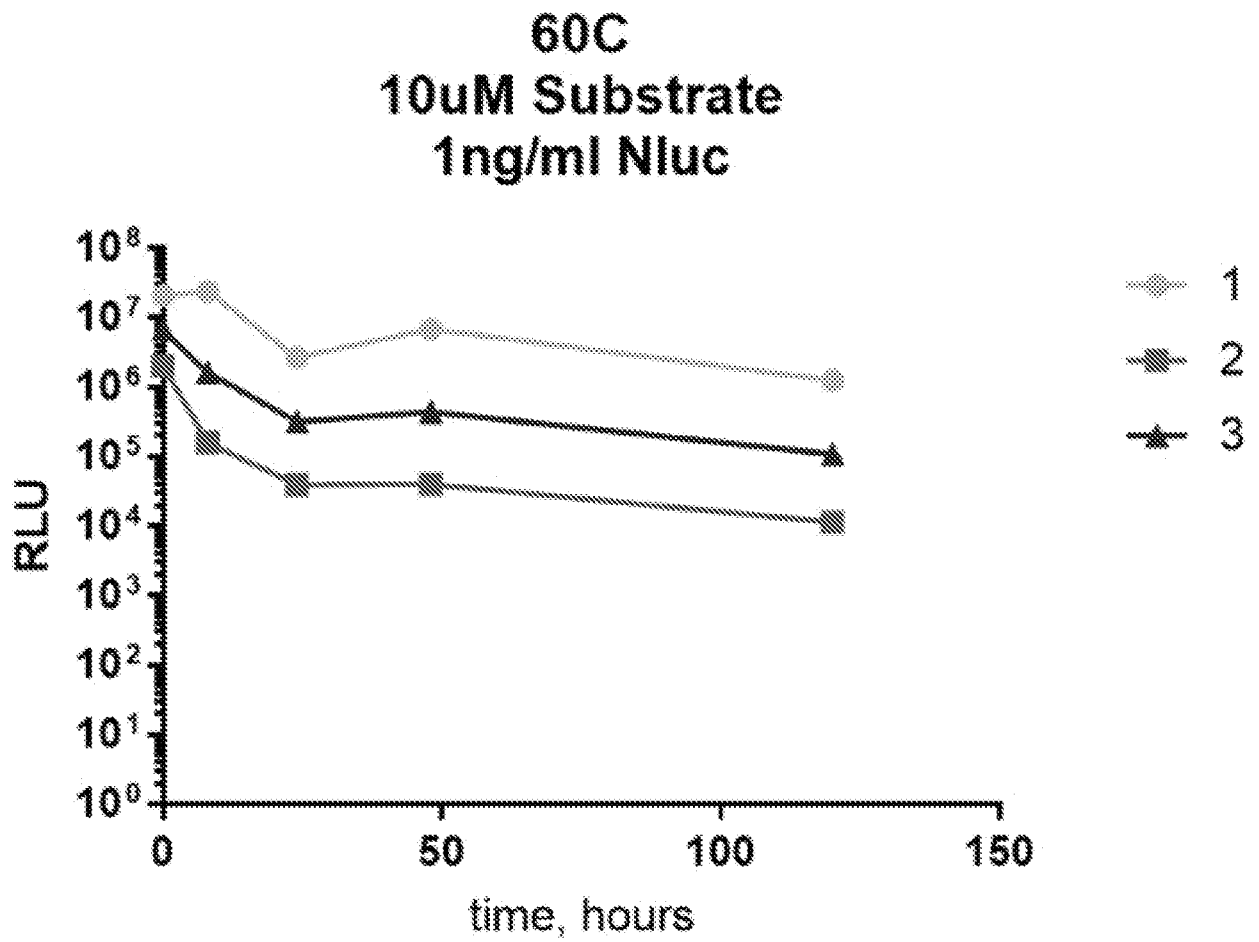


FIG. 19B

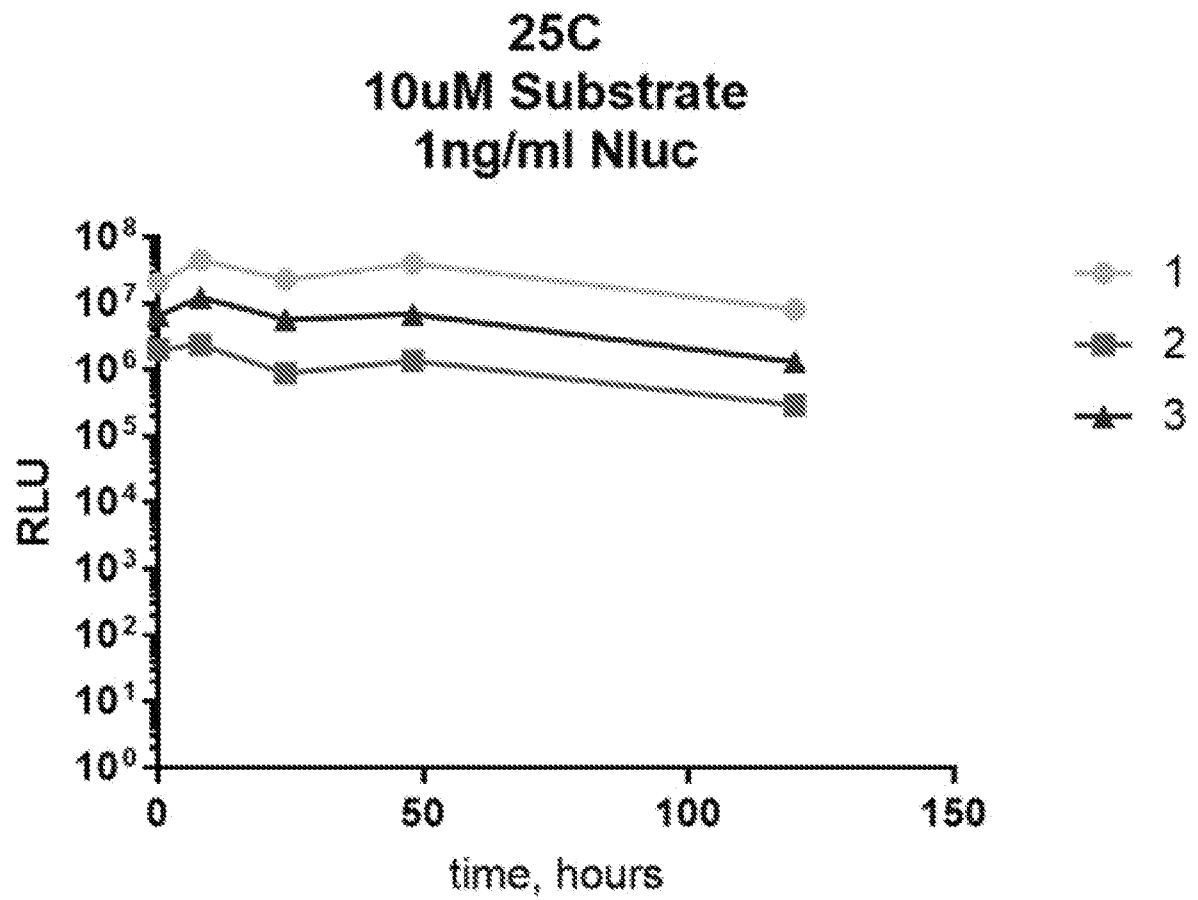


FIG. 19C

60C
10uM Substrate
1ng/ml Nluc

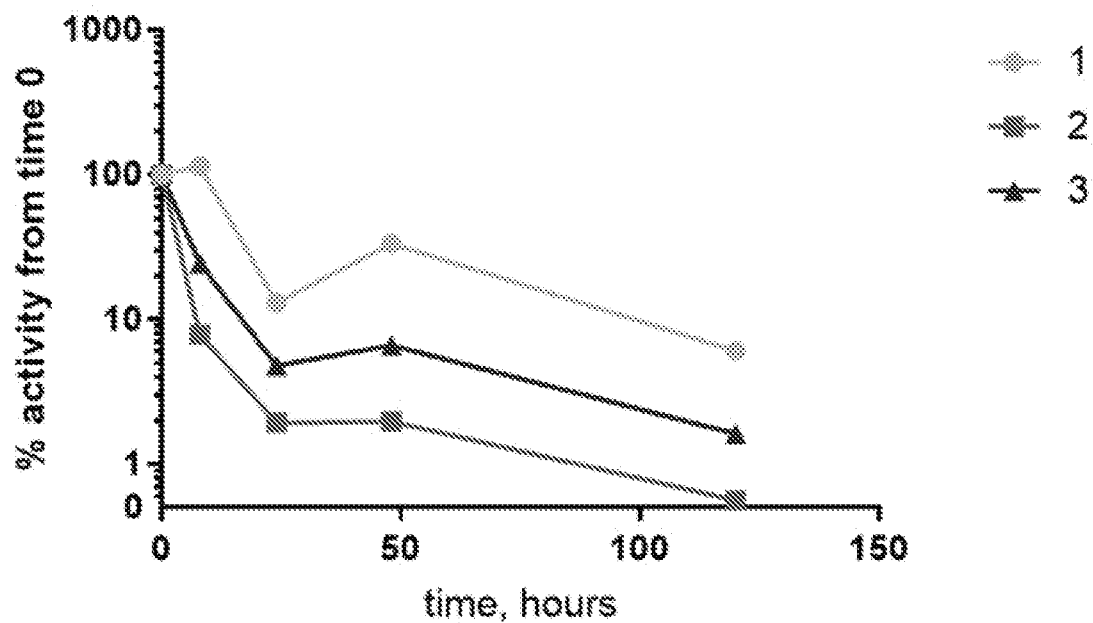


FIG. 19D

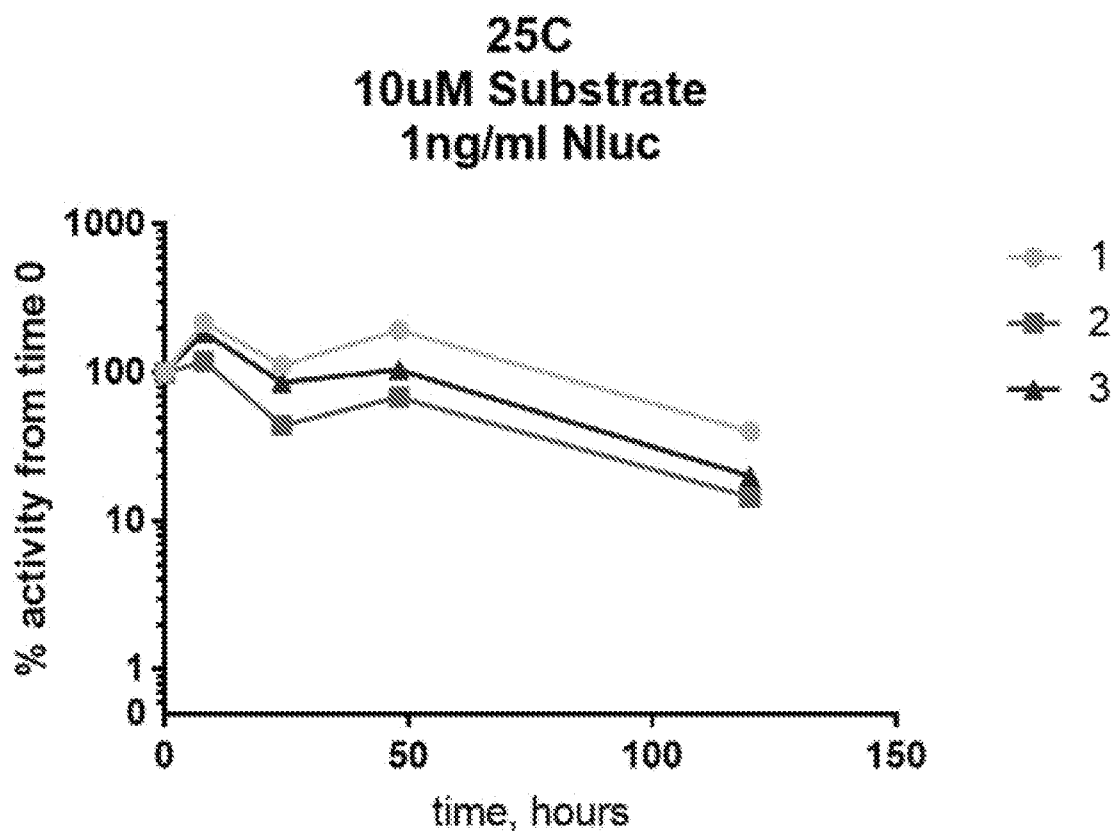


FIG. 20A

Storage Temp 25C

Substrate formulations into paper

2ng/ml Nluc

PBS, pH 7.0 assay buffer

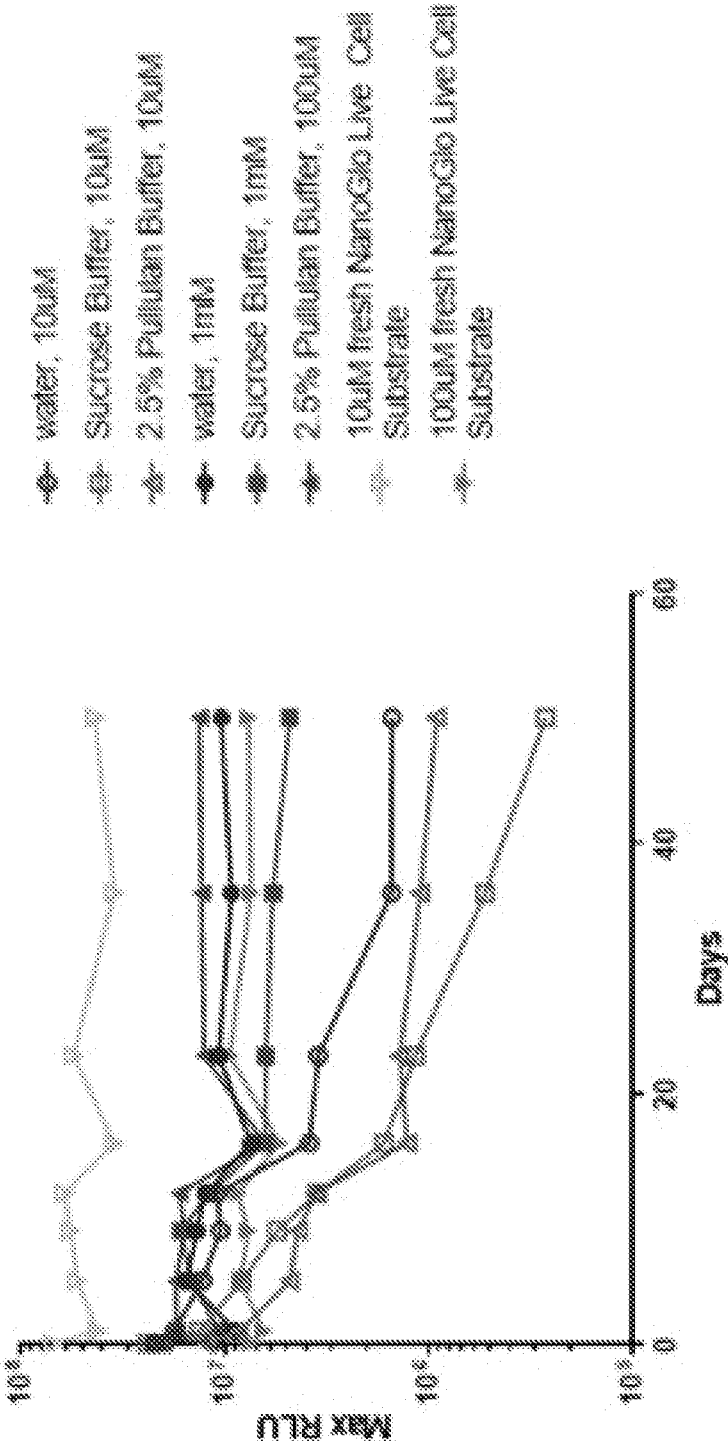


FIG. 20B

Storage Temp 60C

Substrate formulations into paper

2ng/ml NIuc

PBS, pH 7.0 assay buffer

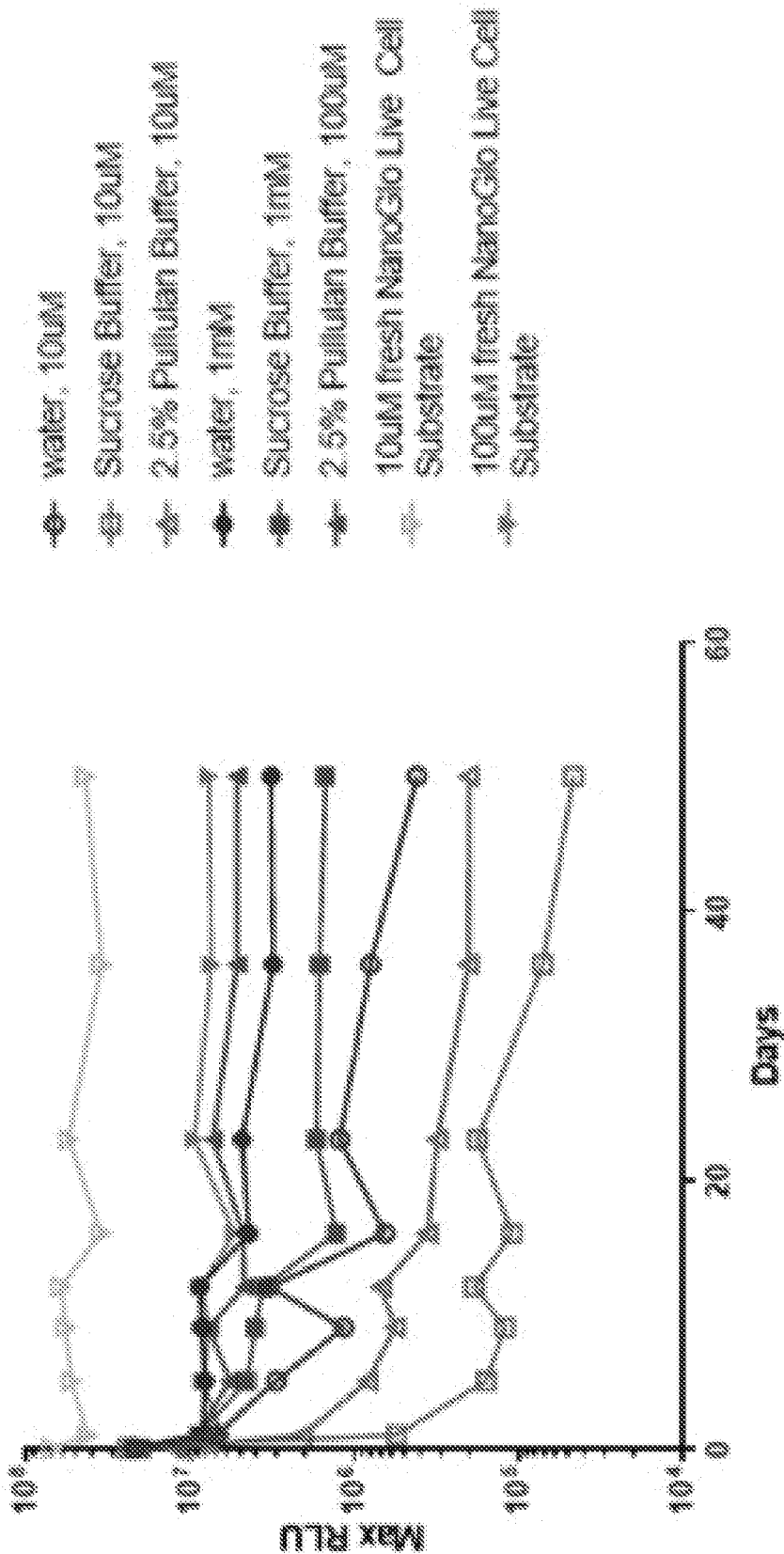


FIG. 20C
Storage Temp 25C
Substrate formulations into paper
2ng/ml Nluc
PBS, pH 7.0 assay buffer

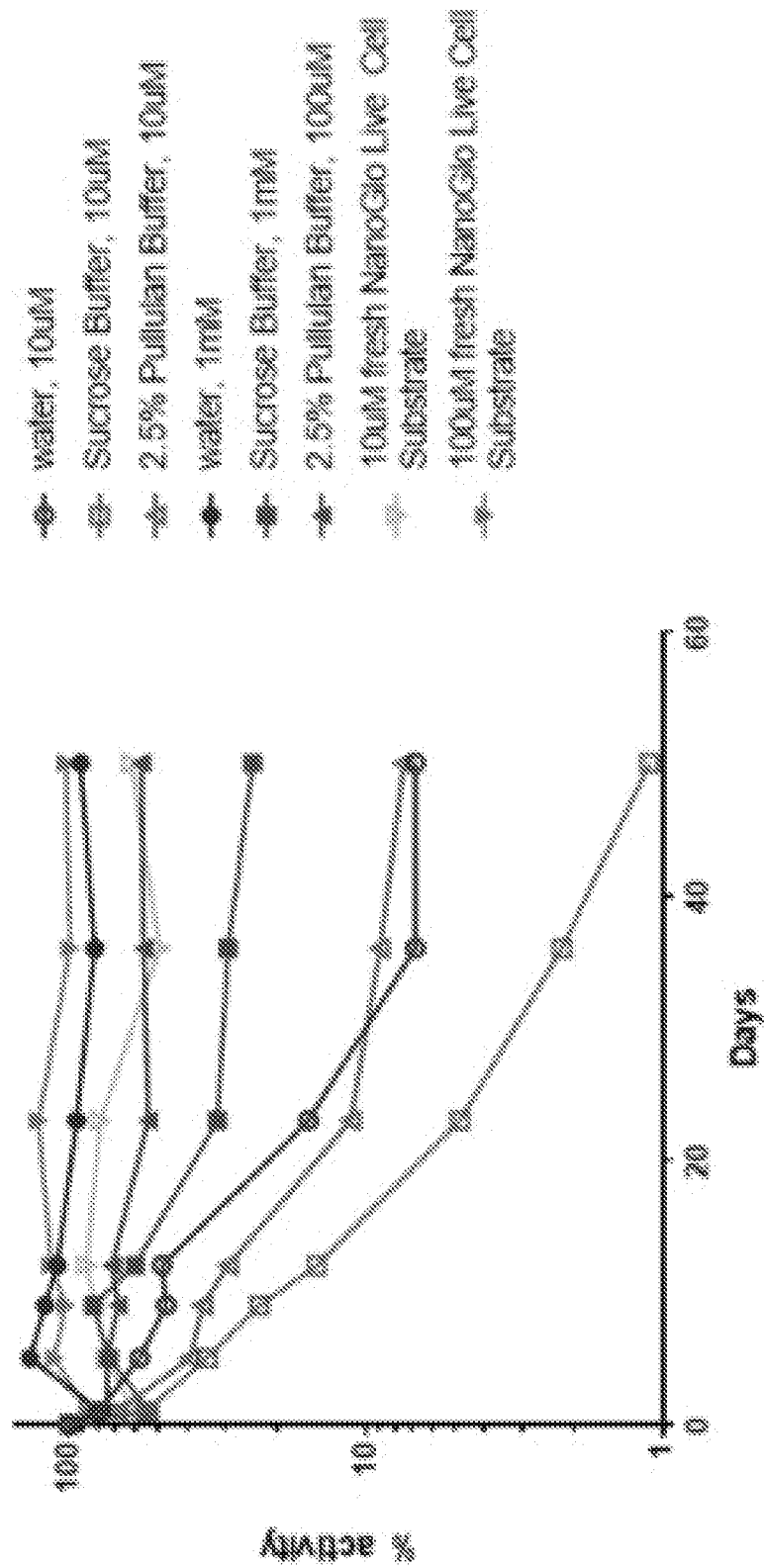


FIG. 20D

Storage Temp 60C
Substrate formulations into paper
2ng/ml Niluc
PBS, pH 7.0 assay buffer

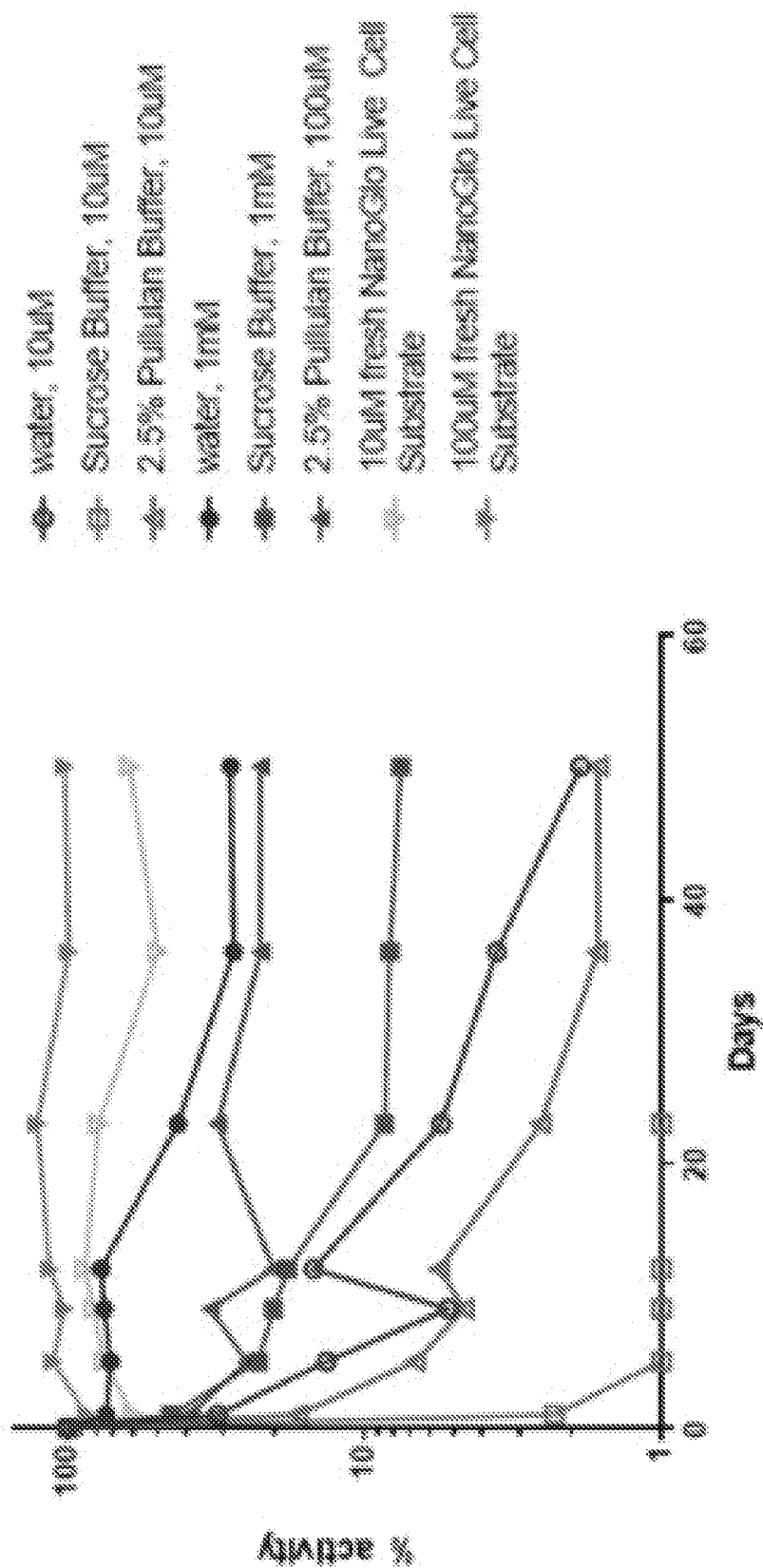


FIG. 21A

**Furimazine Compositions into Paper
Dried Under Vacuum**

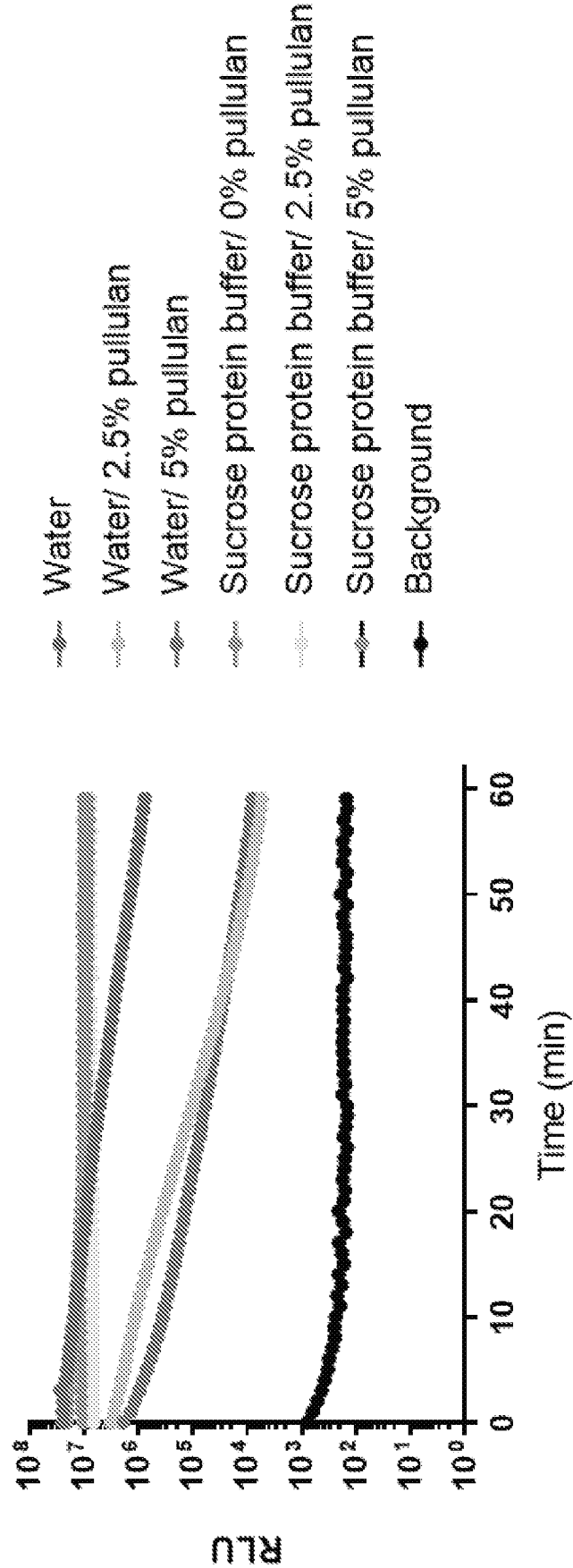


FIG. 21B
Furimazine Compositions into Paper
Dried Under Ambient Air

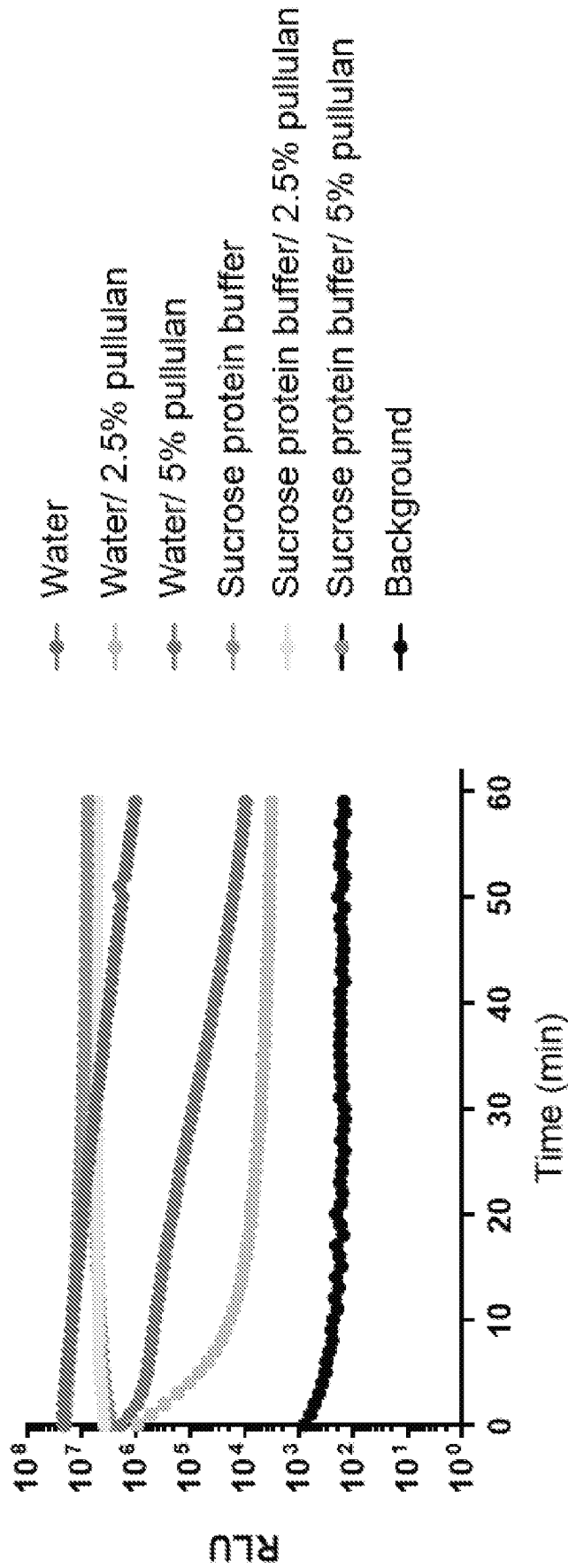


FIG. 21C

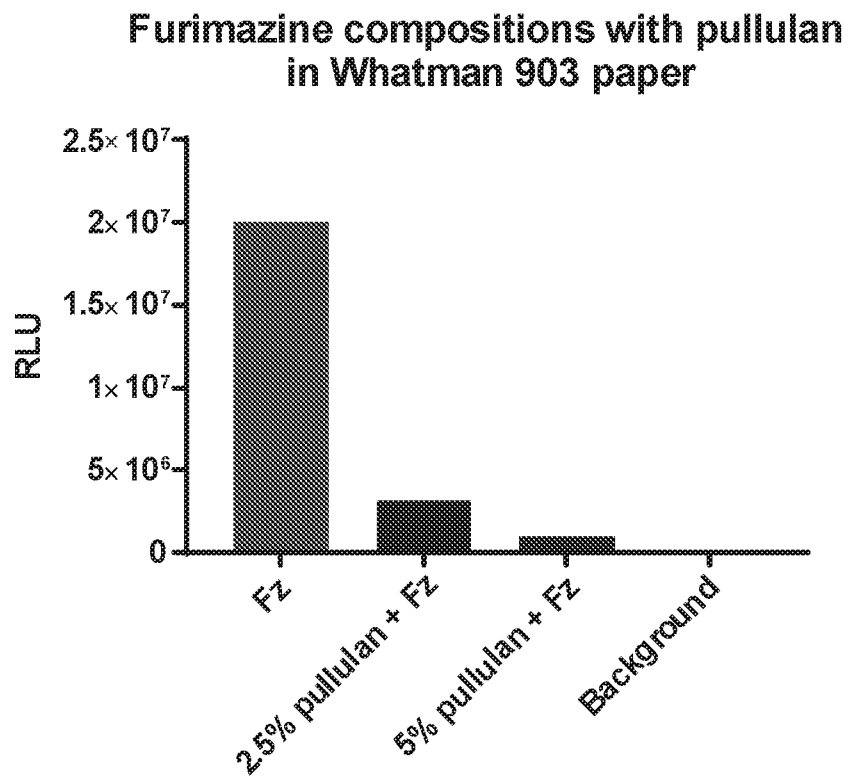


FIG. 22A

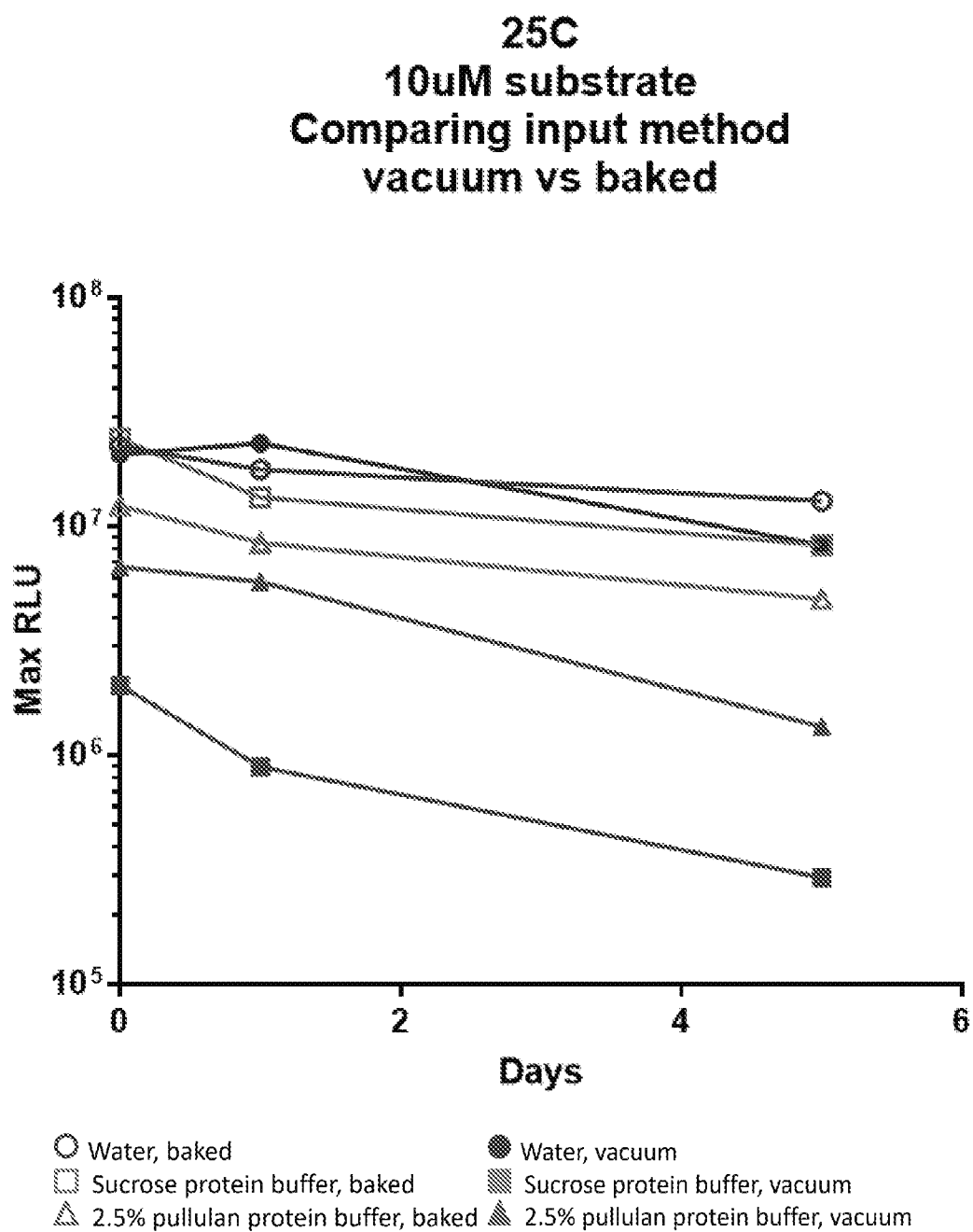


FIG. 22B

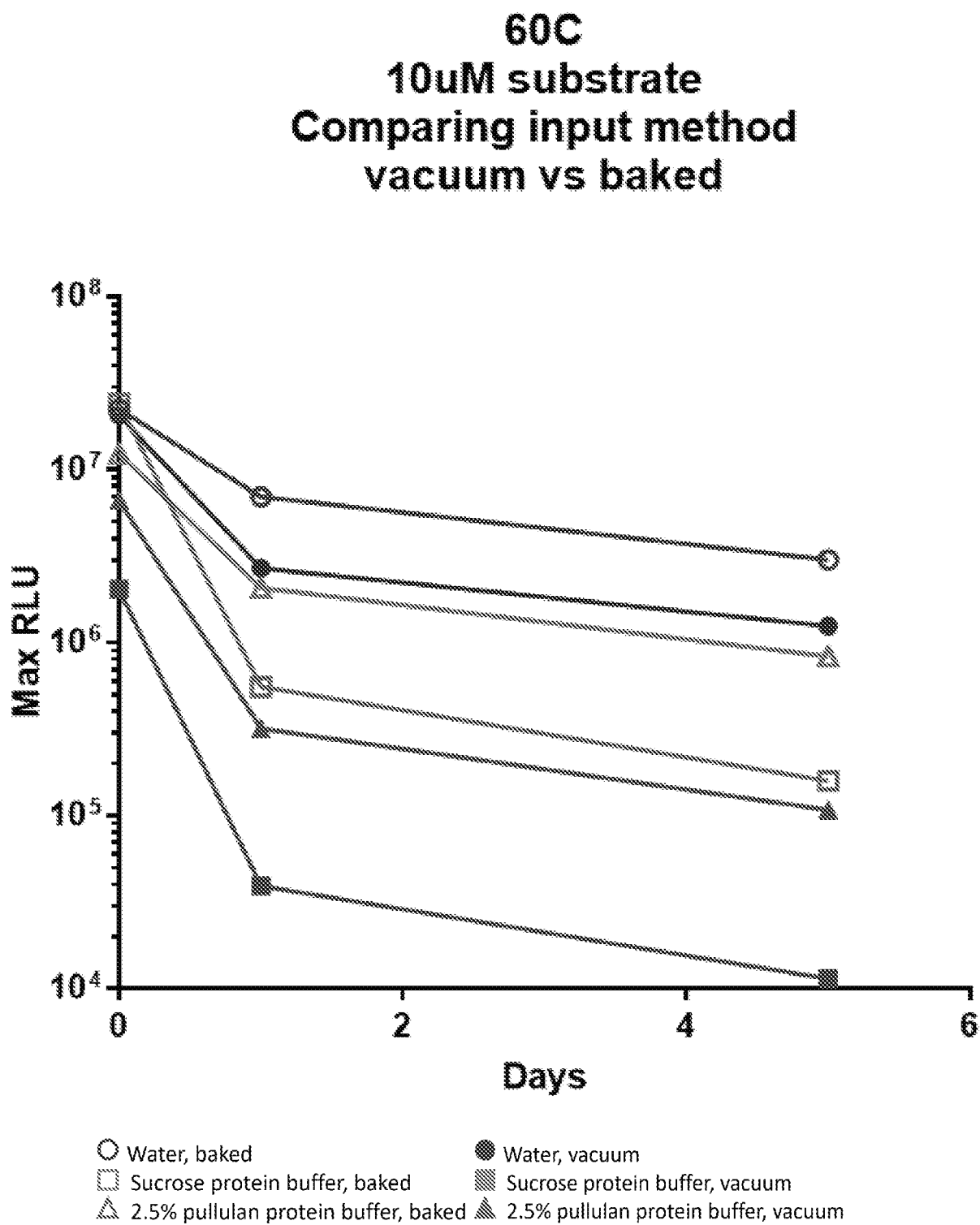


FIG. 22C

25C
10uM substrate
Comparing input method
vacuum vs baked

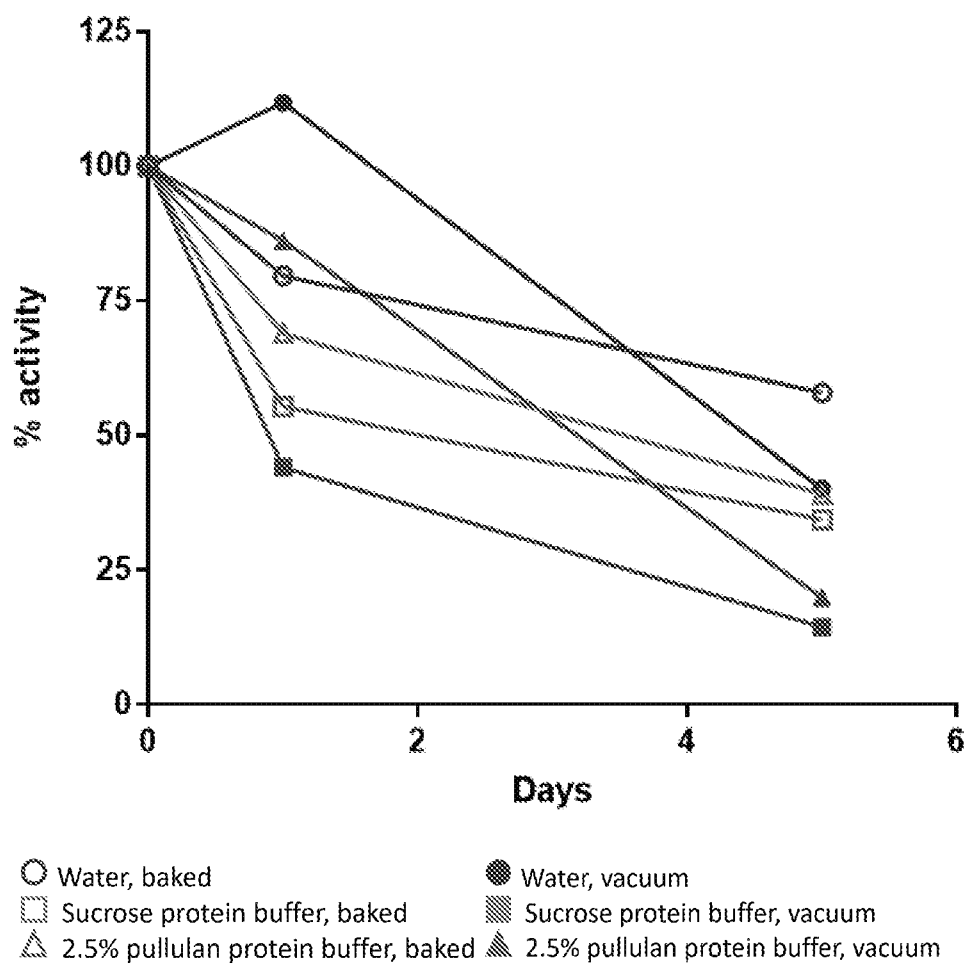


FIG. 22D

60C
10uM substrate
Comparing input method
vacuum vs baked

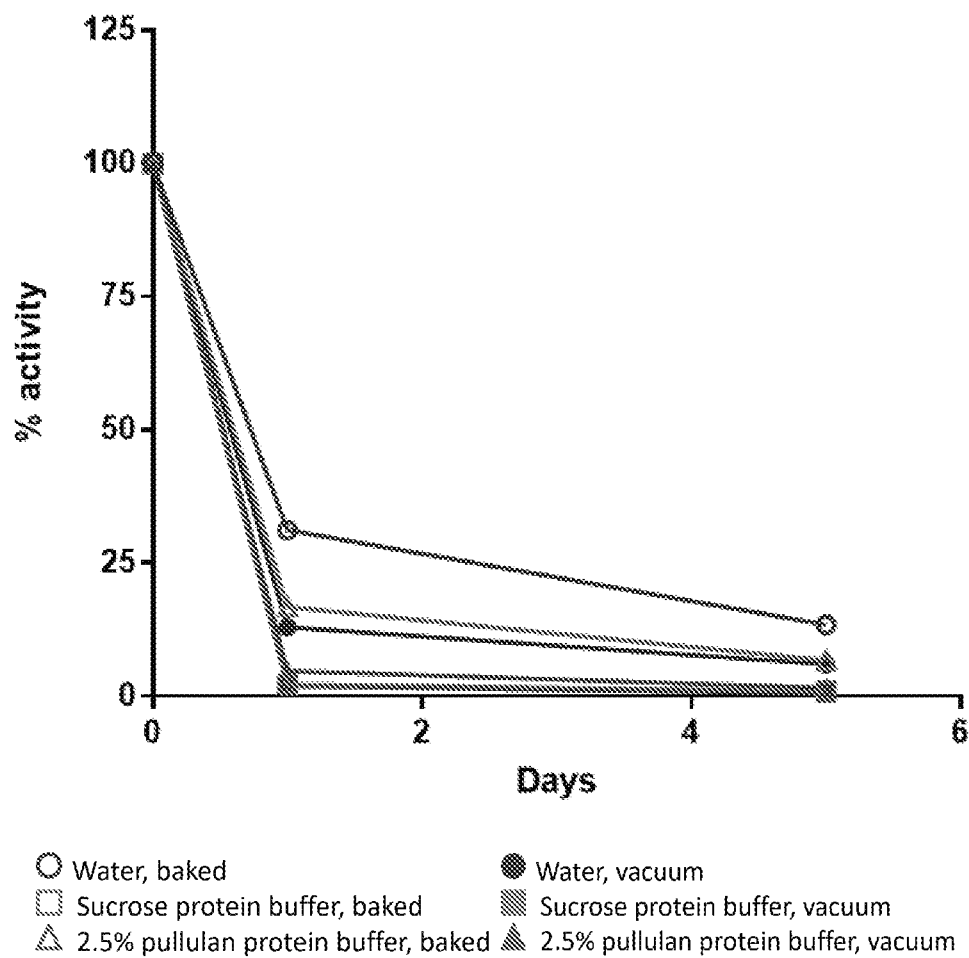
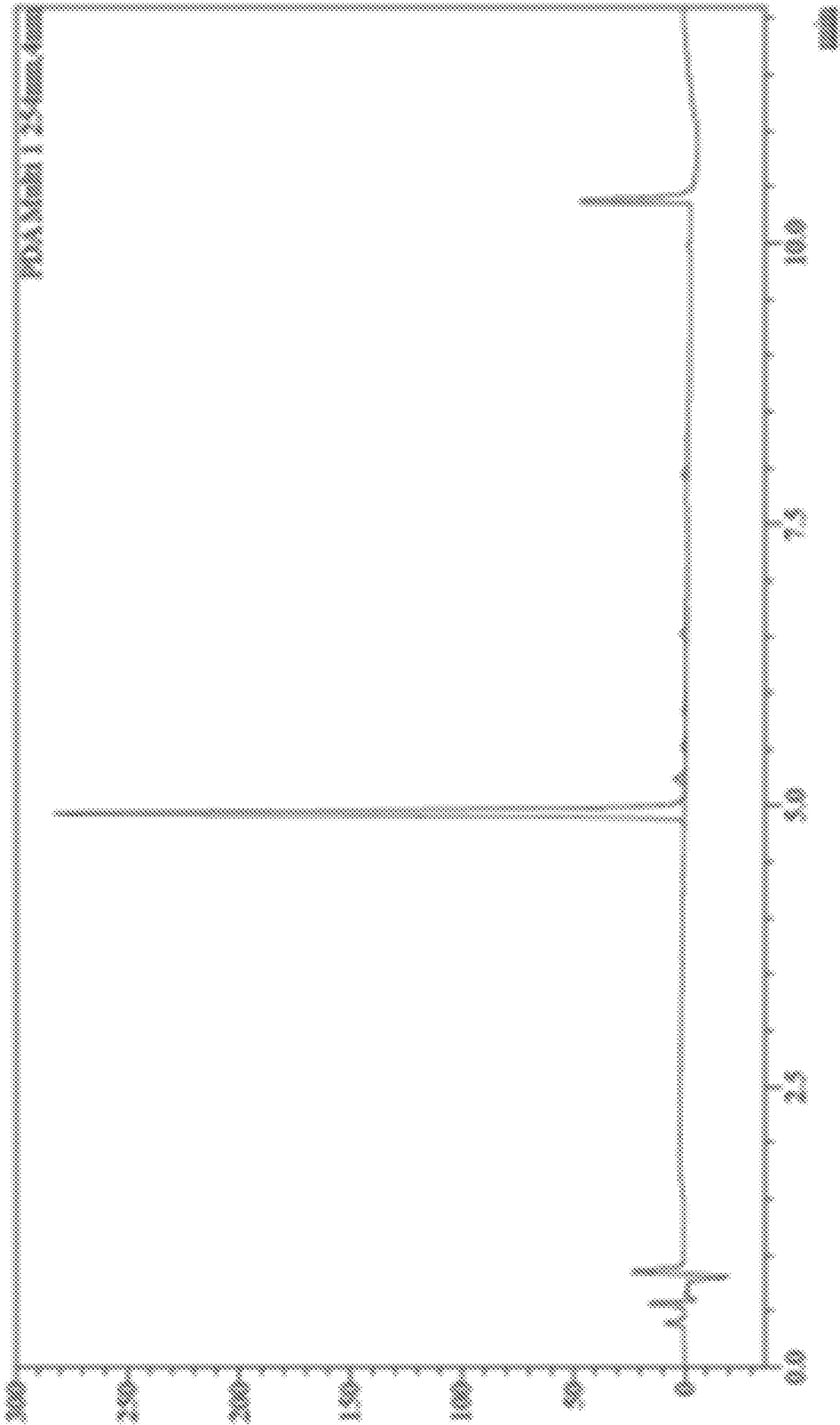


FIG. 23A



Peak#	Ret. Time	Area%	Area
1	4.929	95.945	931311
2	5.235	1.489	14451
3	5.521	0.387	3755
4	5.842	0.434	4212
5	6.522	1.065	10334
6	7.938	0.681	6610
Total		100.000	970673

FIG. 23B

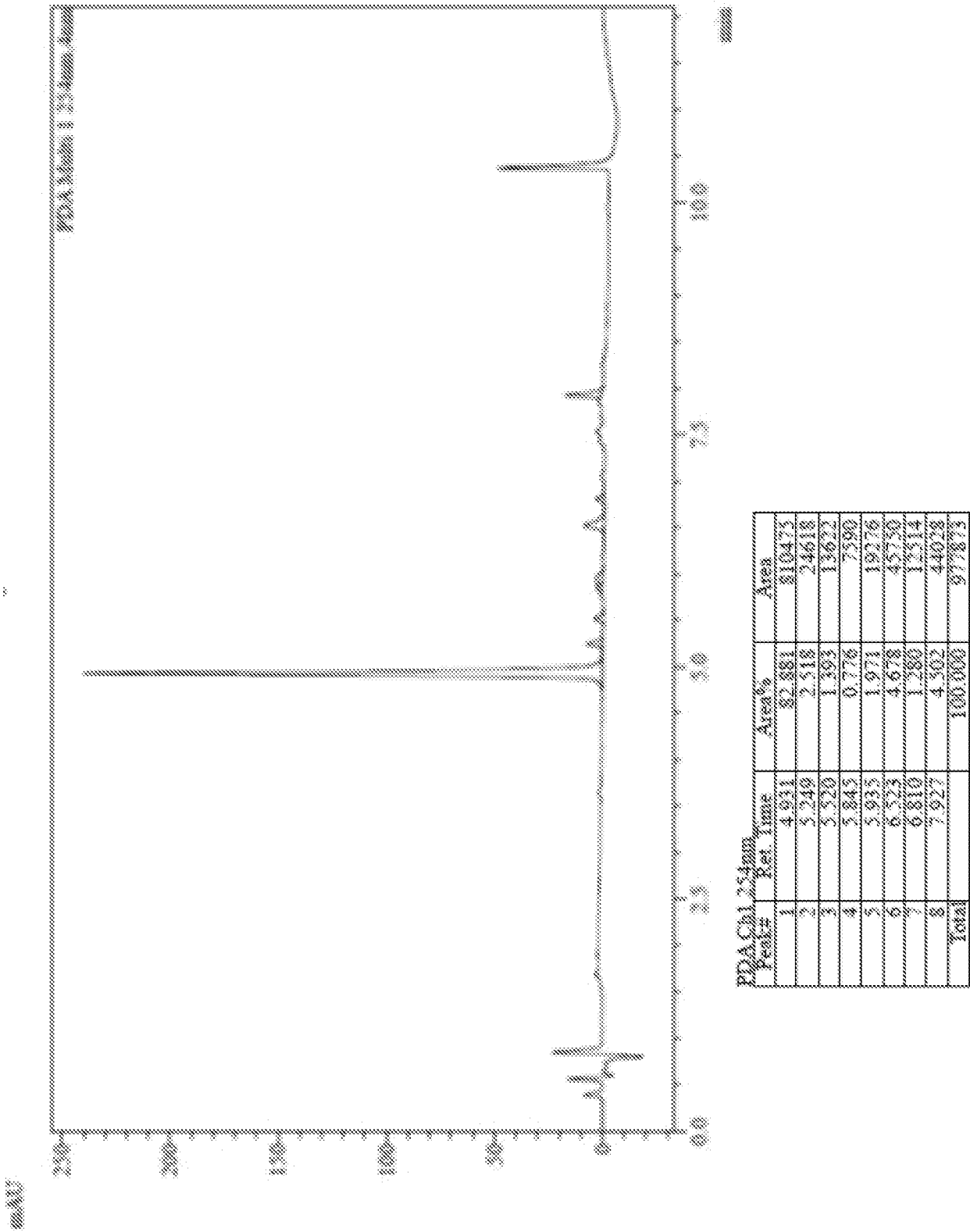
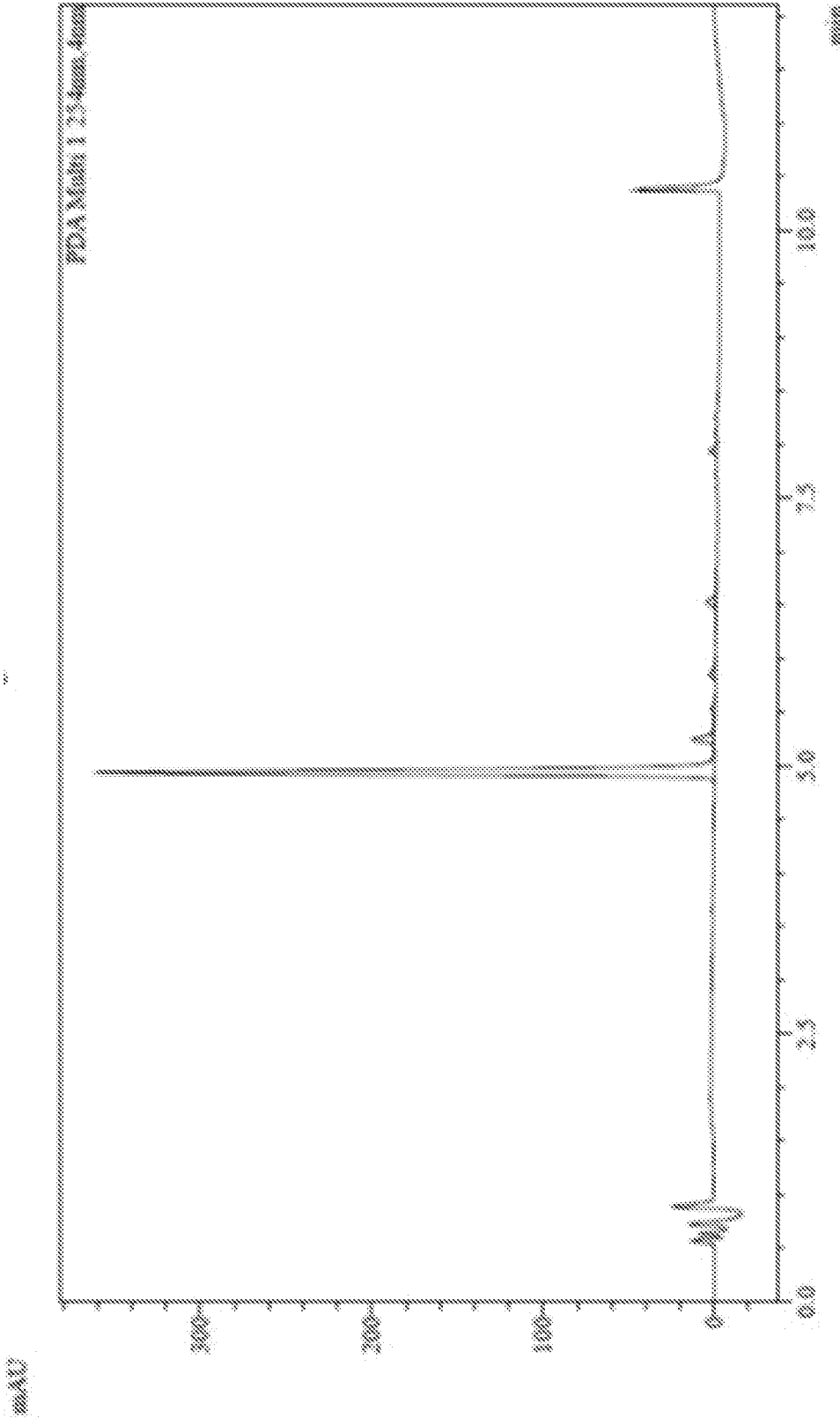


FIG. 24A



Peak#	Ret Time	Area%	Area
1	4.941	91.987	1140316
2	5.250	3.756	46356
3	5.532	0.493	6109
4	5.853	1.084	13432
5	6.530	1.796	22165
6	7.936	0.883	10968
Total		100.000	1239646

FIG. 24B

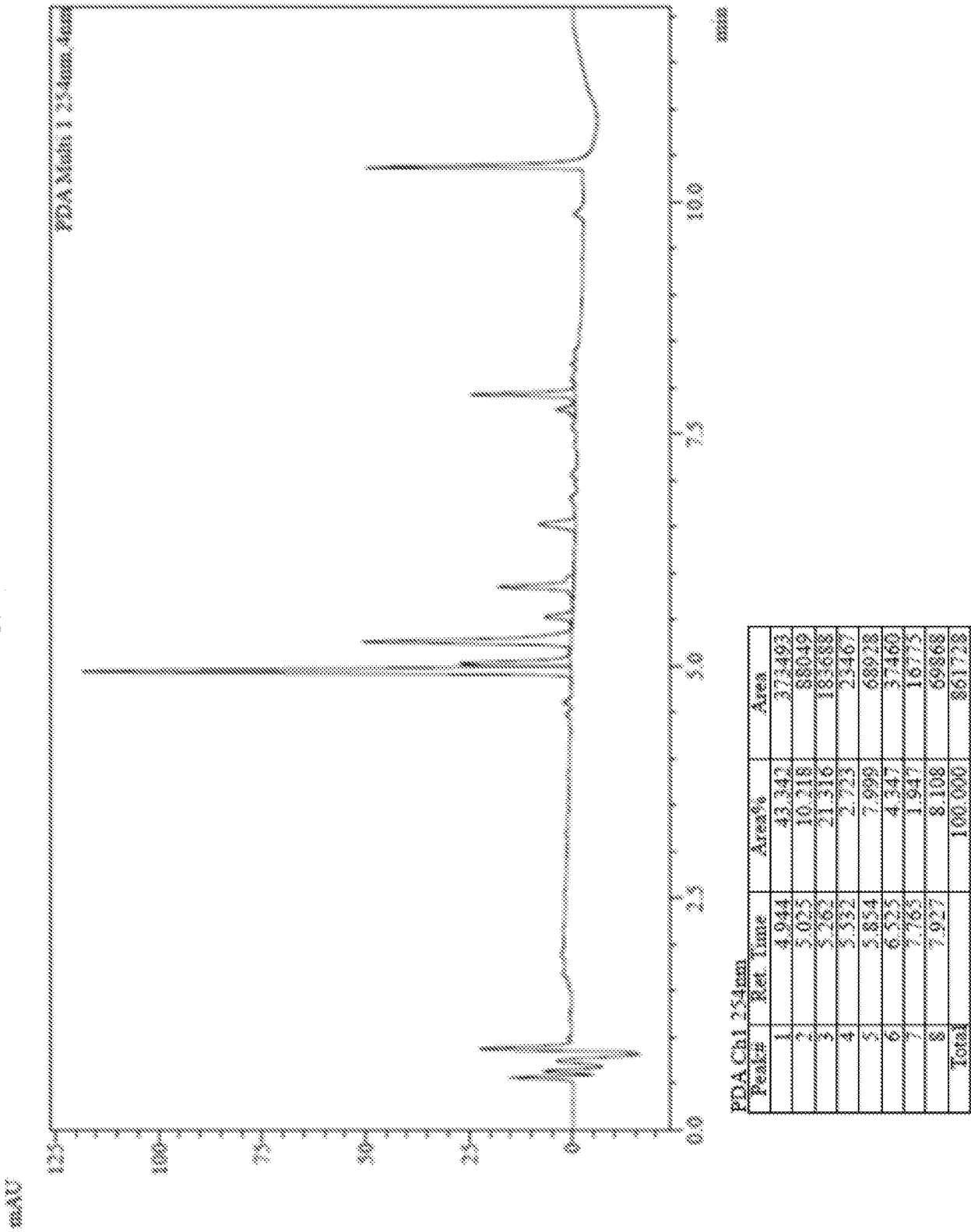


FIG. 25A

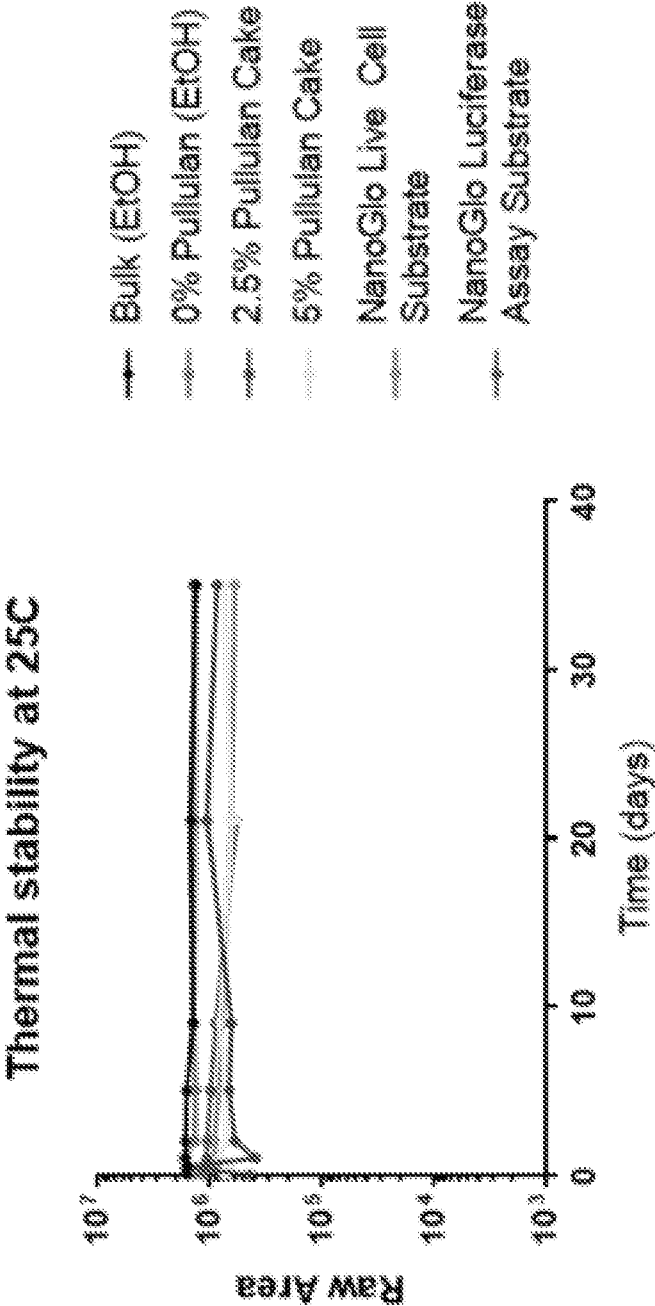


FIG. 25B

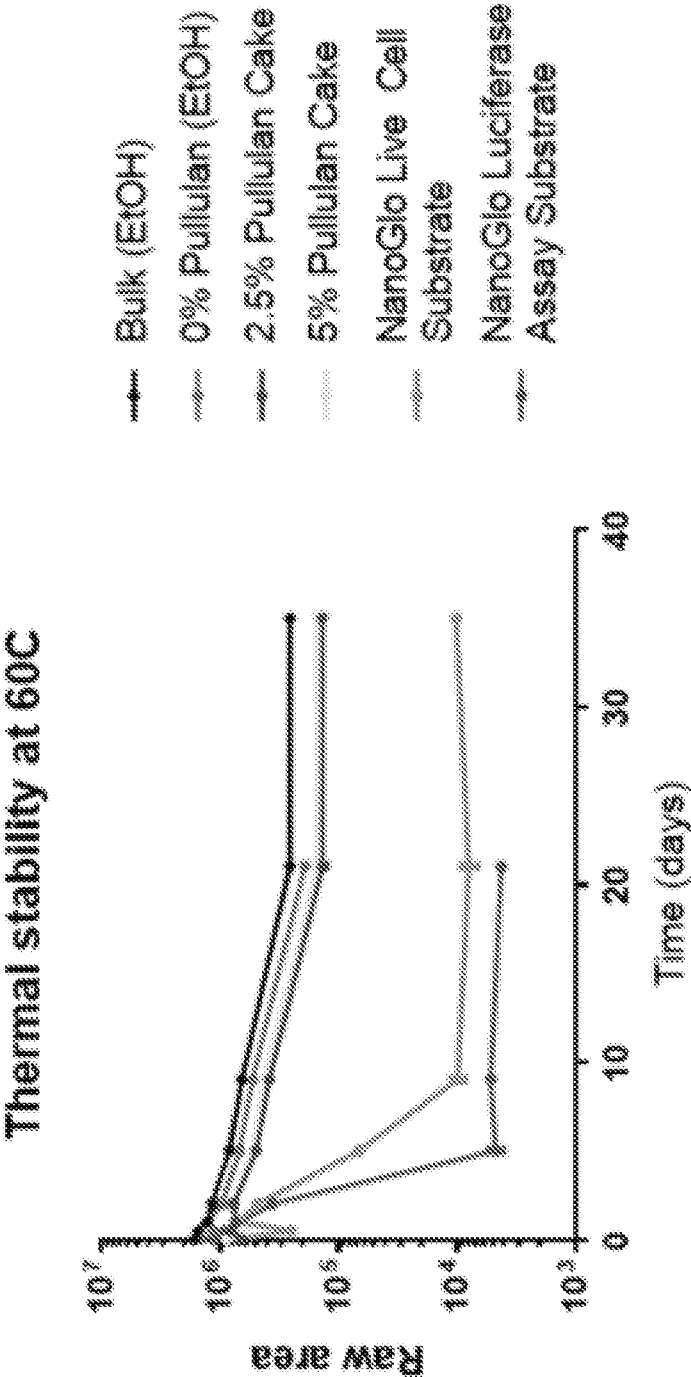


FIG. 25C

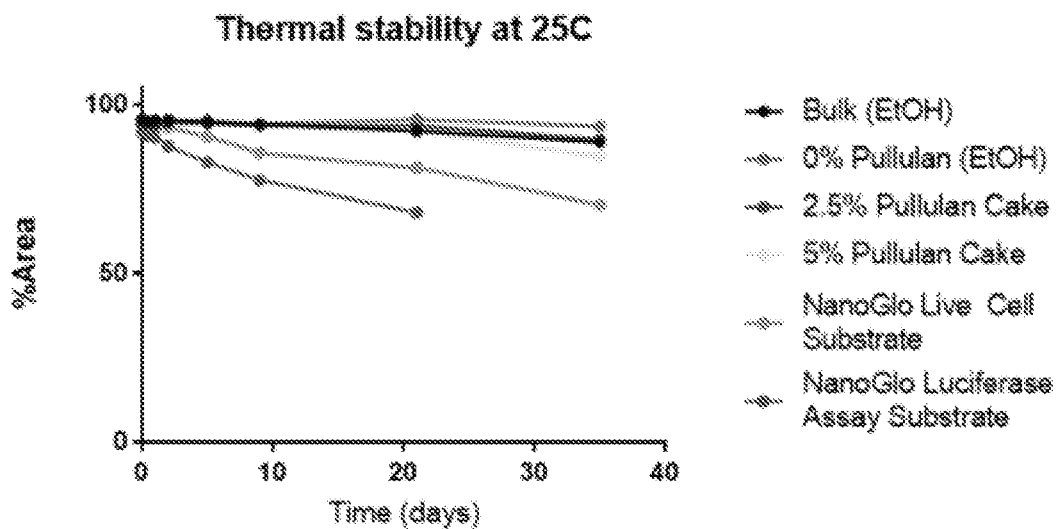


FIG. 25D

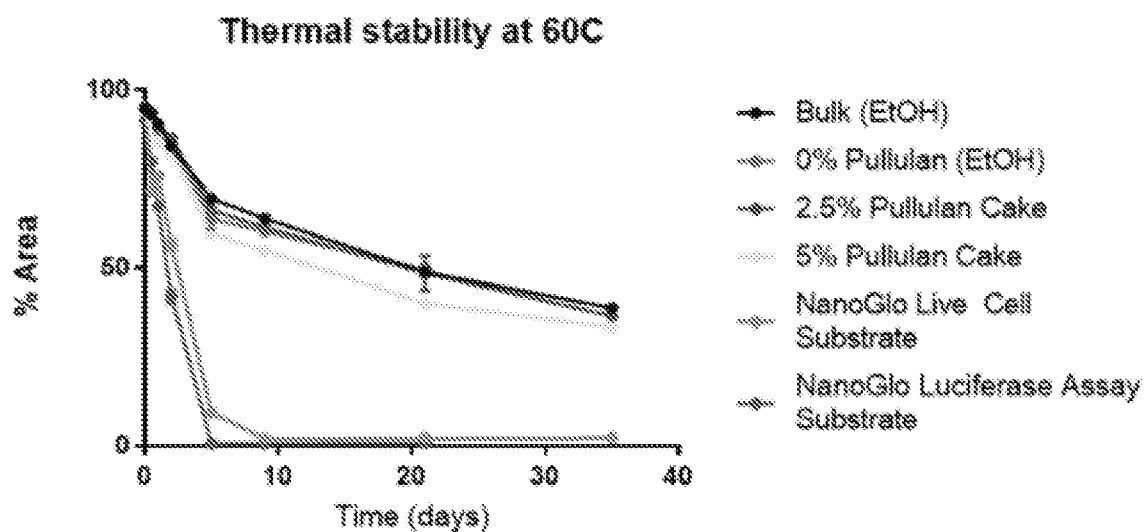


FIG. 26A

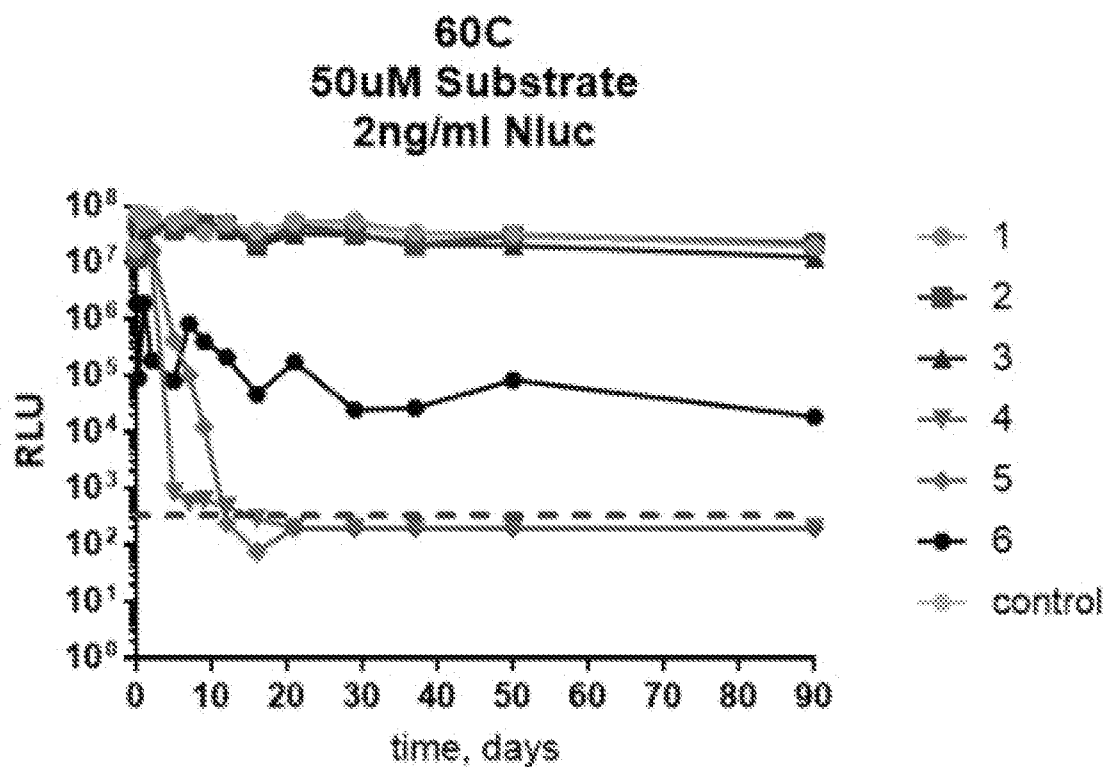


FIG. 26B

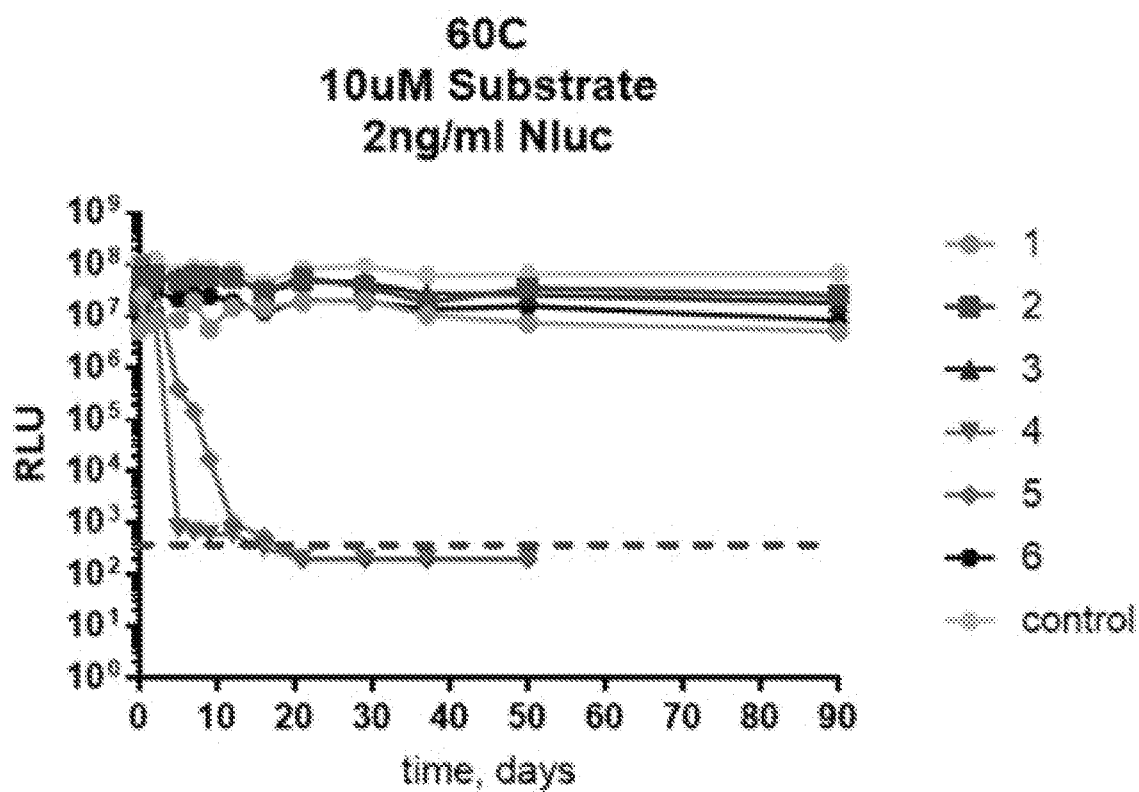


FIG. 26C

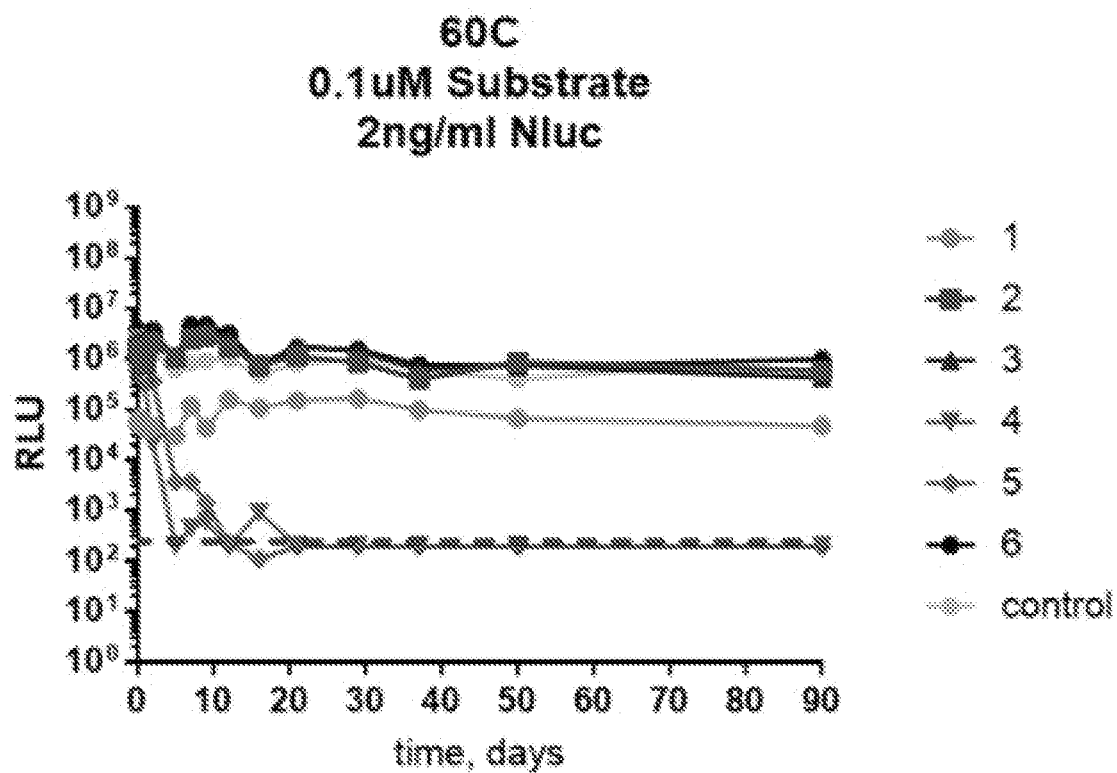


FIG. 26D

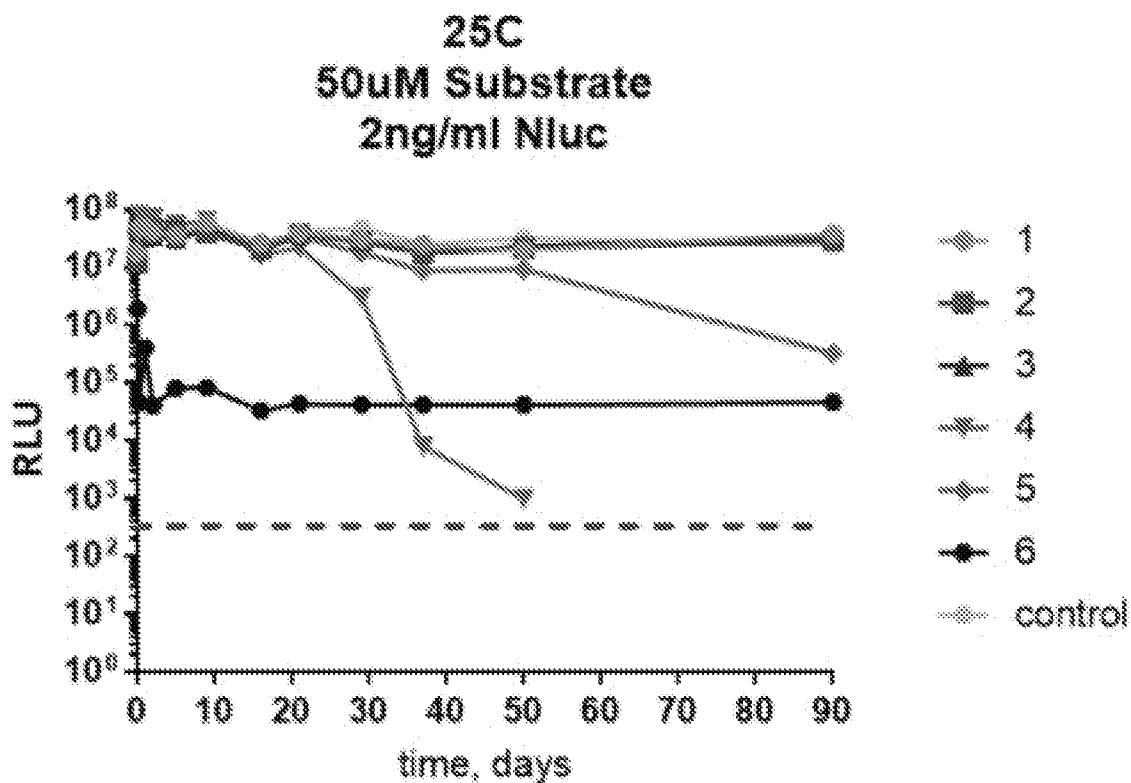


FIG. 26E

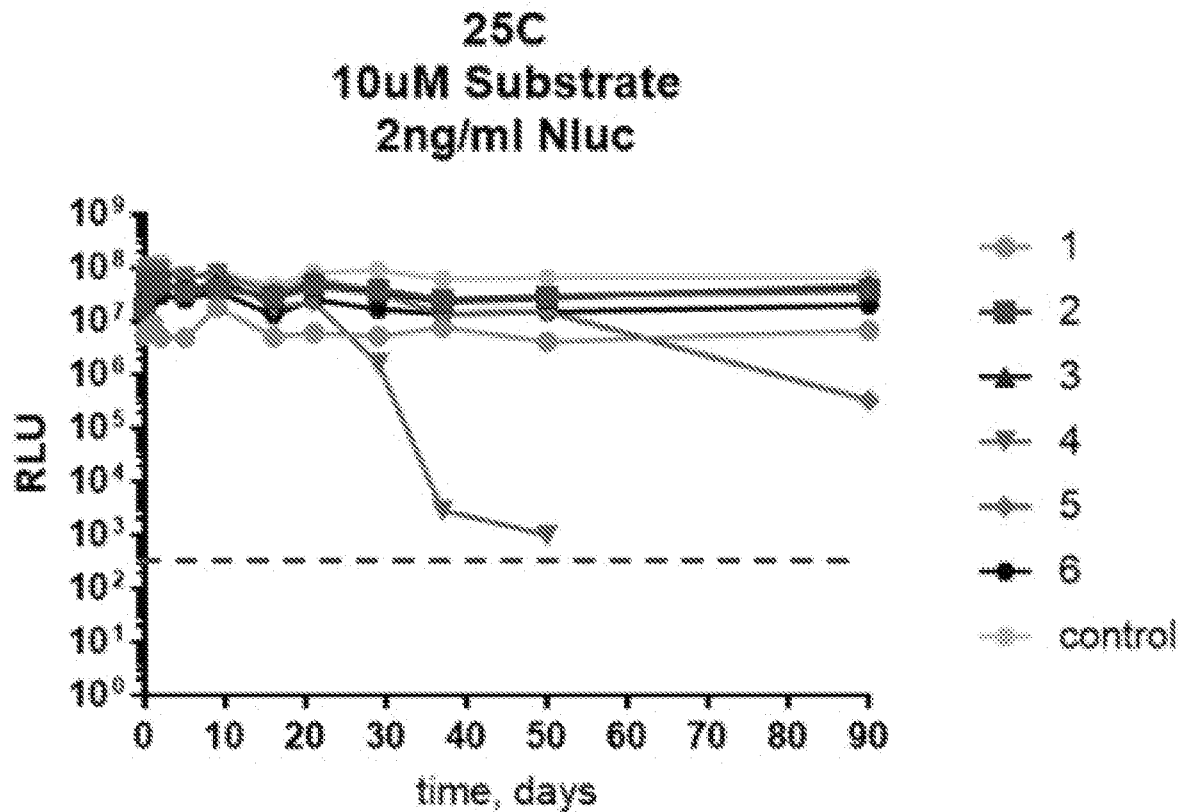


FIG. 26F

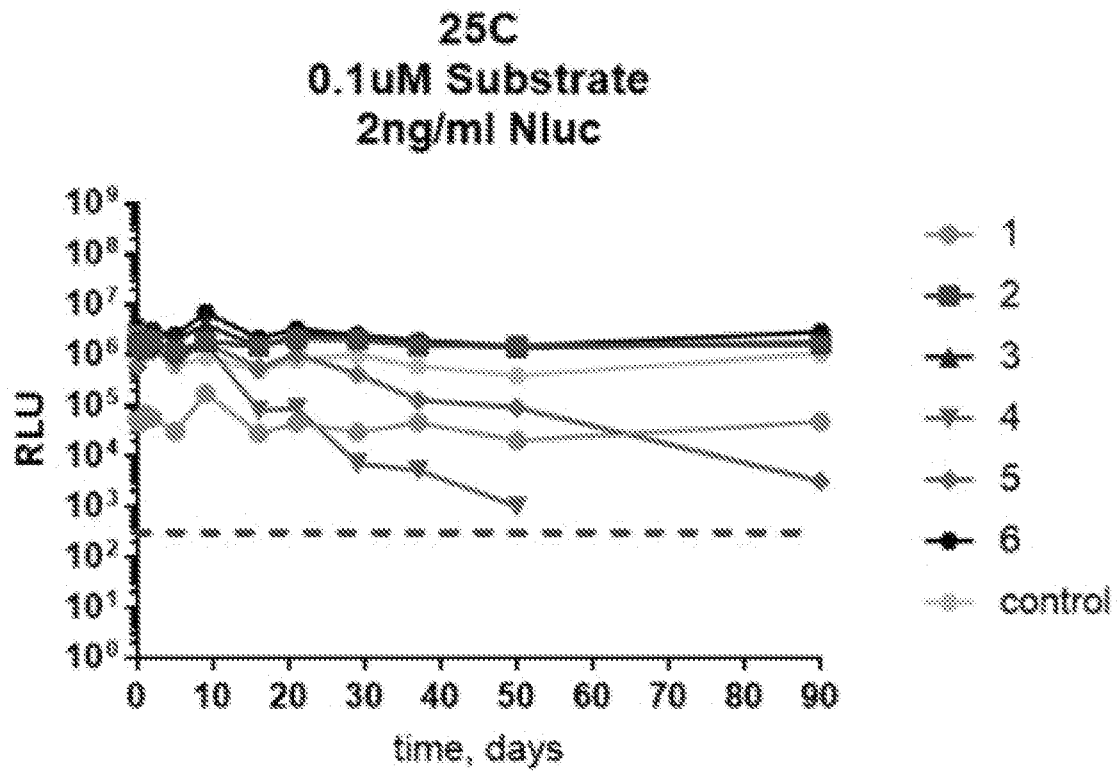


FIG. 27A

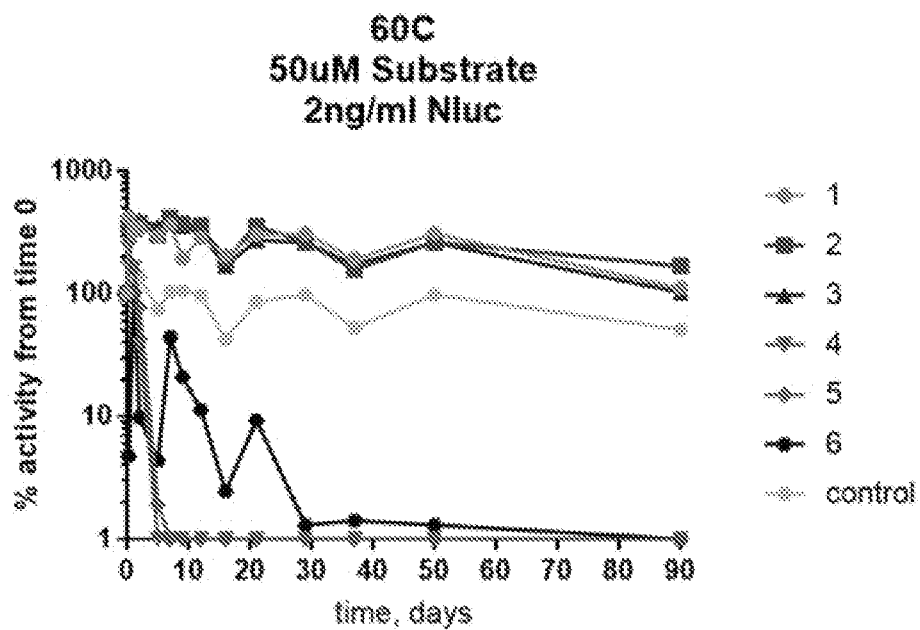


FIG. 27B

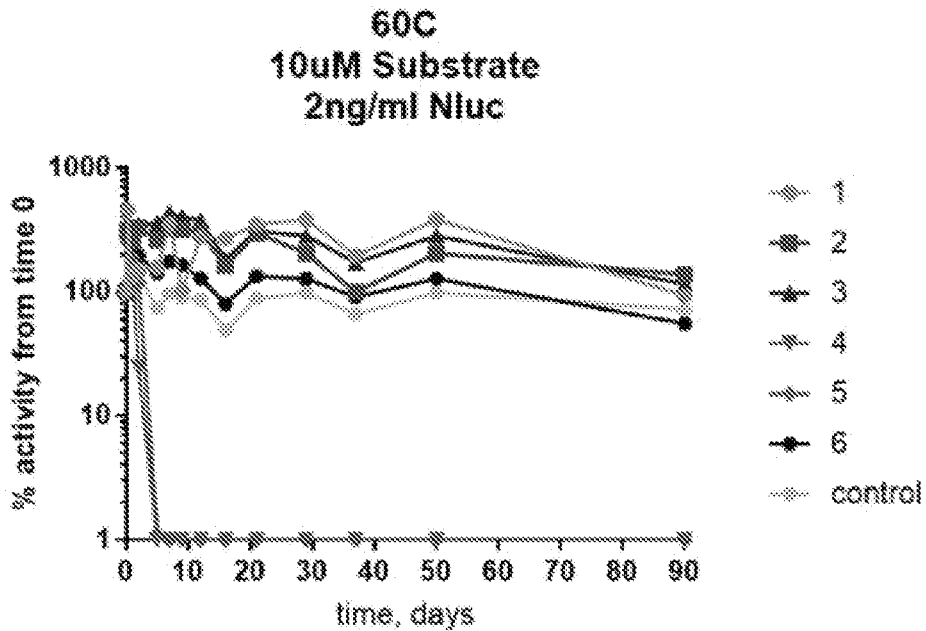


FIG. 27C

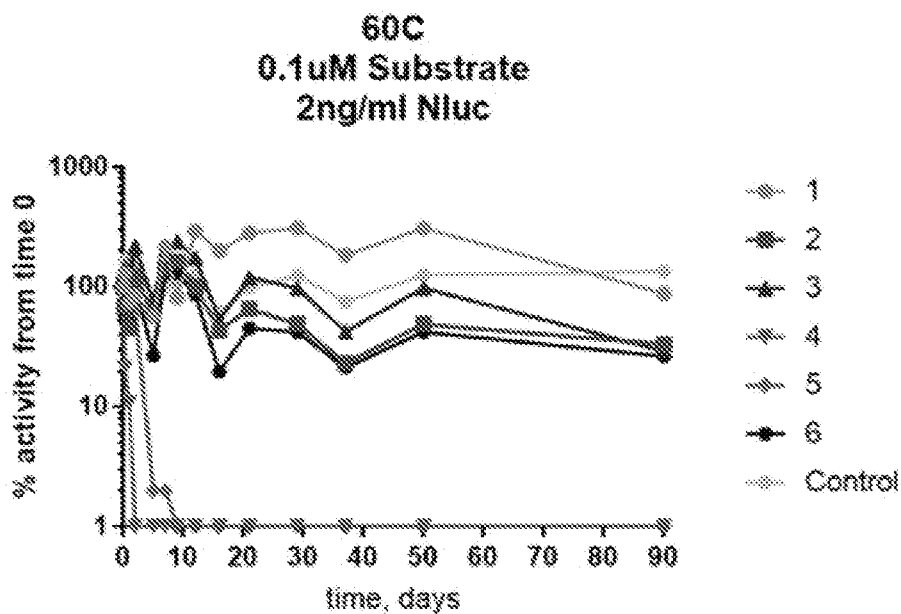


FIG. 27D

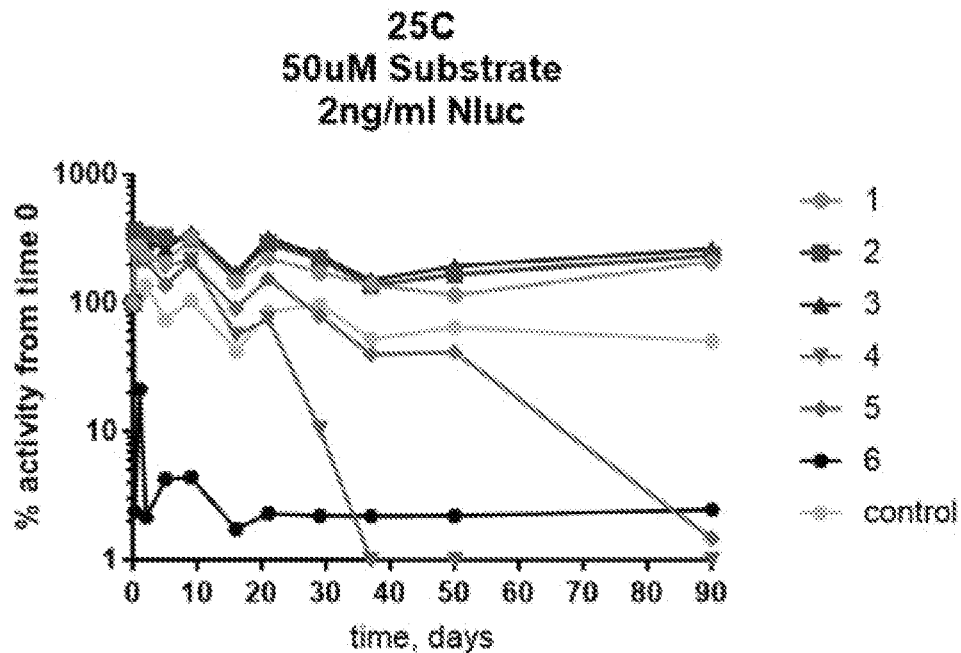


FIG. 27E

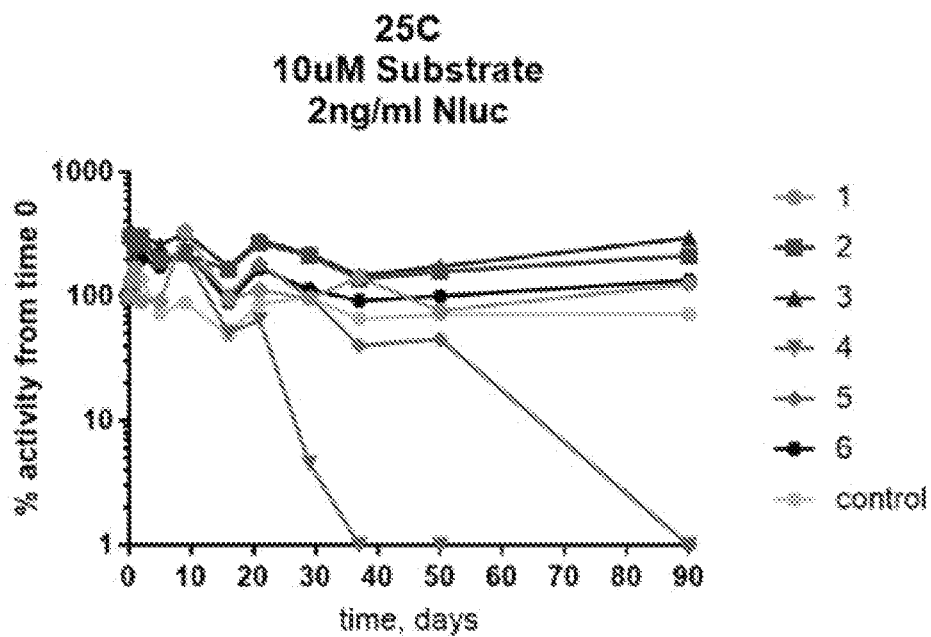


FIG. 27F

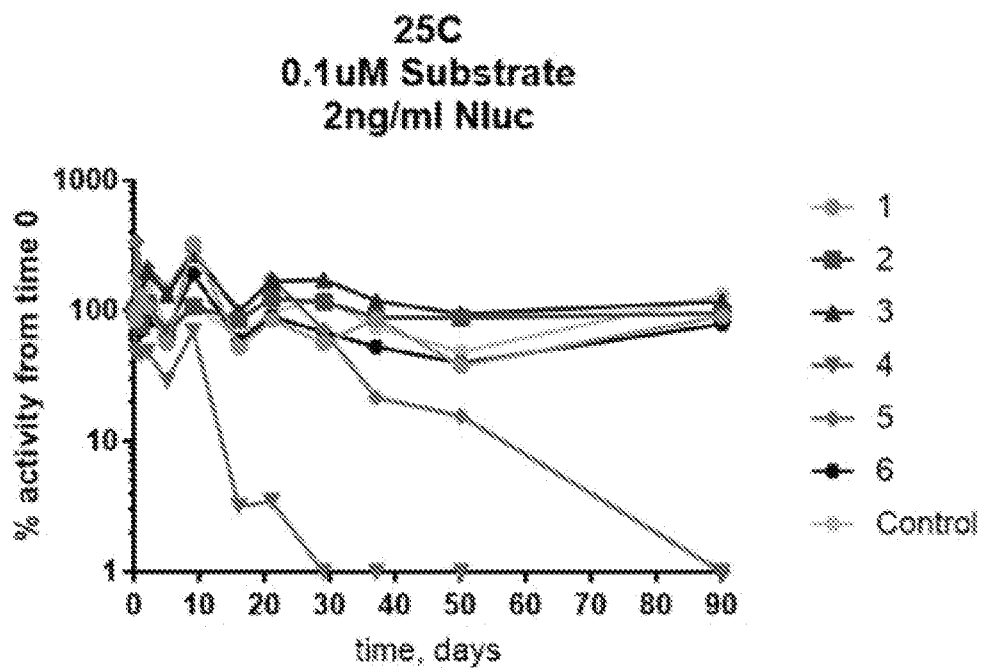


FIG. 28A

Formulated Furimazine Film-Coated Microtiter Plates
2ng/ml Nluc in PBS
no pre-equilibration

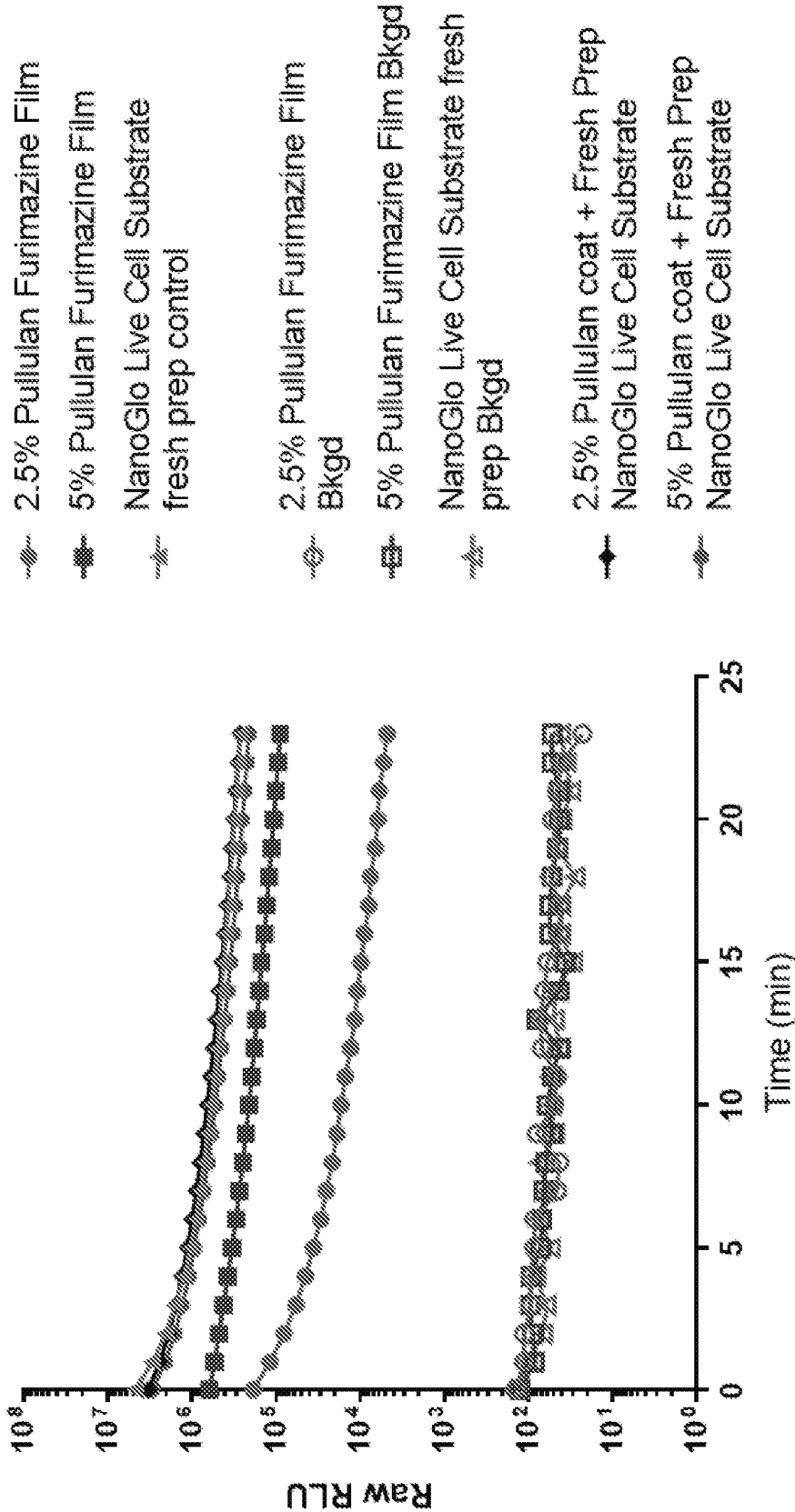


FIG. 28B

Formulated Furimazine Film-Coated Microtiter Plates
2ng/ml Nluc in PBS
no pre-equilibration

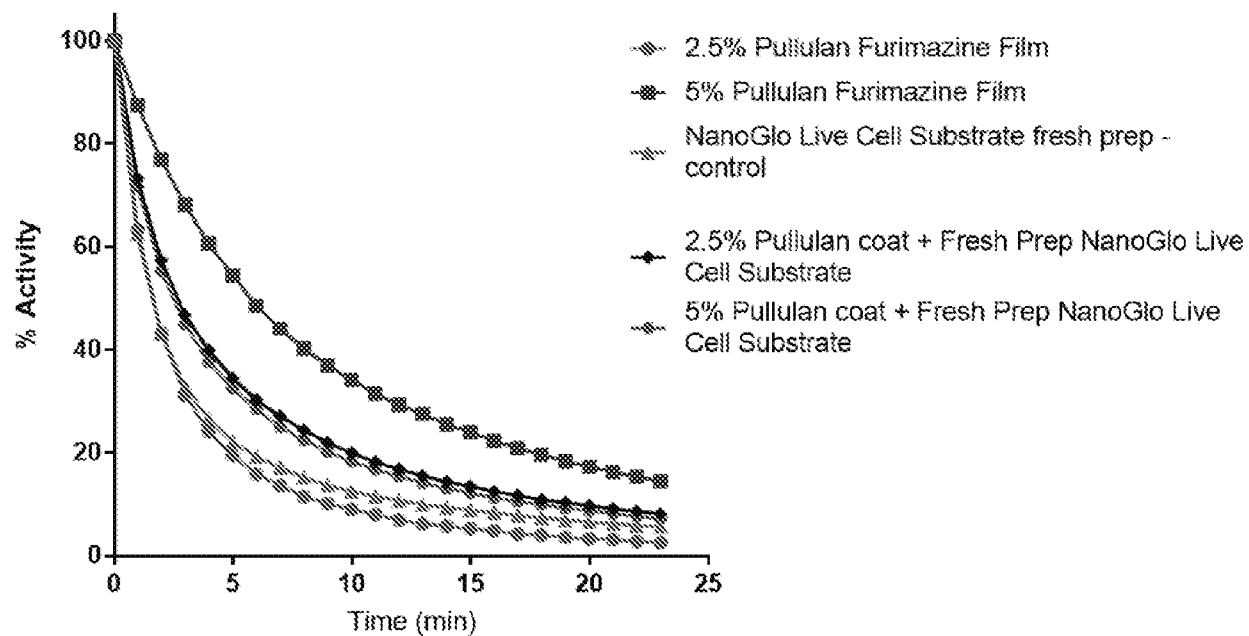


FIG. 28C

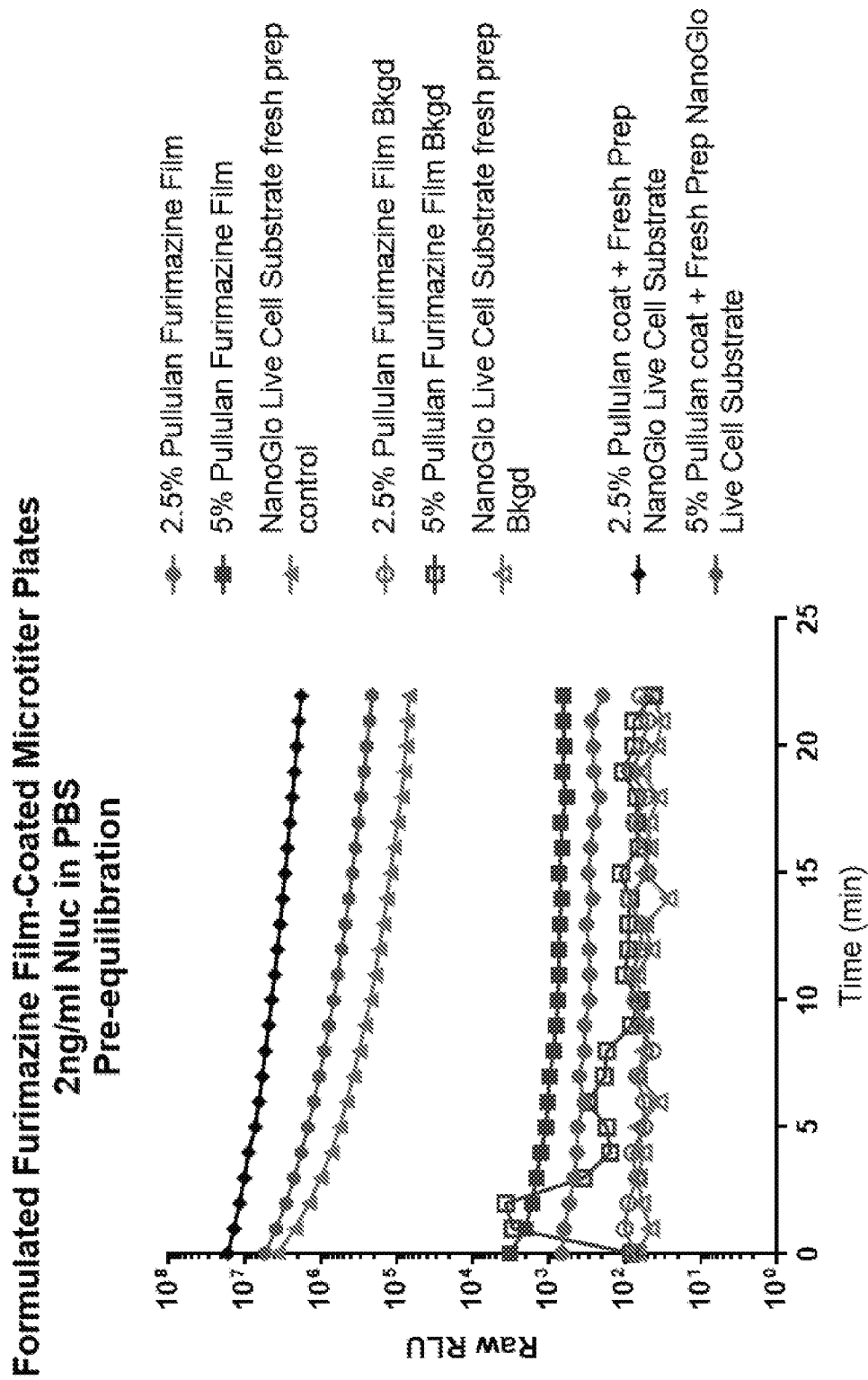


FIG. 29

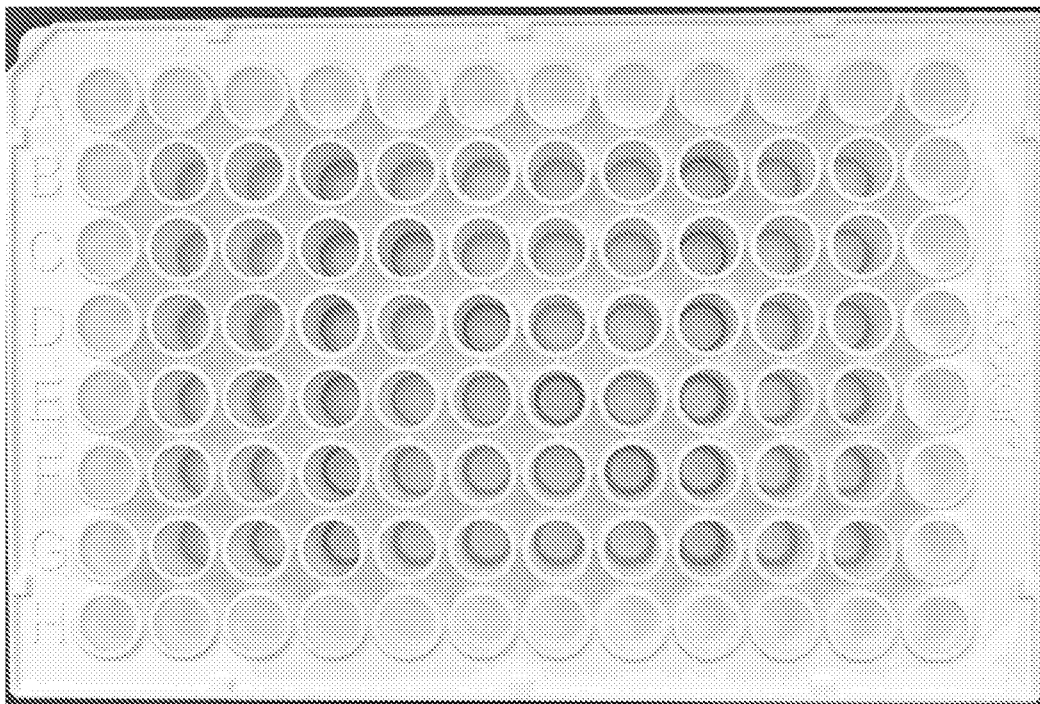


FIG. 30A

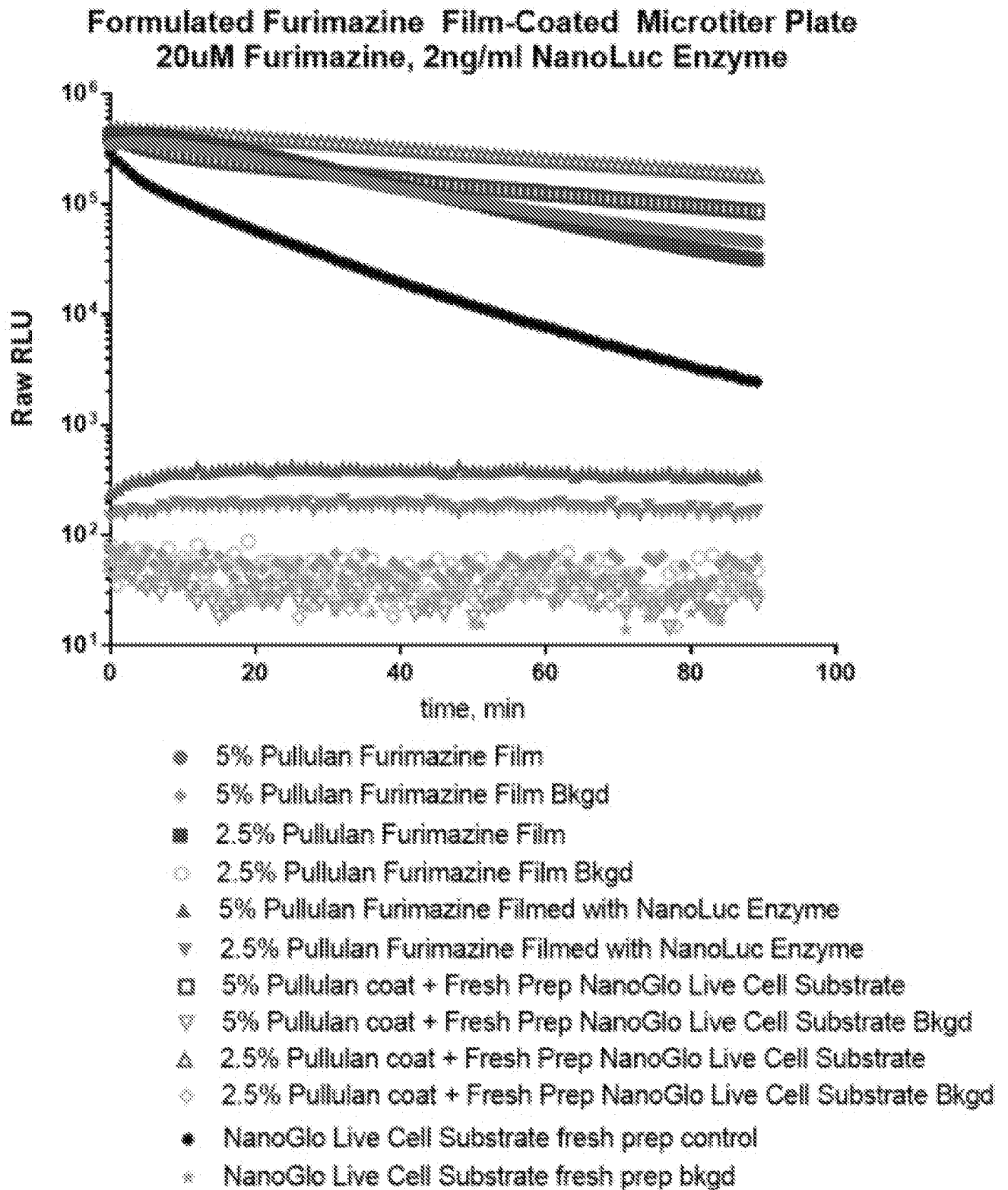
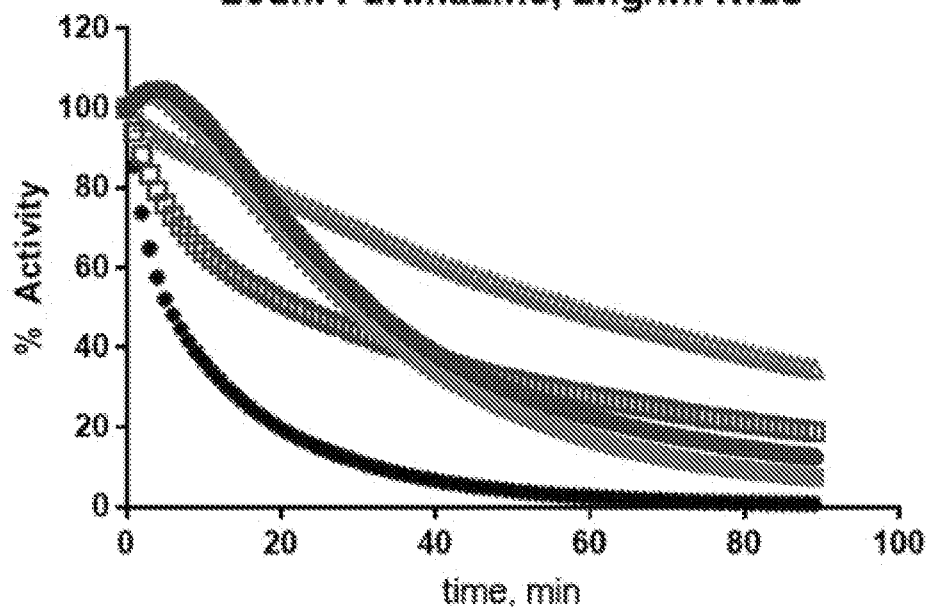


FIG. 30B

Formulated Furimazine Film-coated Microtiter Plate
20uM Furimazine, 2ng/ml Nluc



- 5% Pullulan Furimazine Film
- ▨ 2.5% Pullulan Furimazine Film
- 5% Pullulan coat + Fresh Prep NanoGlo Live Cell Substrate Bkgd
- △ 2.5% Pullulan coat + Fresh Prep NanoGlo Live Cell Substrate
- NanoGlo Live Cell Substrate fresh prep control

FIG. 30C

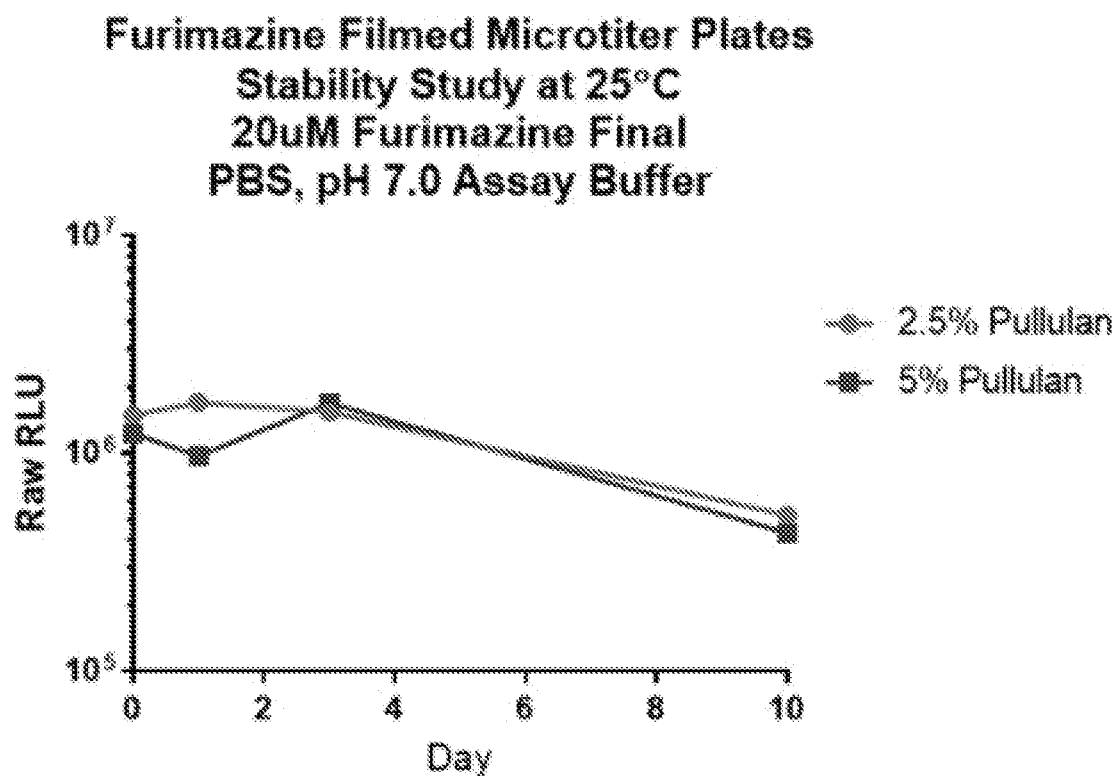


FIG. 31A

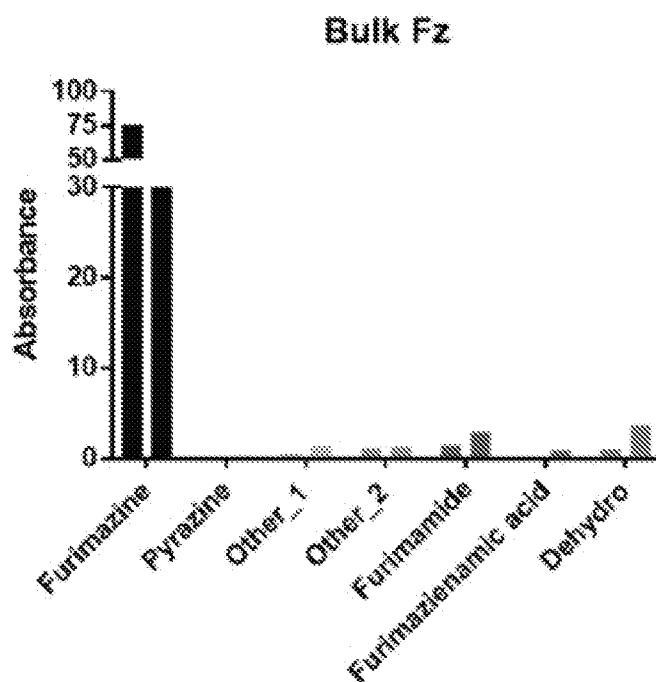


FIG. 31B

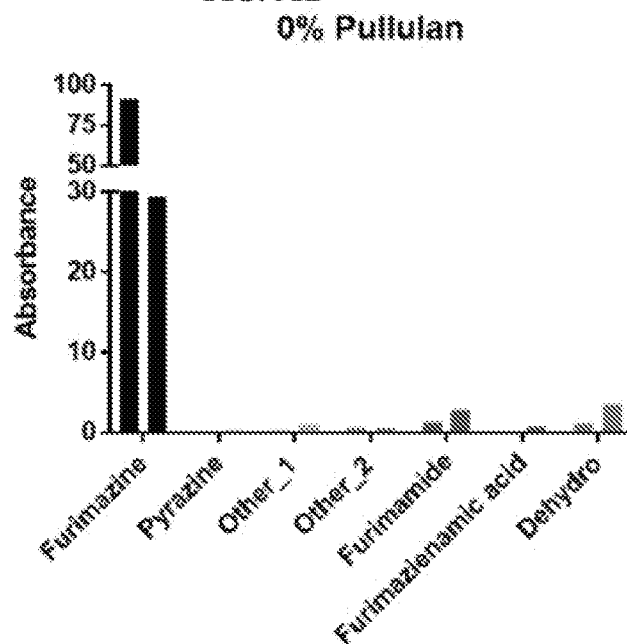


FIG. 31C

2.5% Pullulan

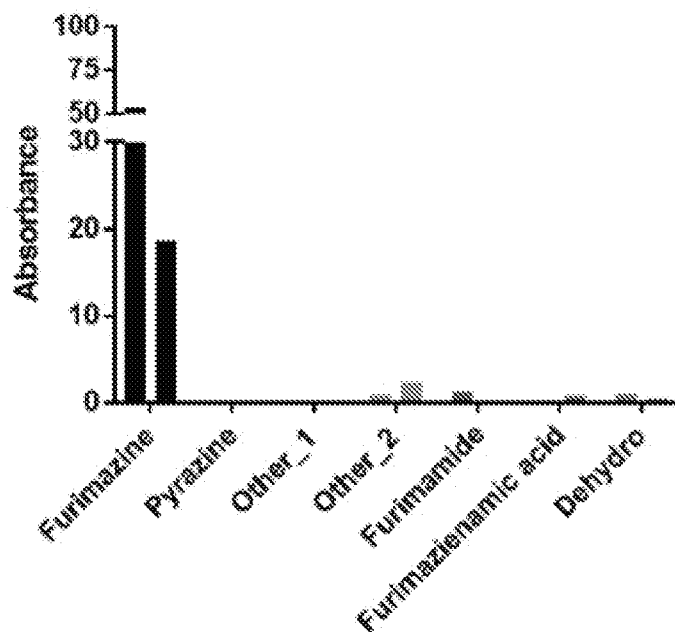


FIG. 31D

5% Pullulan

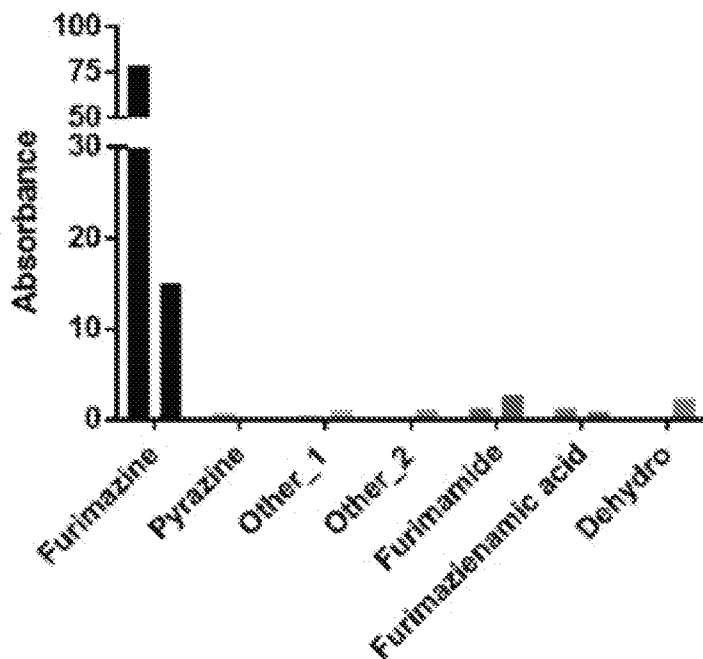


FIG. 31E

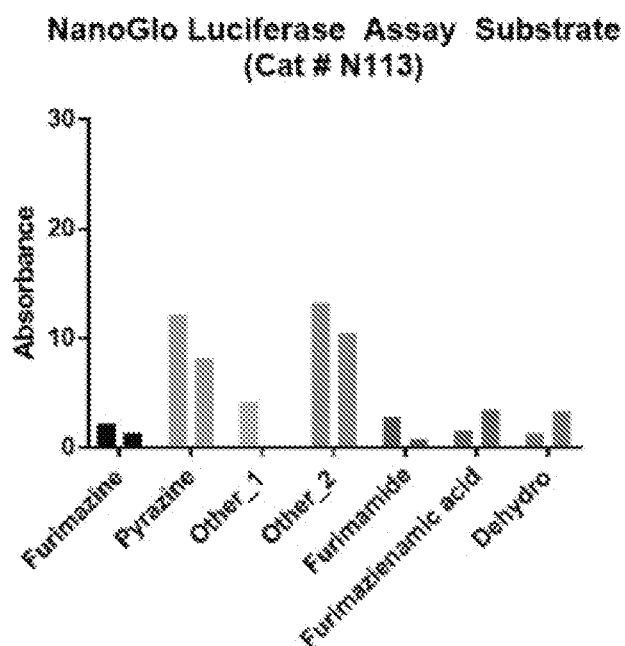


FIG. 31F

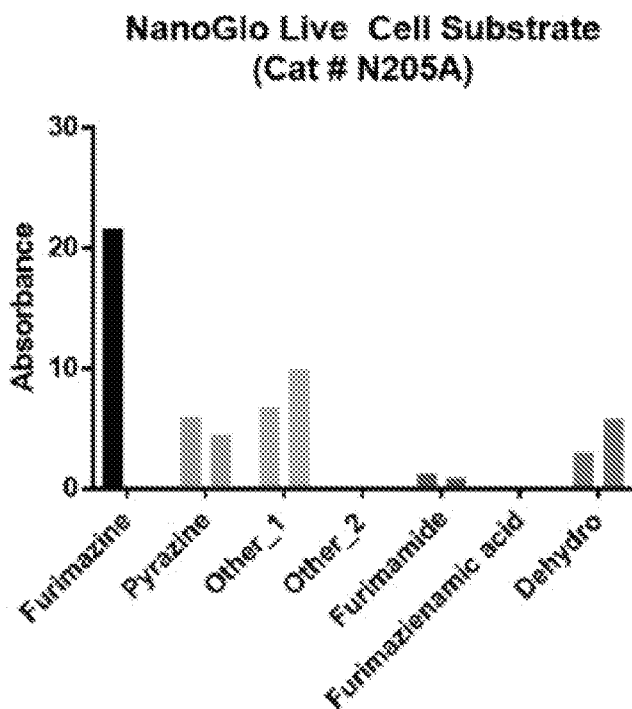
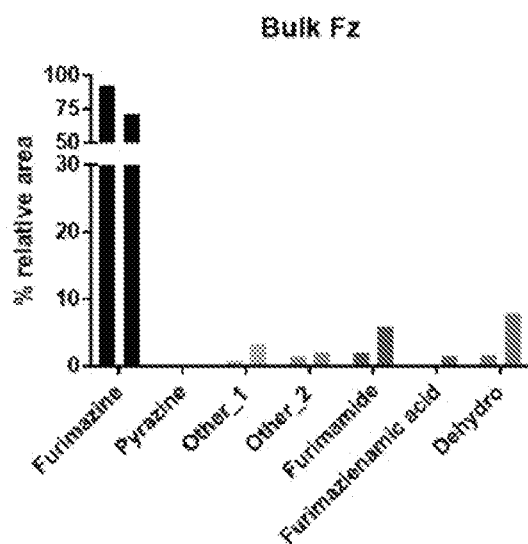
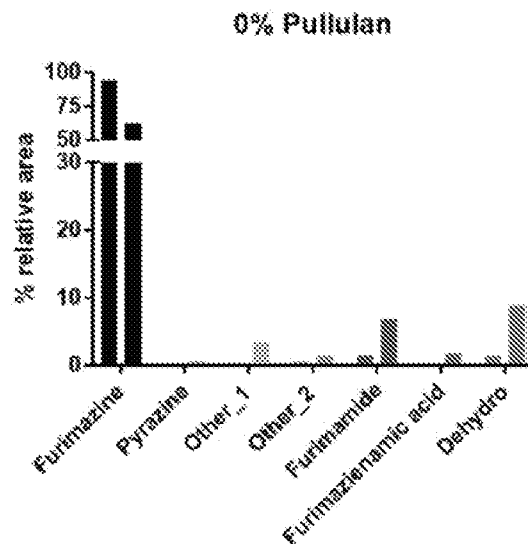


FIG. 32

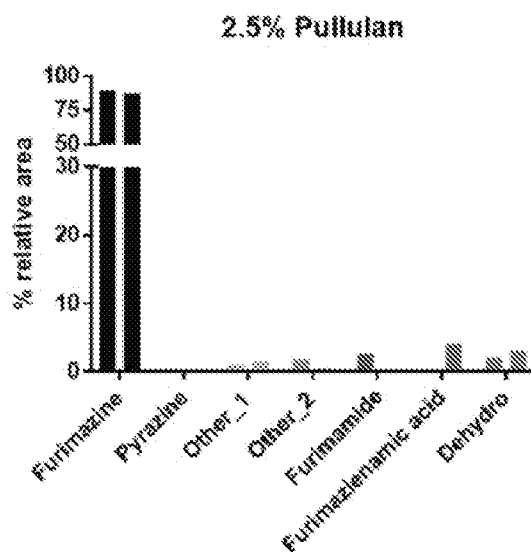
A.



B.



C.



D.

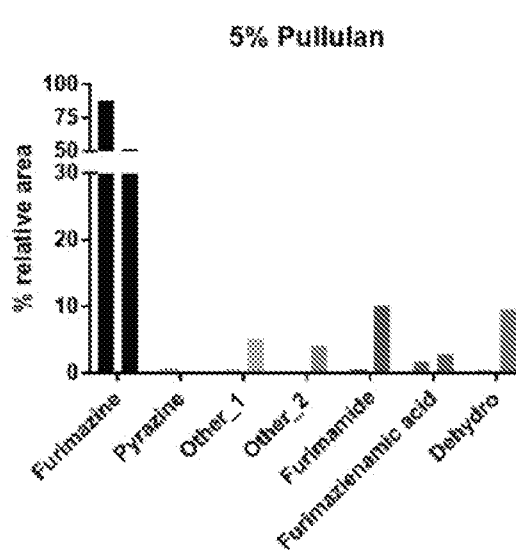
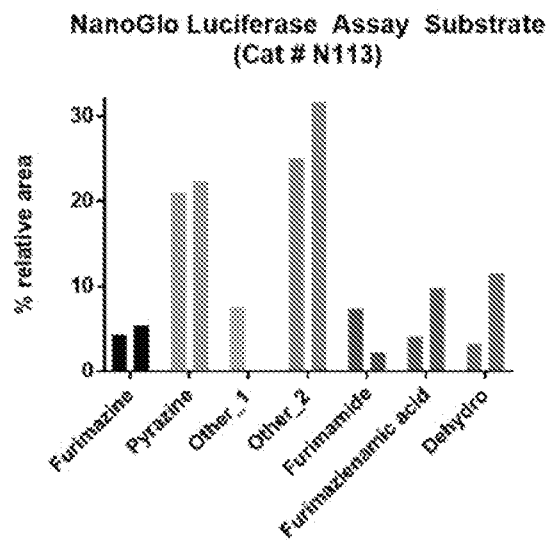


FIG. 32 (cont'd)

E.



F.

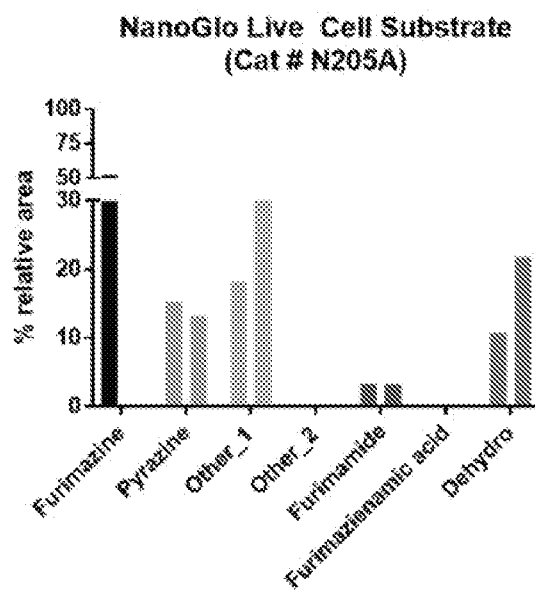


FIG. 33A-B

Formulated Furimazine Compositions
Storage: 6 month in ambient
temperature and not protected
from light
10uM final substrate
1ng/ml Niuc
PBS, pH 7.0 assay buffer

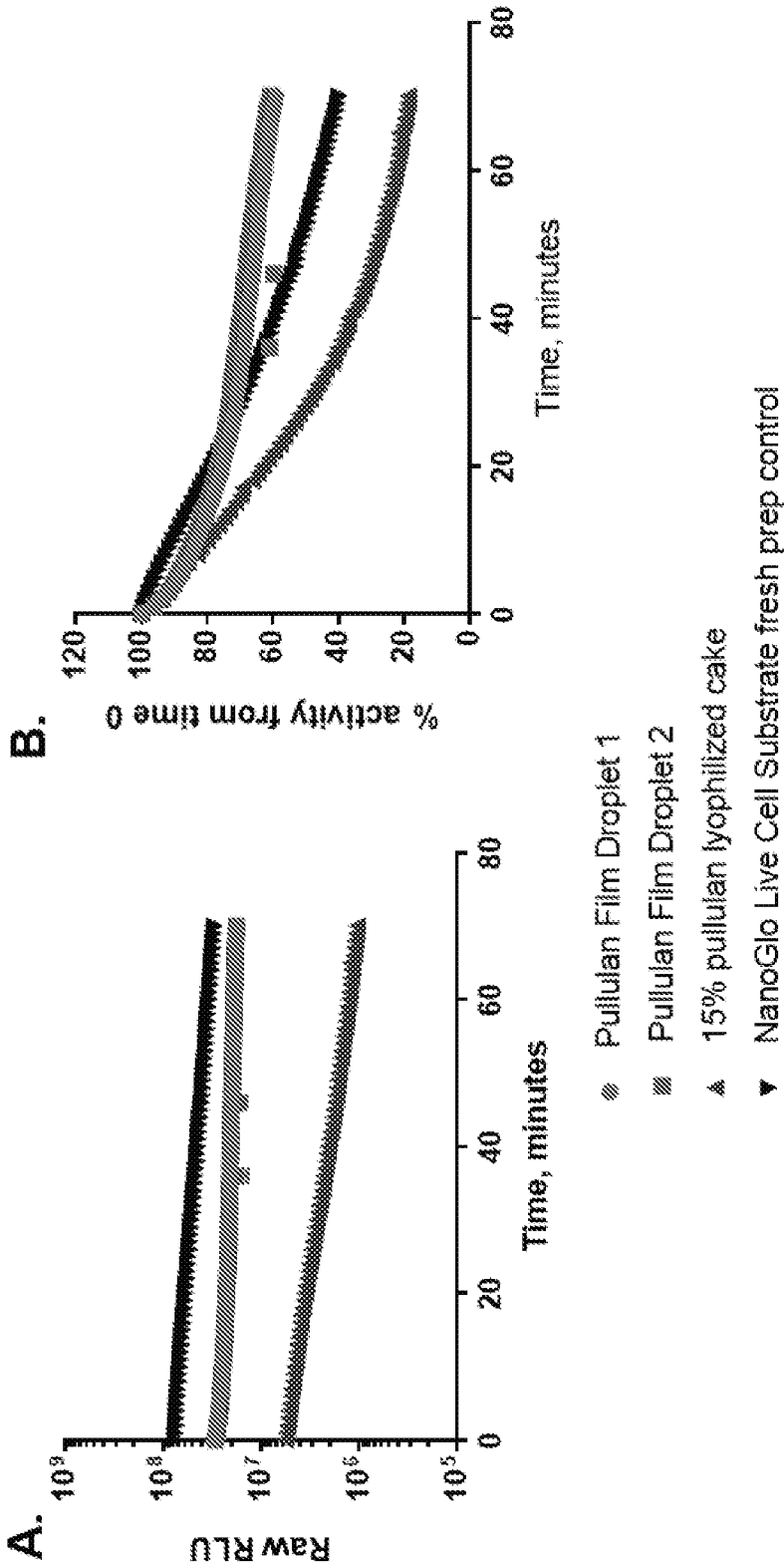


FIG. 34

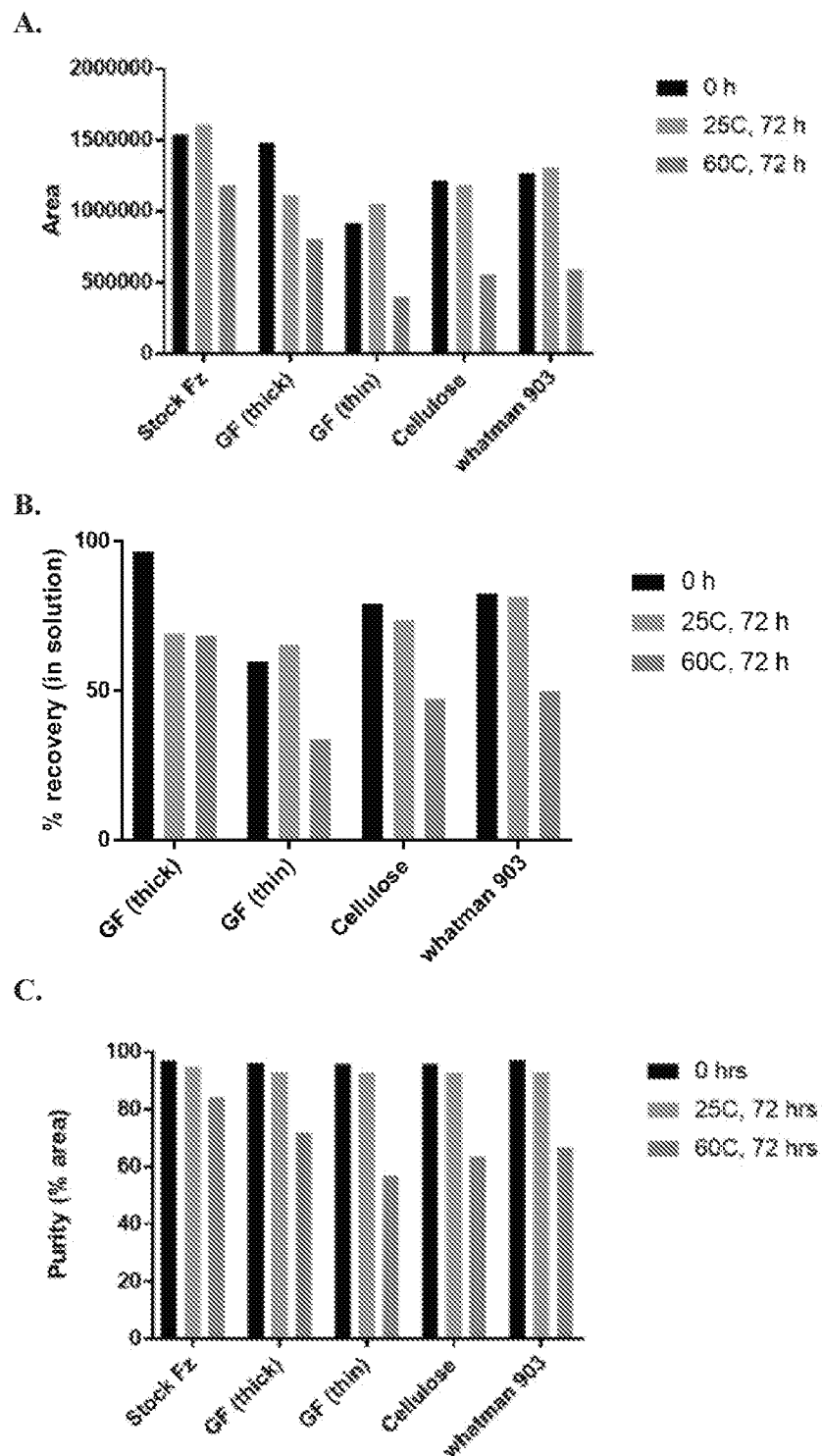
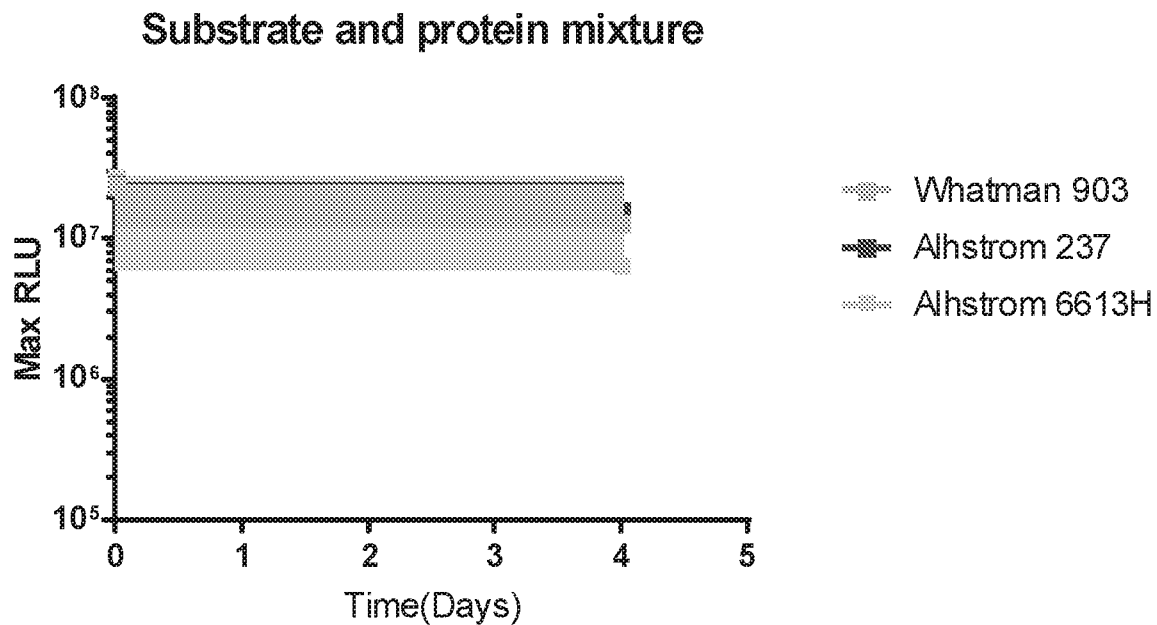


FIG. 35

A.



B.

Substrate and reporter protein on Whatman 903 surface

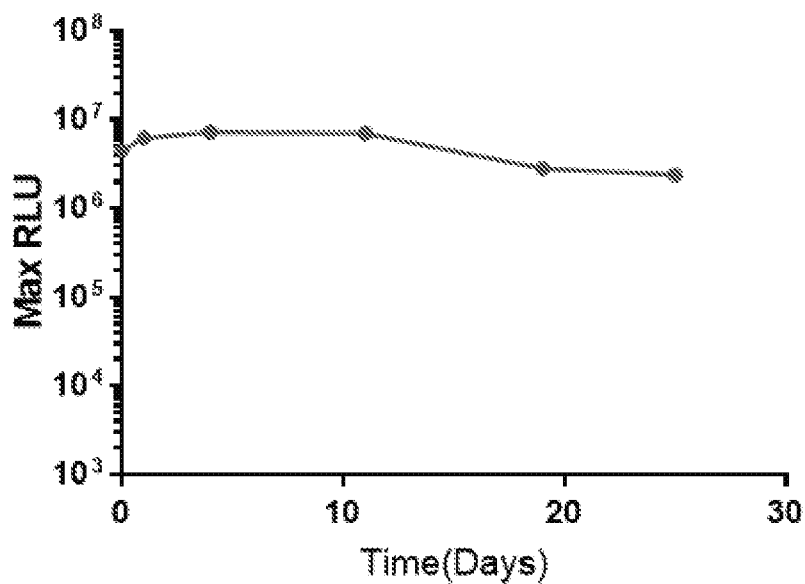
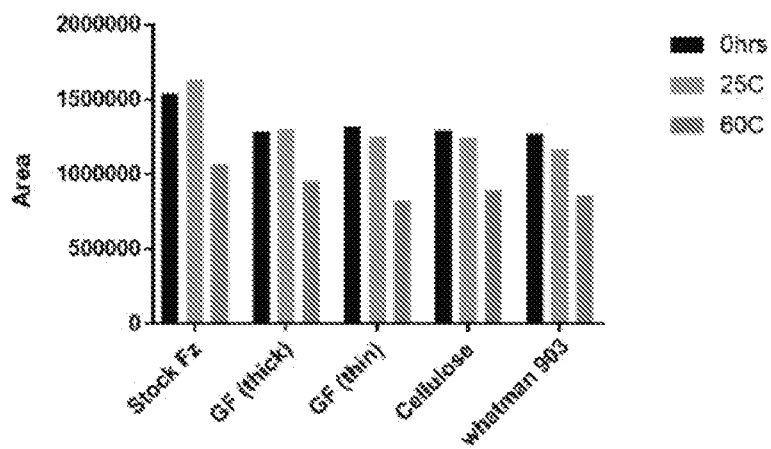
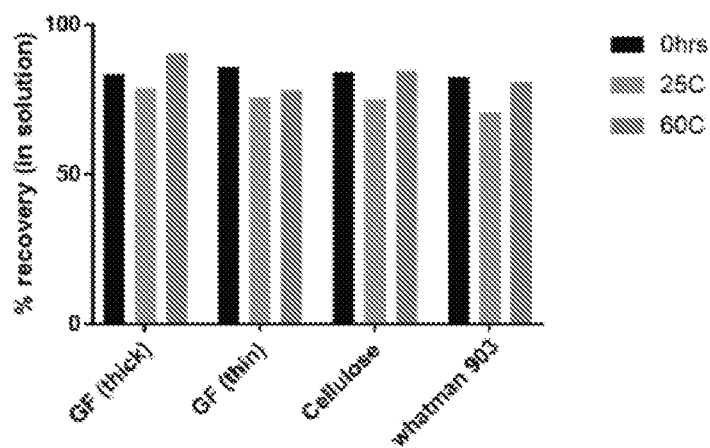


FIG. 36

A.



B.



C.

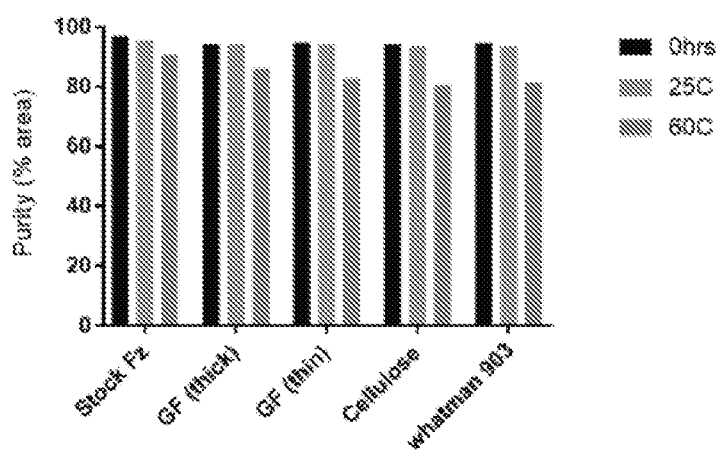
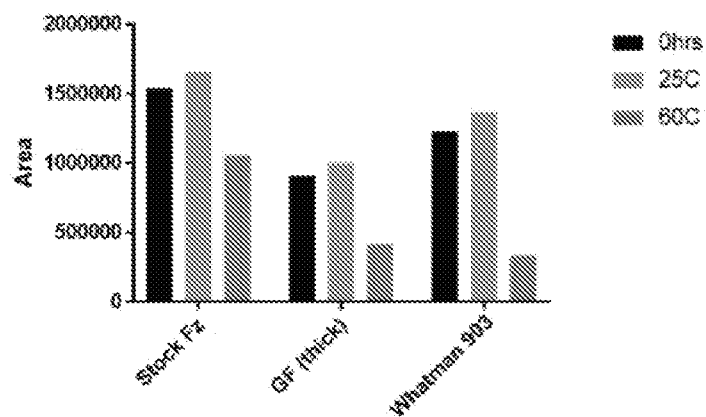
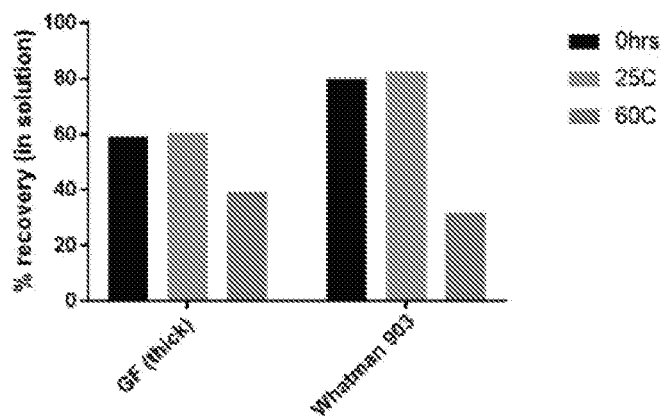


FIG. 37

A.



B.



C.

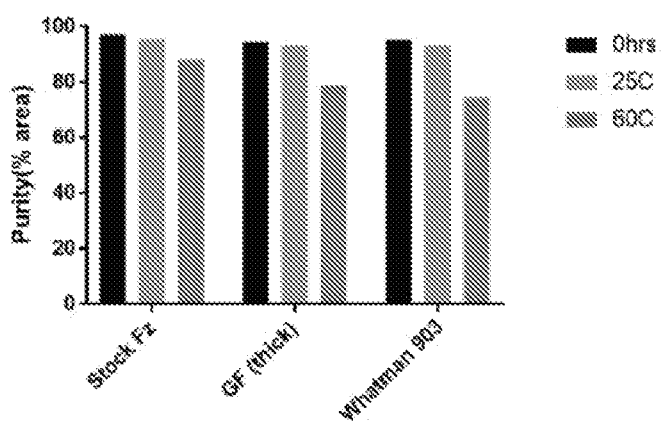
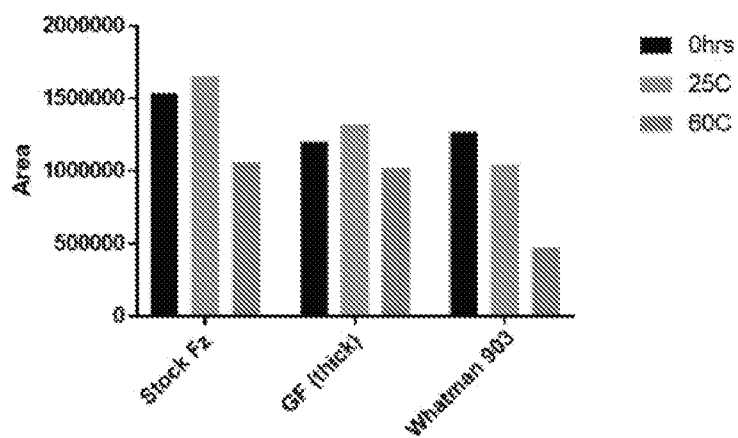
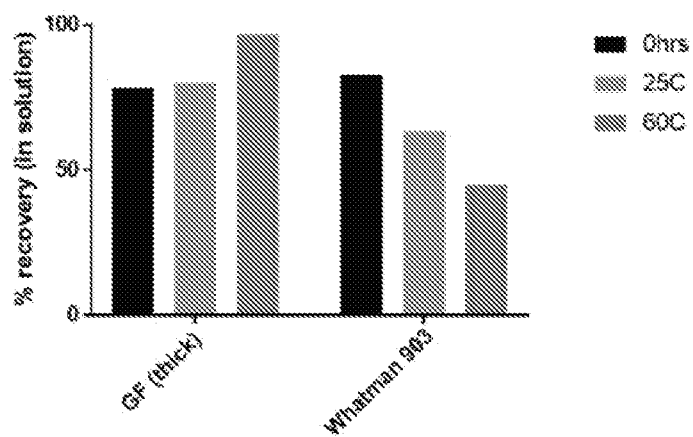


FIG. 38

A.



B.



C.

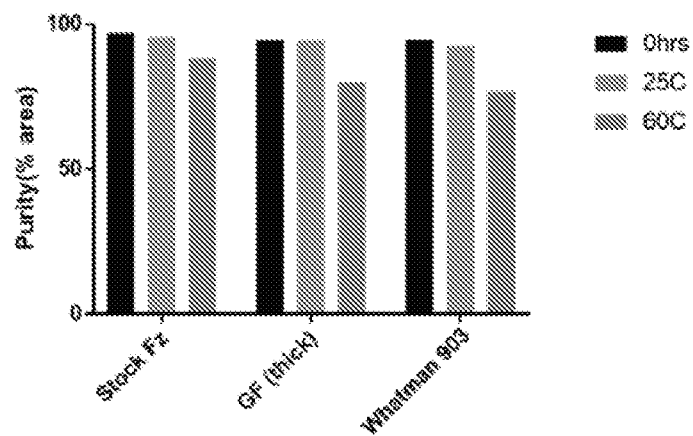


FIG. 39A

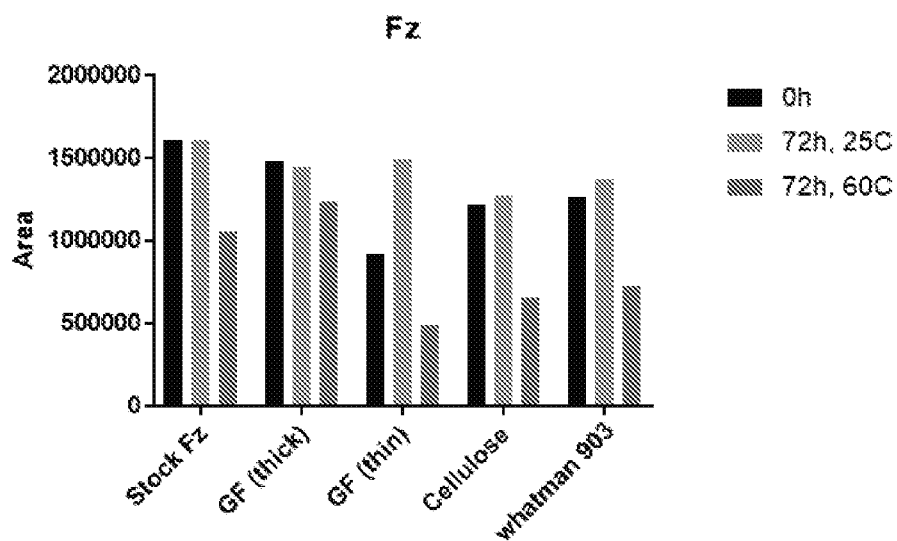


FIG. 39B

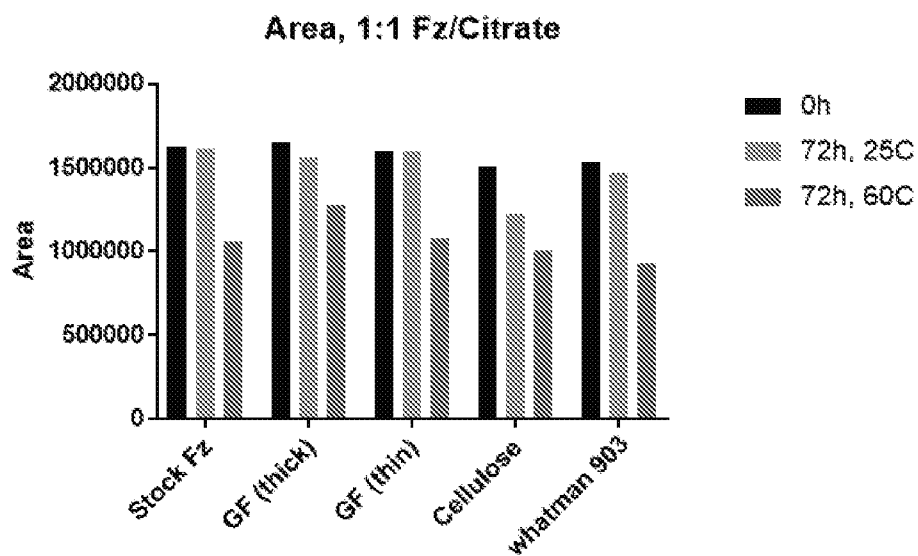


FIG. 39C

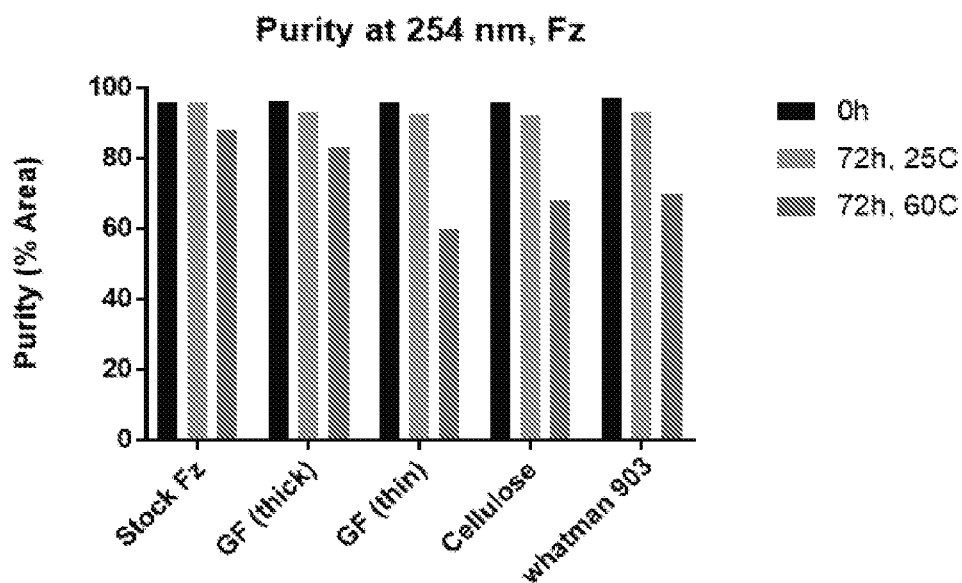


FIG. 39D

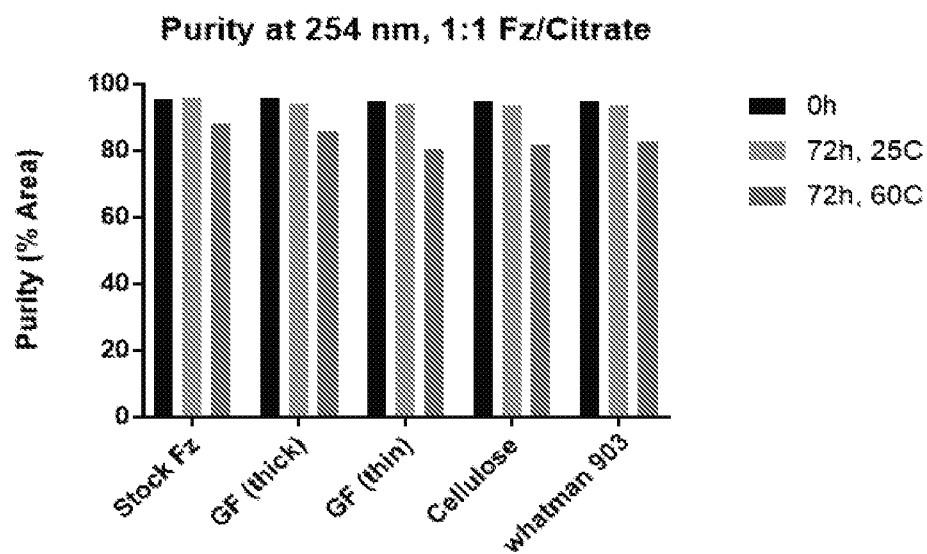


FIG. 40A

Whatman 903 protein saver card punches at 25C
PBS, pH 7.0
2 ng/ml NIuc

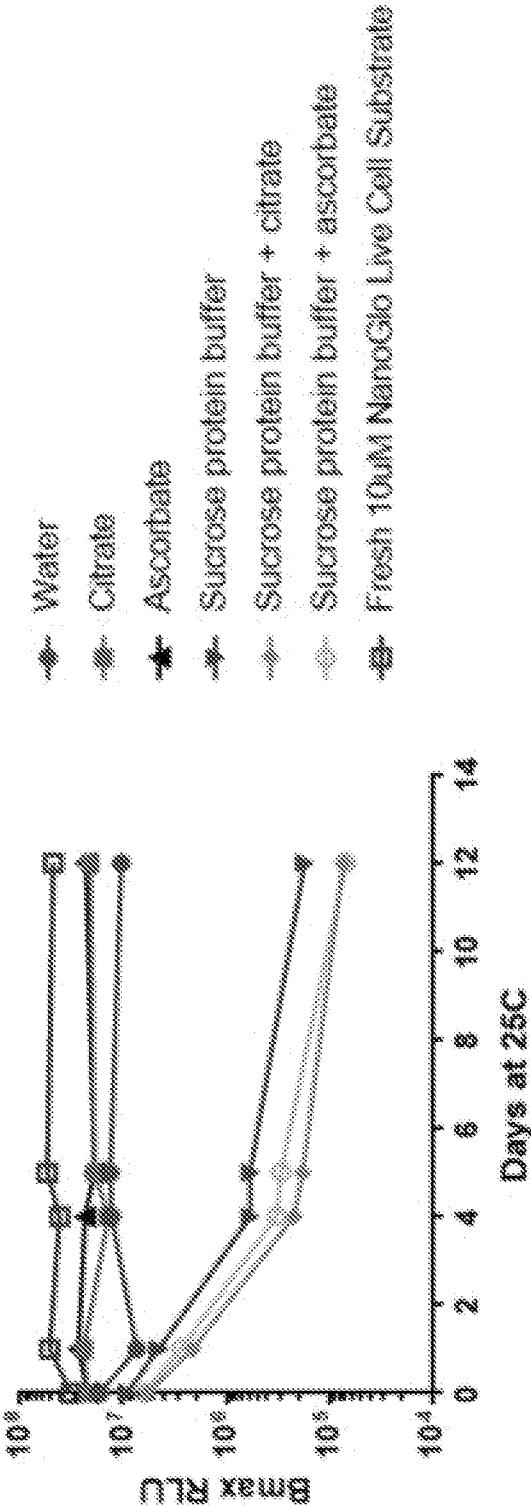


FIG. 40B

Whatman 903 protein saver card punches at 25C

PBS, pH 7.0

2 ng/ml Nluc

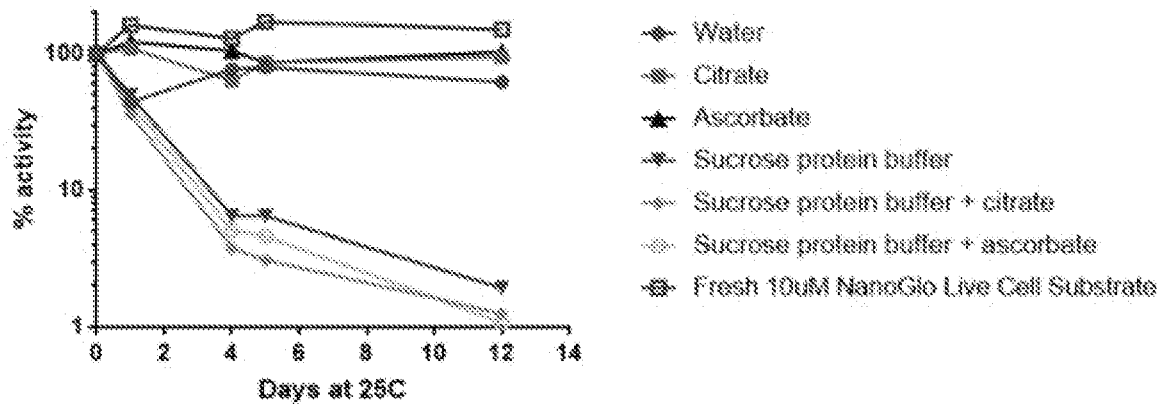


FIG. 40C

Day 12

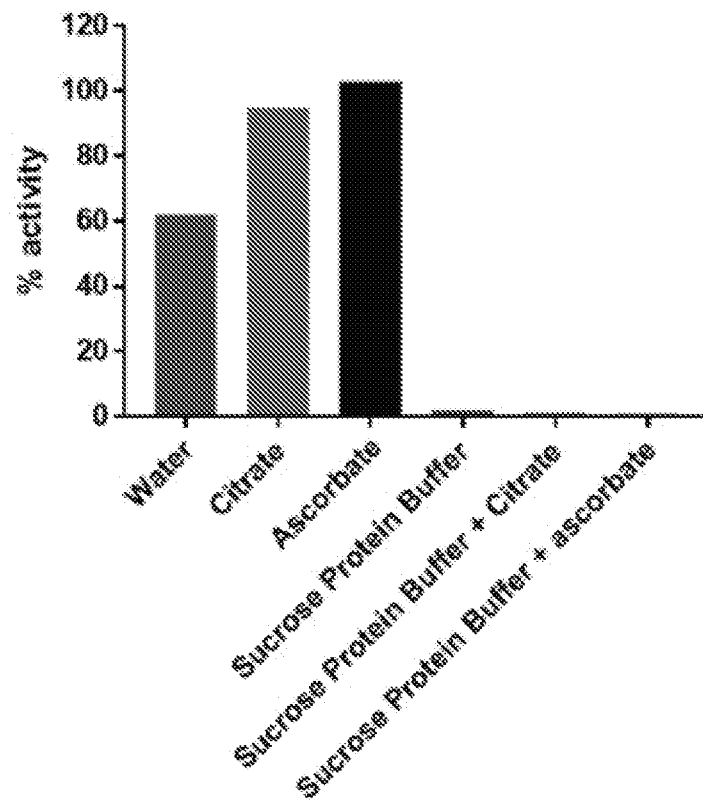
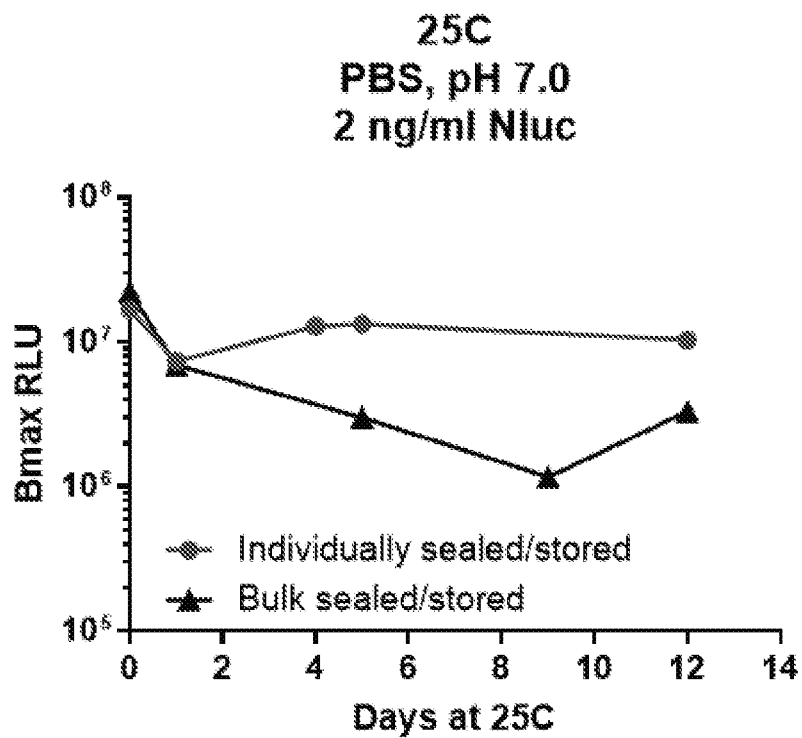


FIG. 41

A.



B.

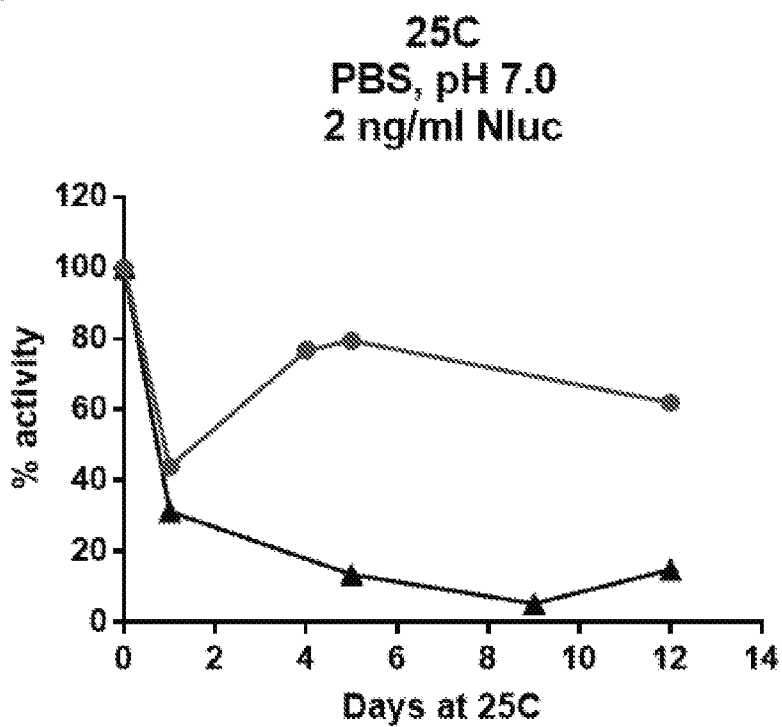


FIG. 42A

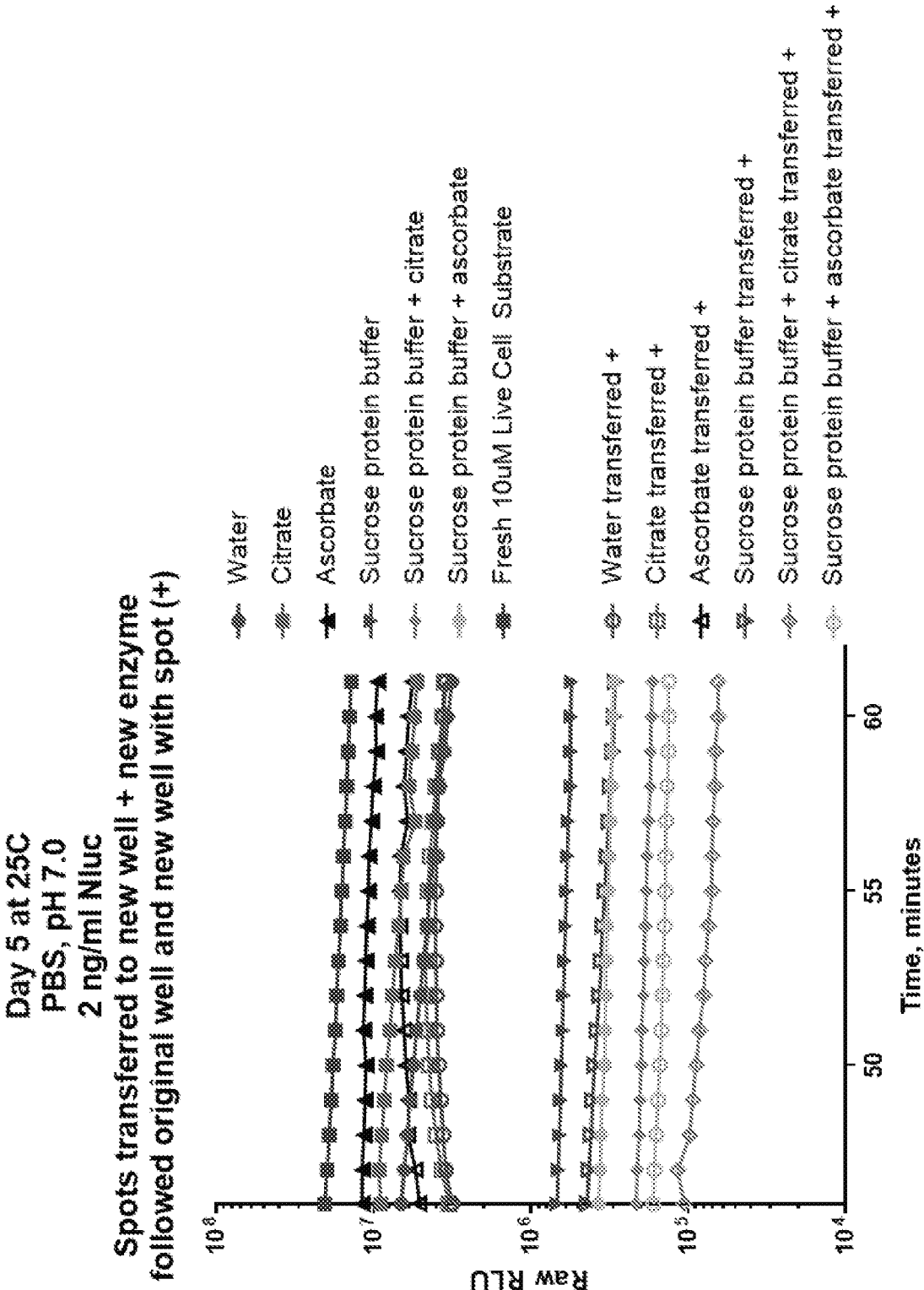


FIG. 42B

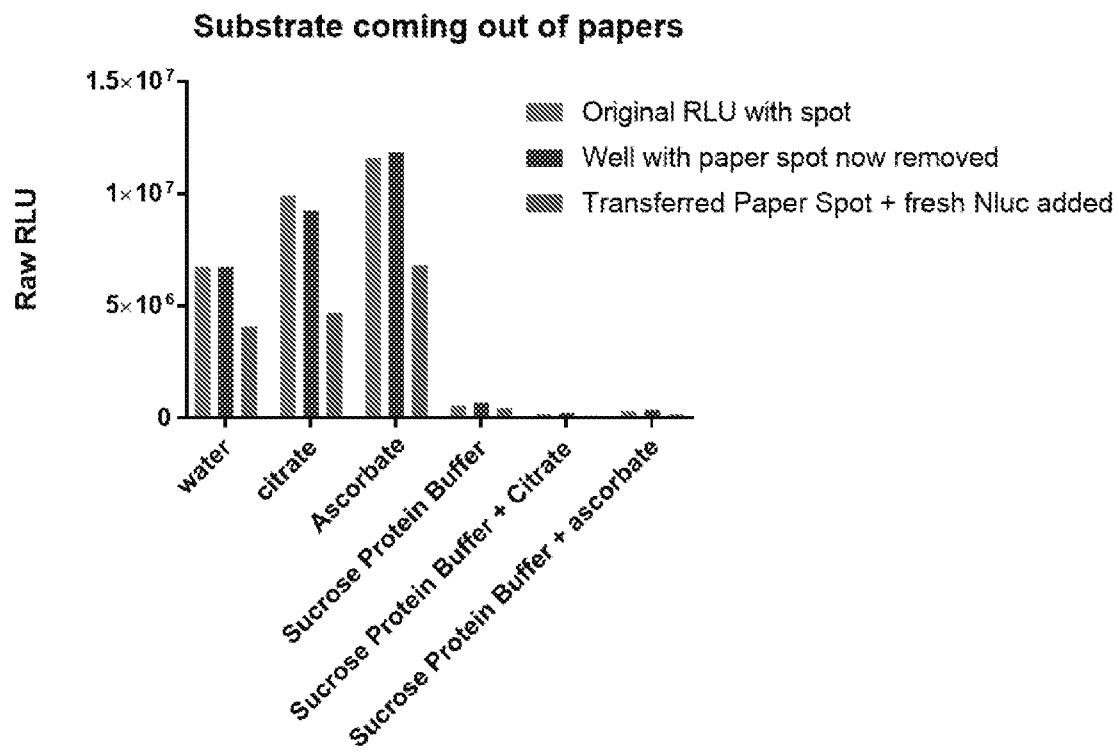


FIG. 42C

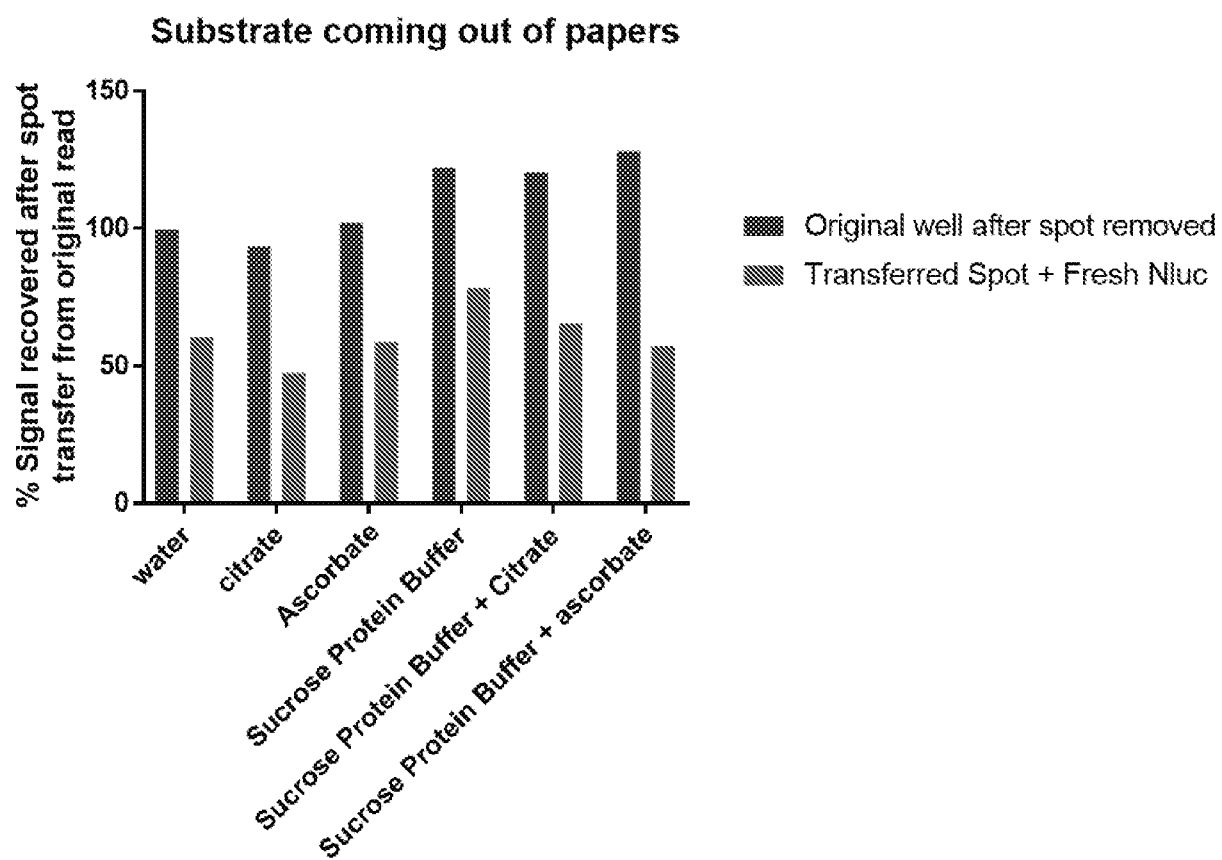
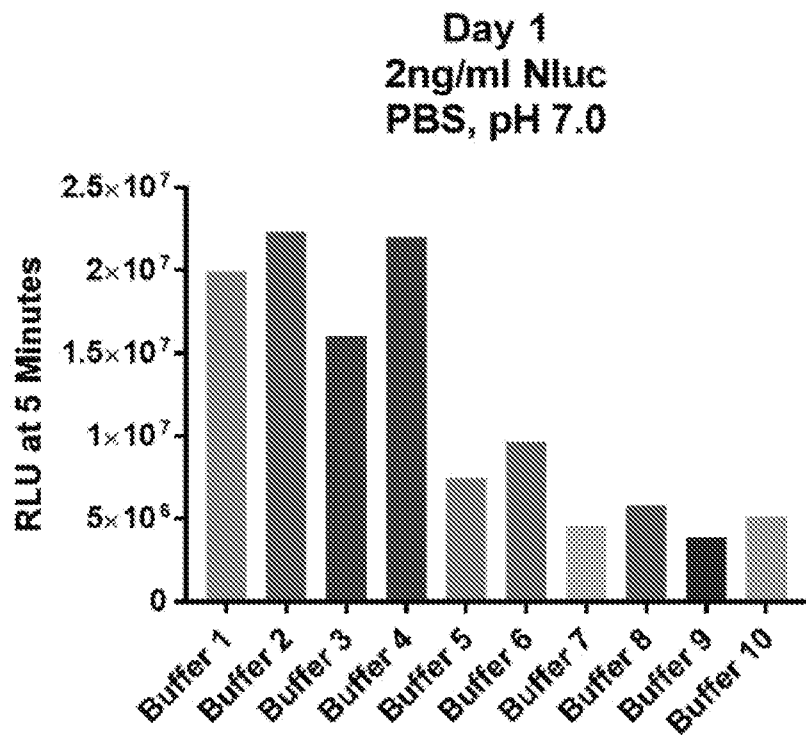


FIG. 43

A.



B.

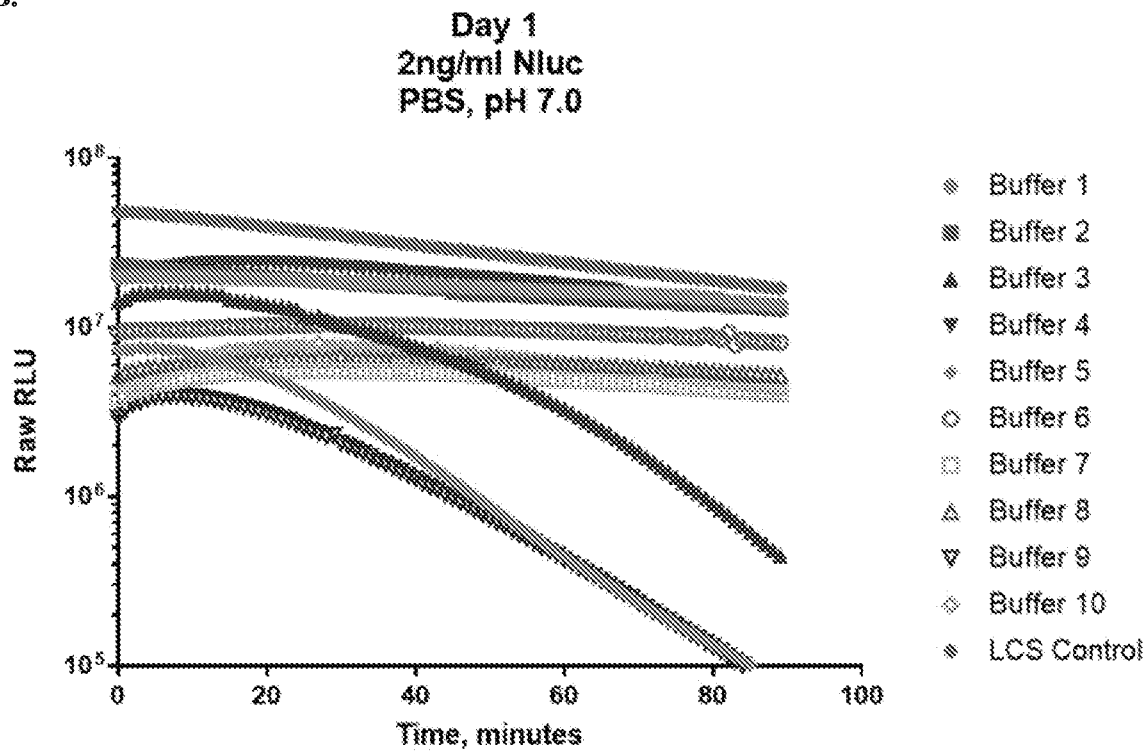


FIG. 43 (cont'd)

C.

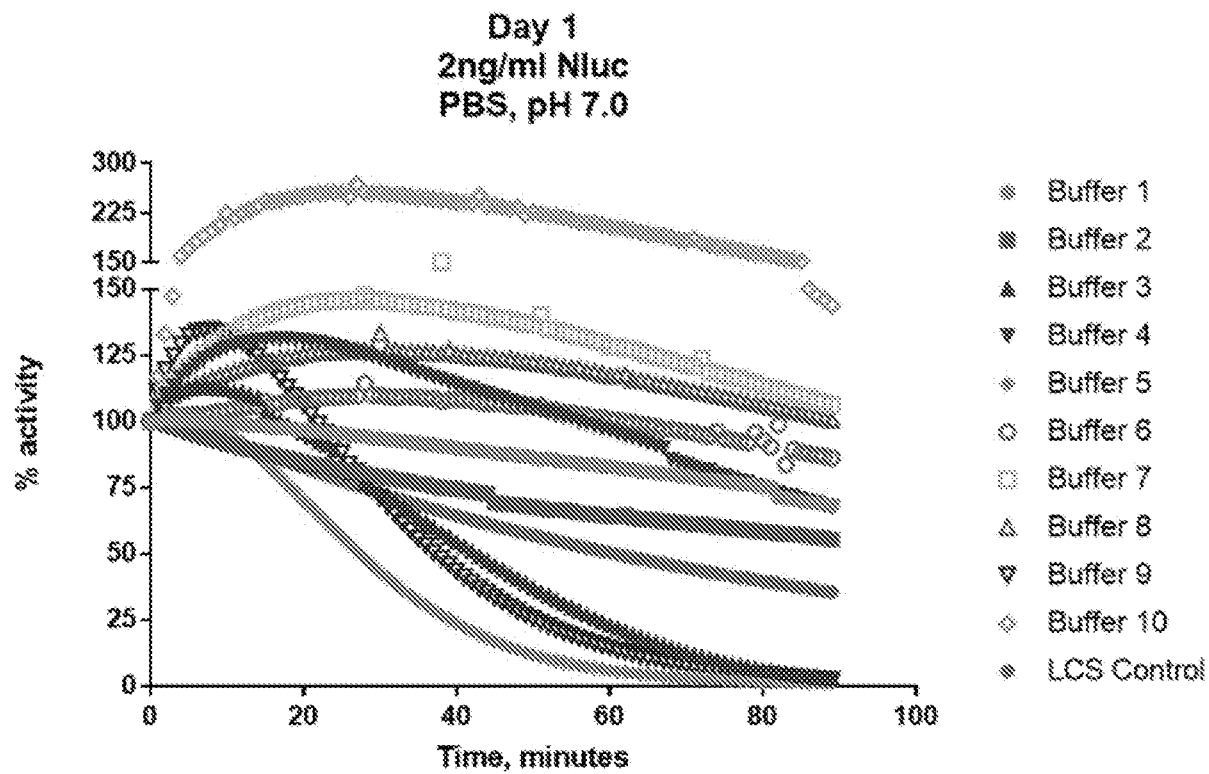
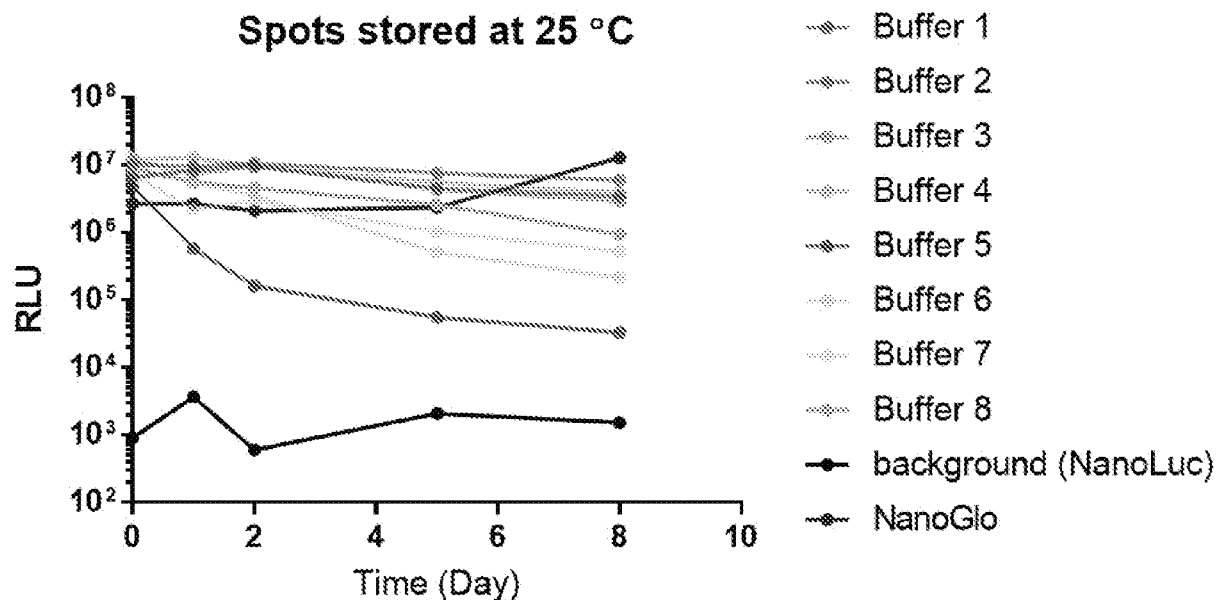


FIG. 44

A.



B.

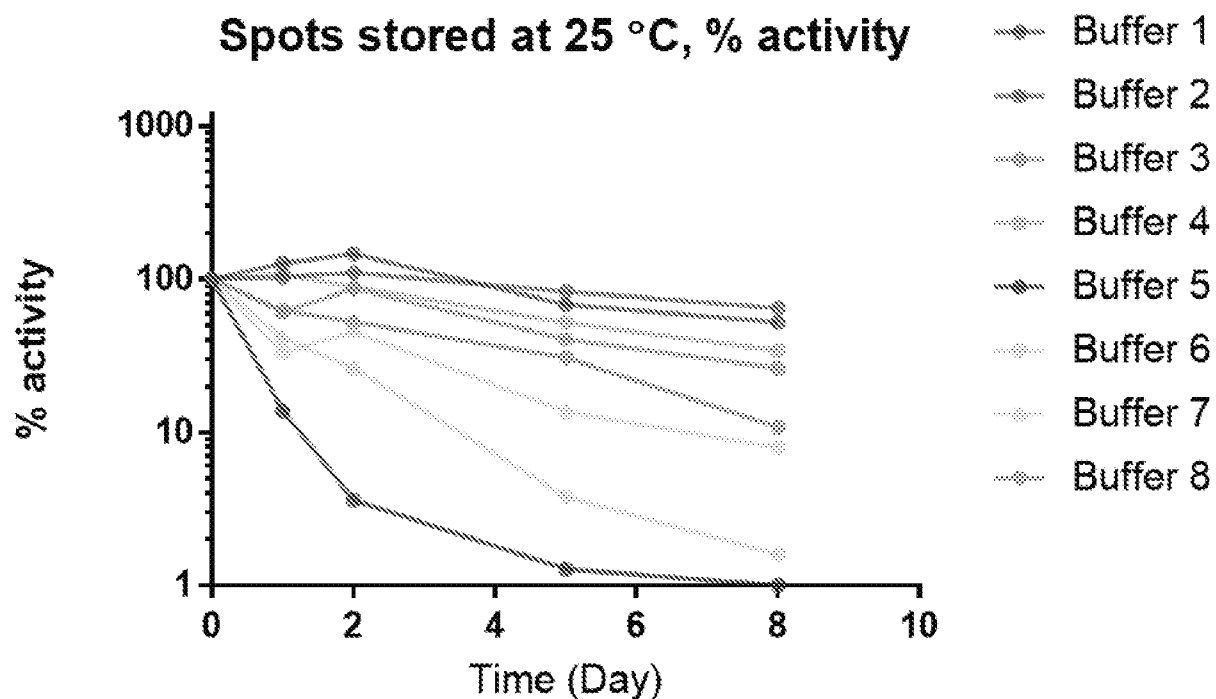


FIG. 44 (cont'd)

C.

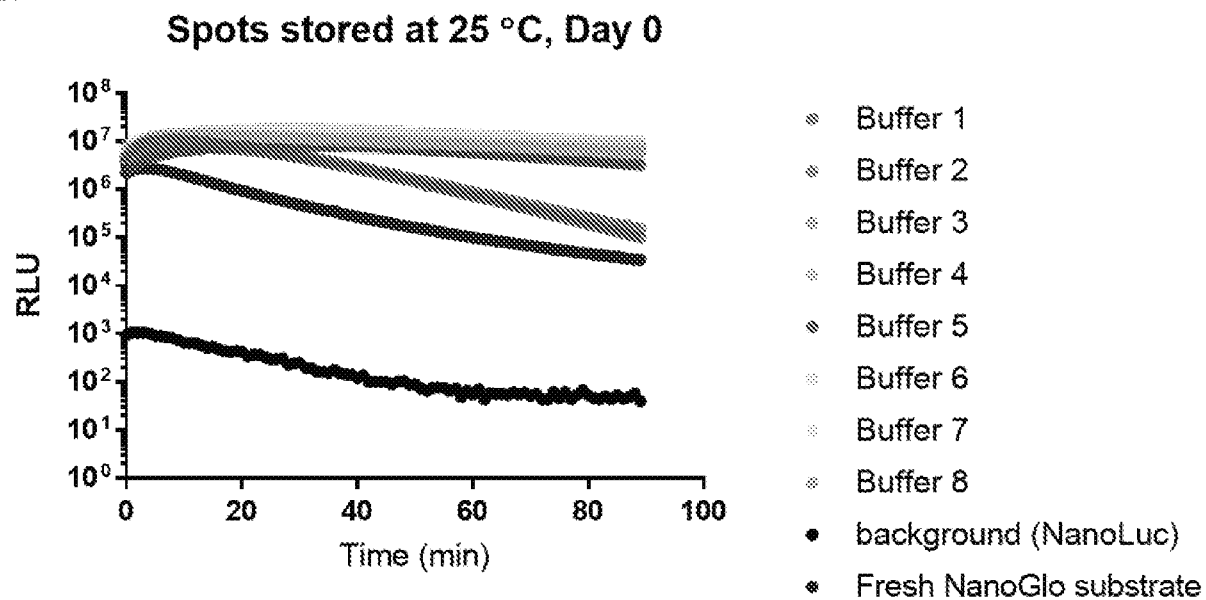
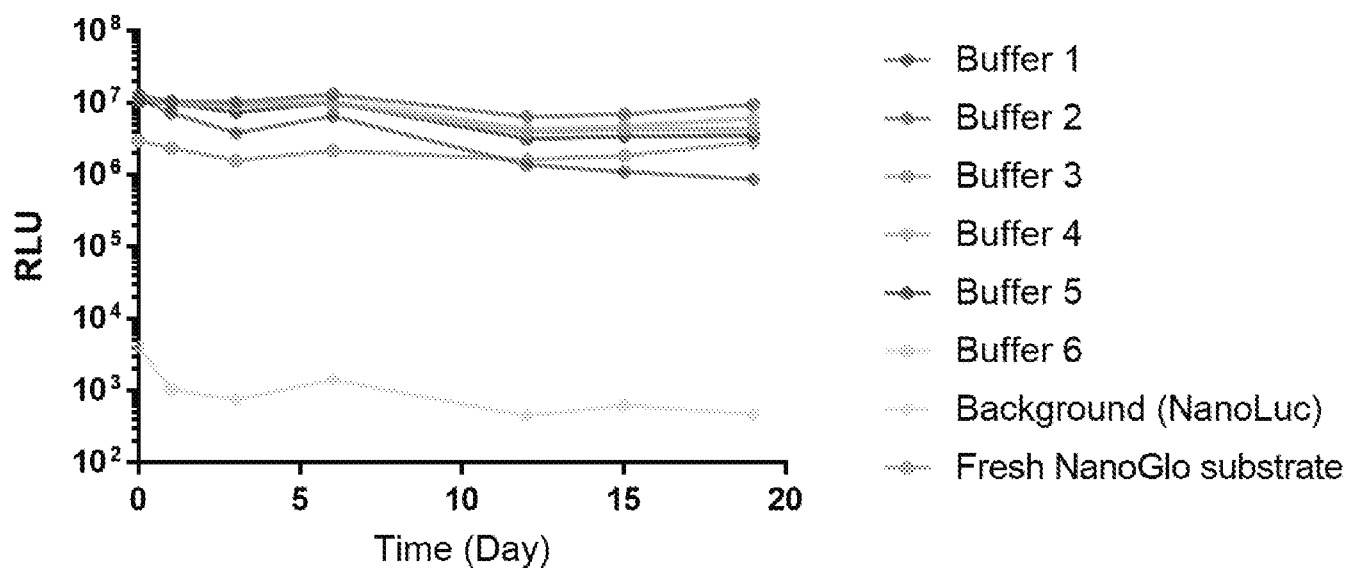


FIG. 45

A.

Spots stored at 25 °C, Summary

B.

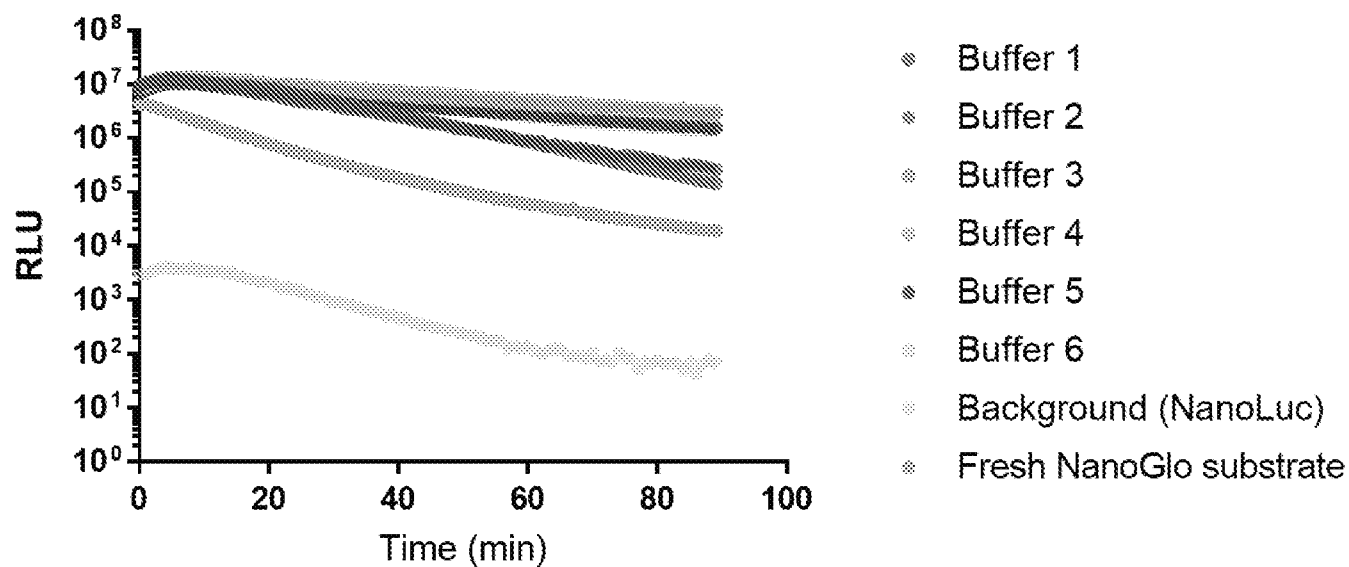
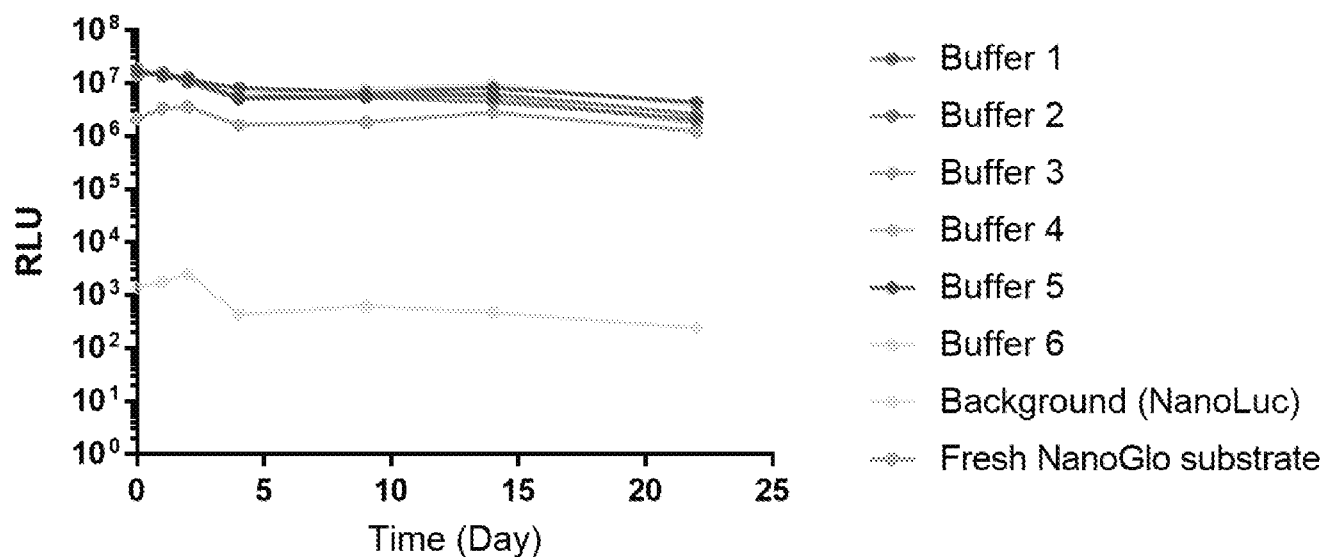
Spots stored at 25 °C, Day 0

FIG. 46

A.

Spots stored at 25 °C, Summary

B.

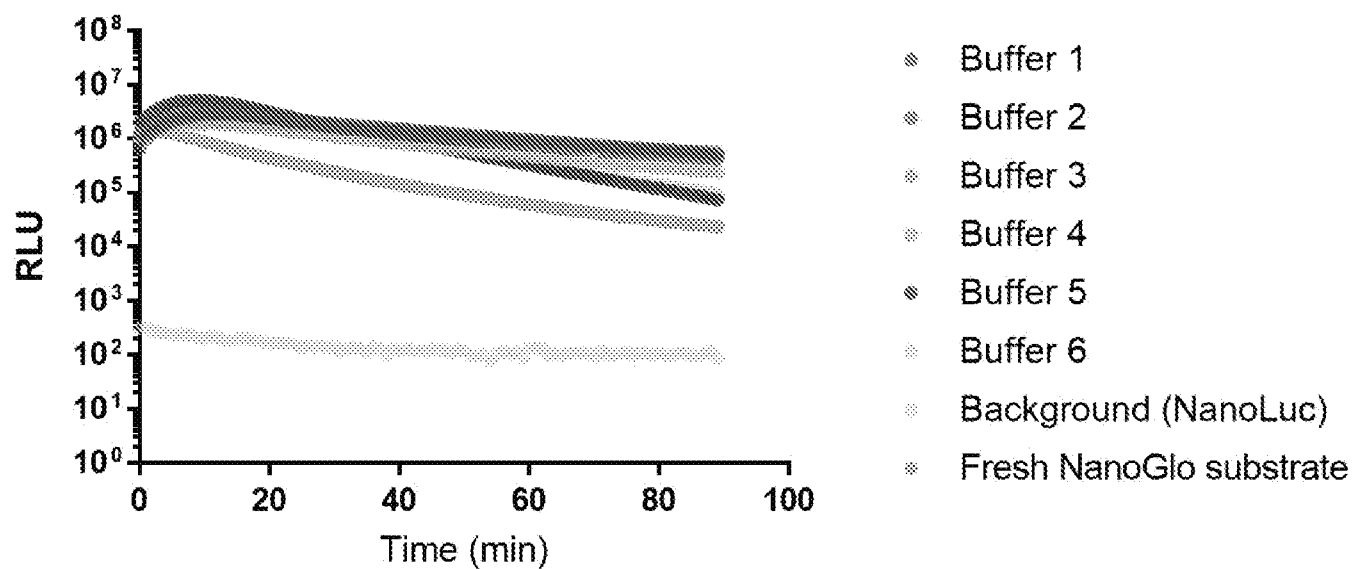
Spots stored at 25 °C, Day 22

FIG. 47A

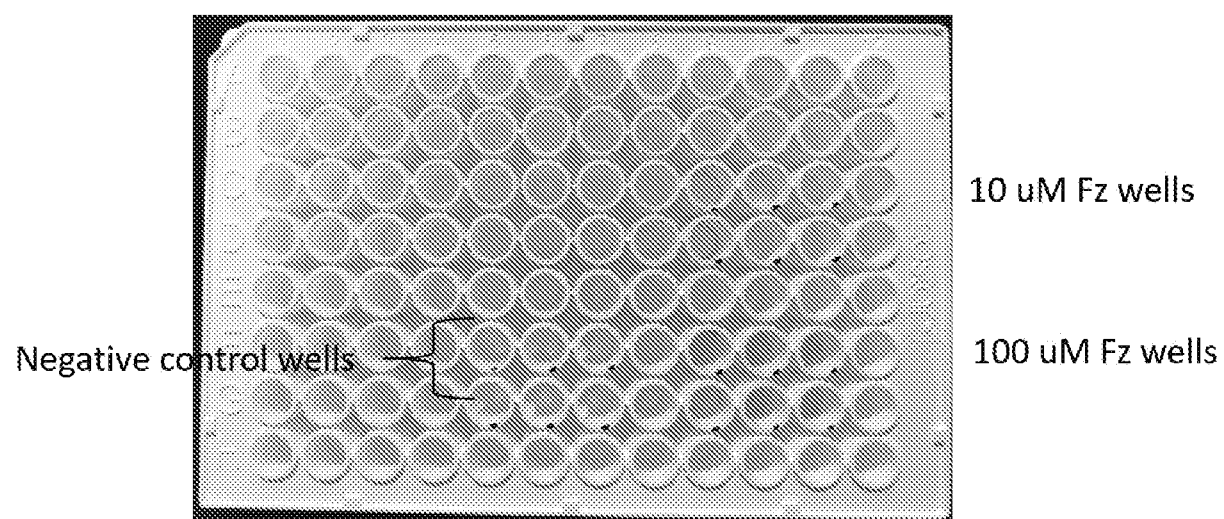


FIG. 47B

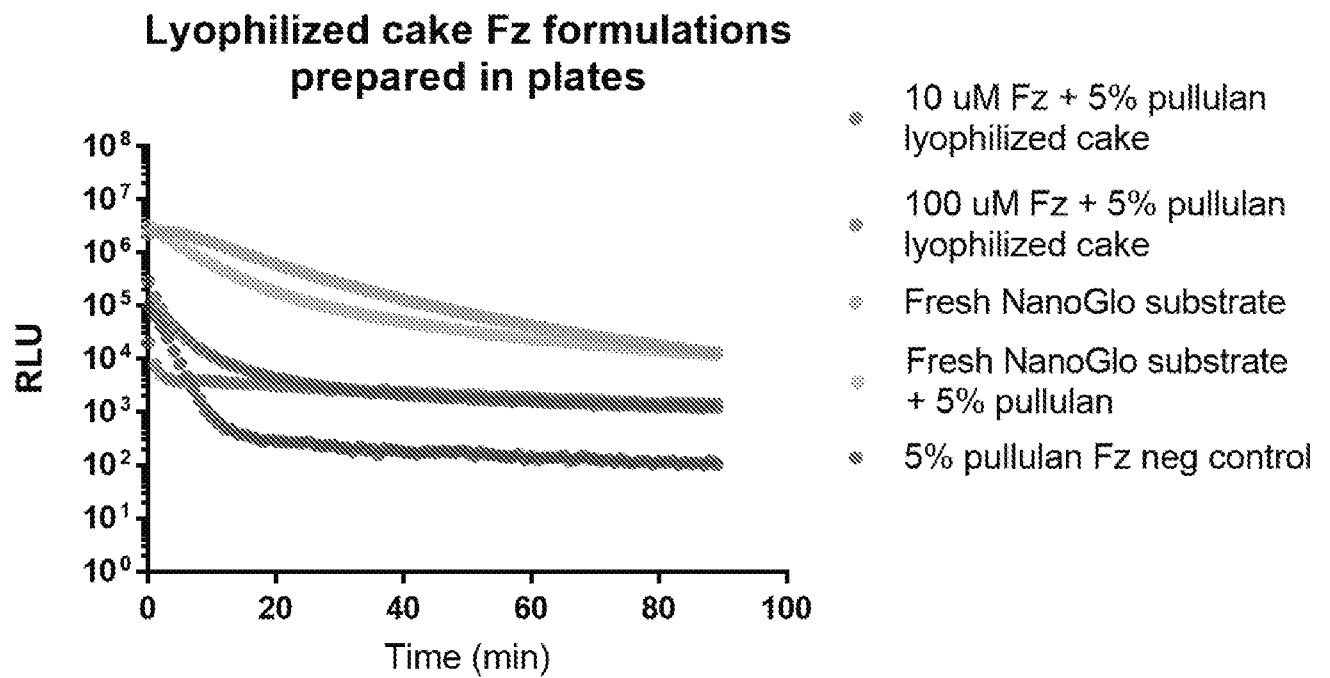


FIG. 48

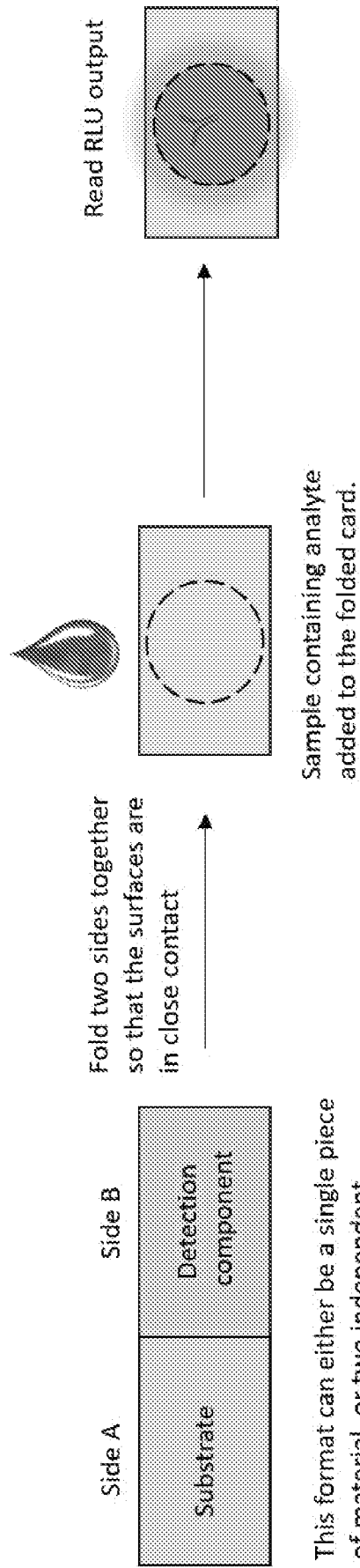


FIG. 49

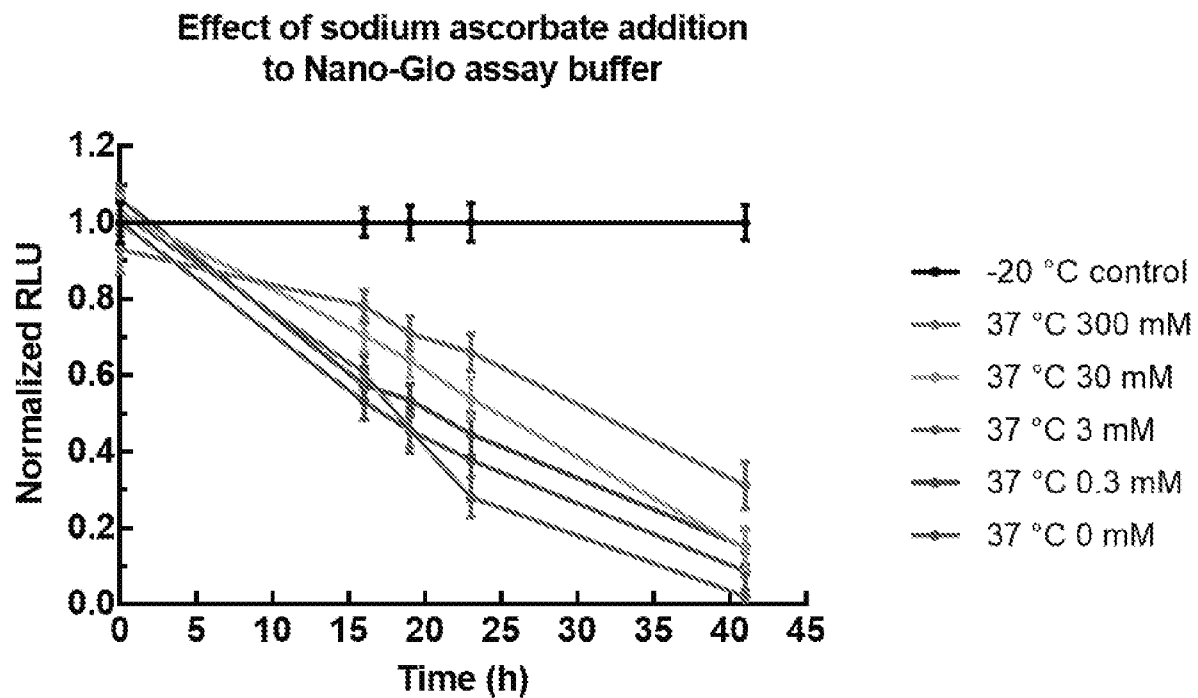


FIG. 50

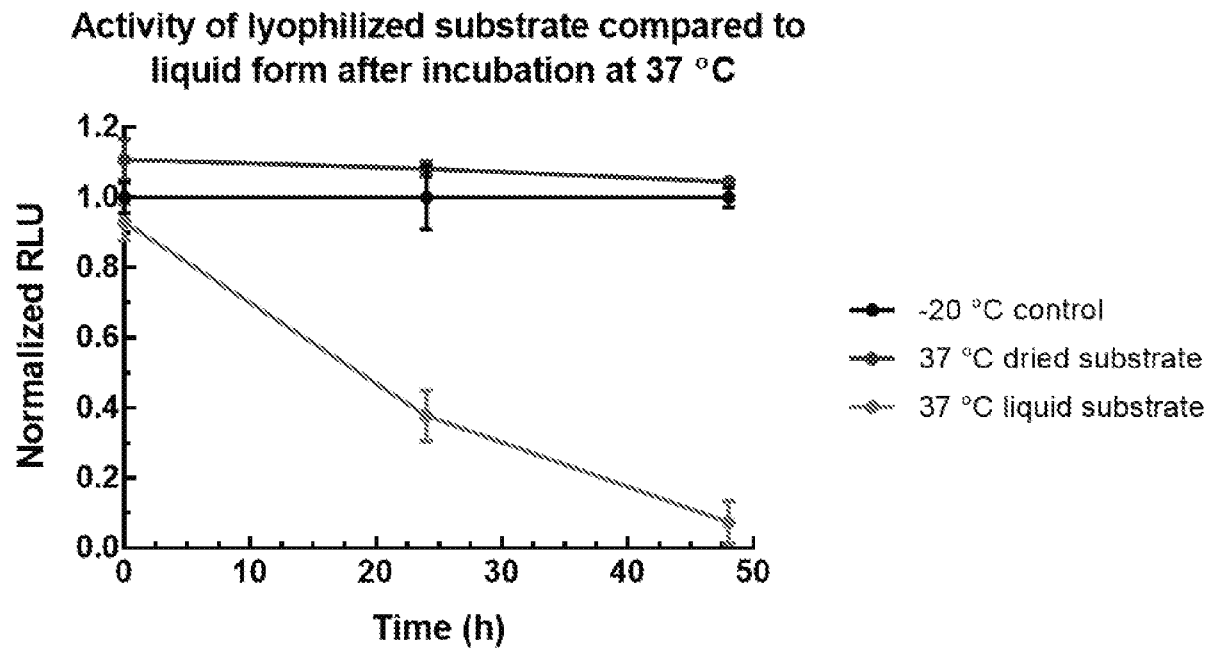
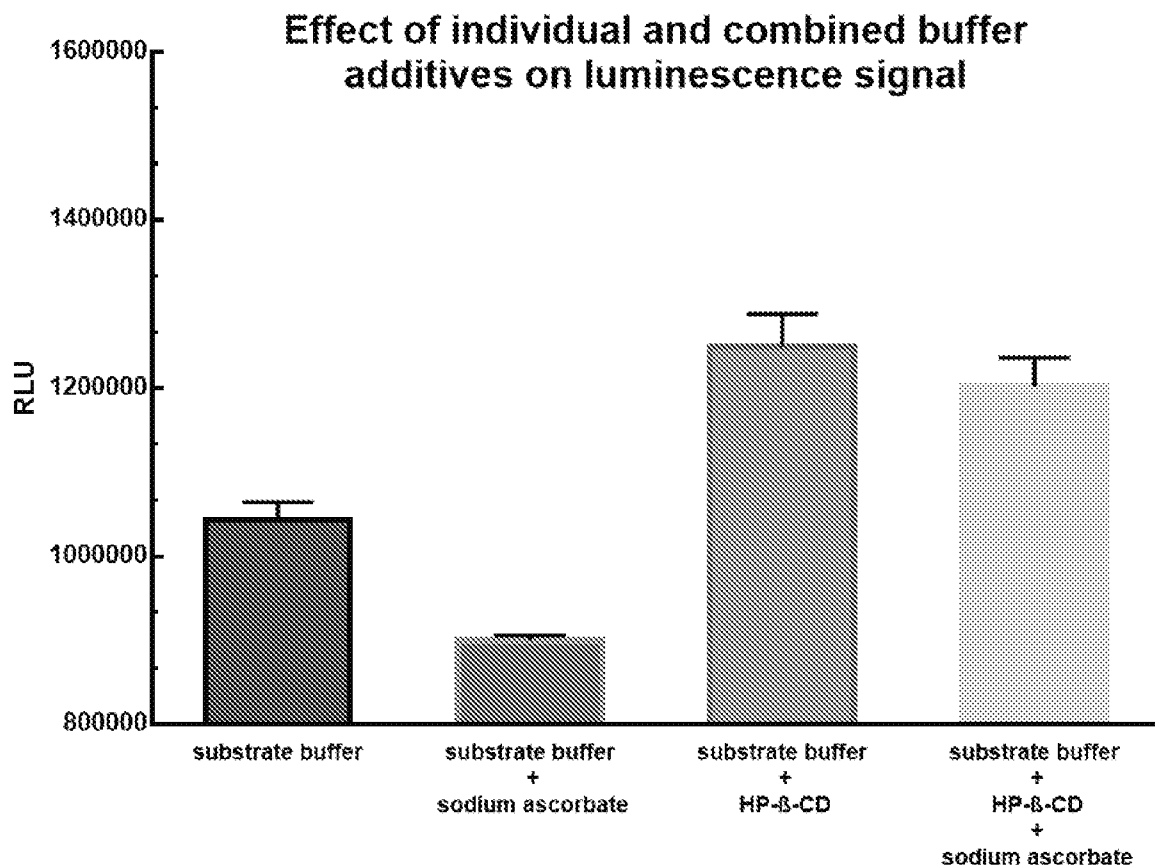


FIG. 51

A.



B.

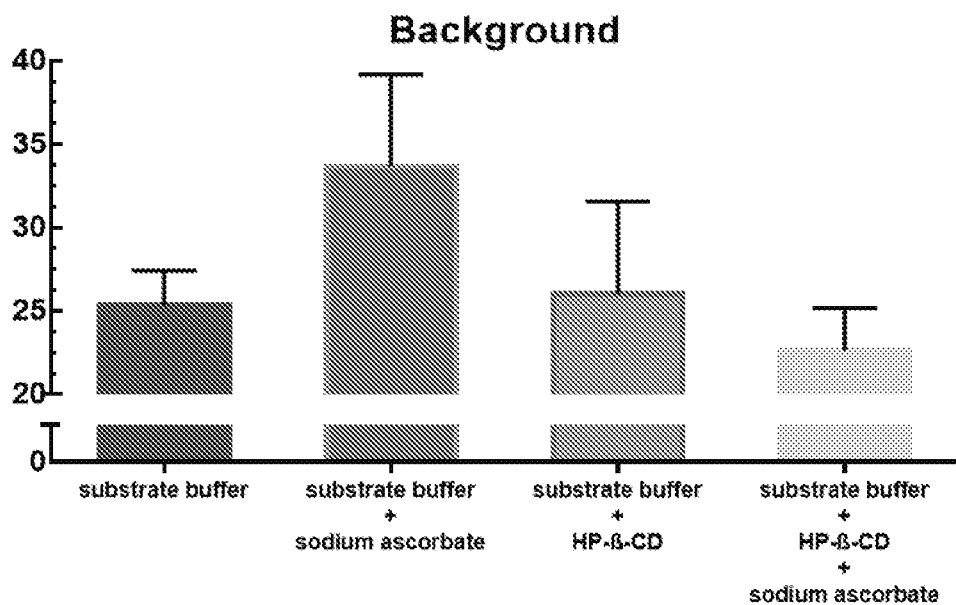


FIG. 52

Long term stability of pulullan encapsulated substrate at room temperature and 37 °C

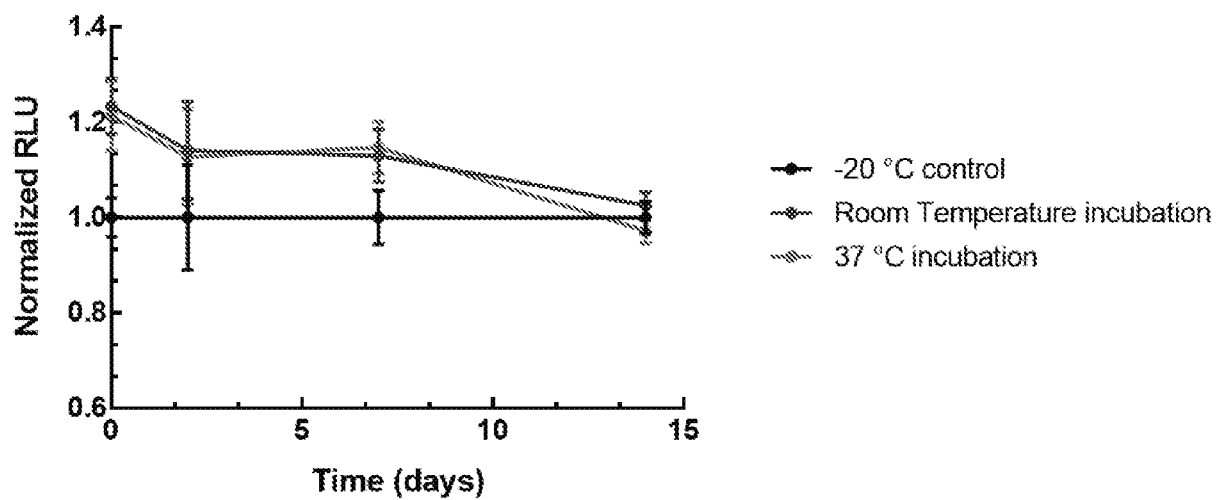


FIG. 53

Condition 1

Condition 2

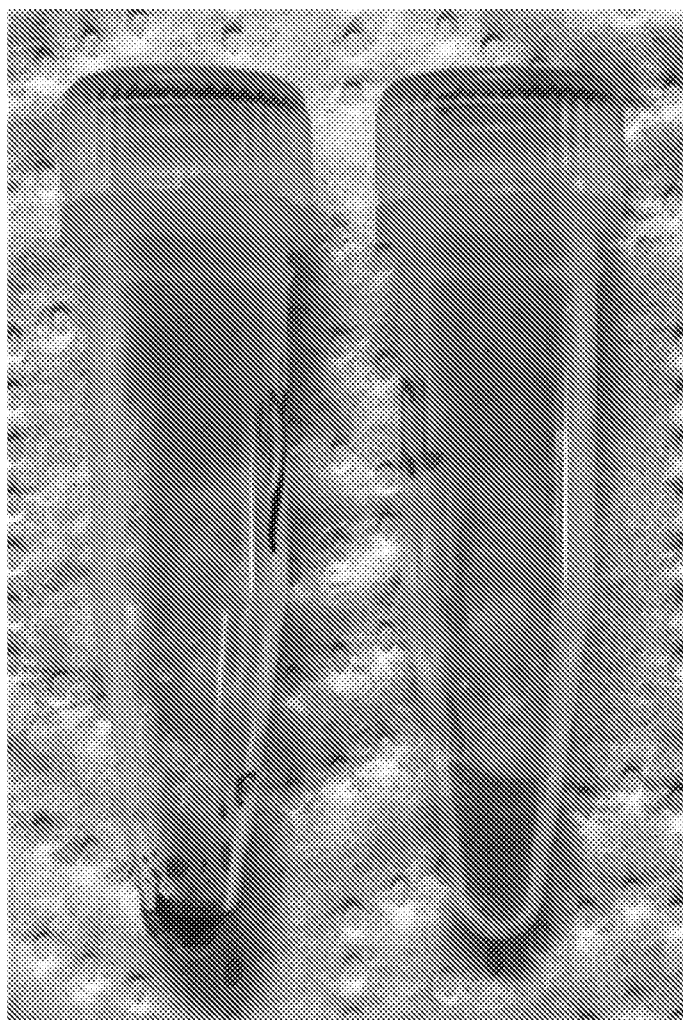


FIG. 54A

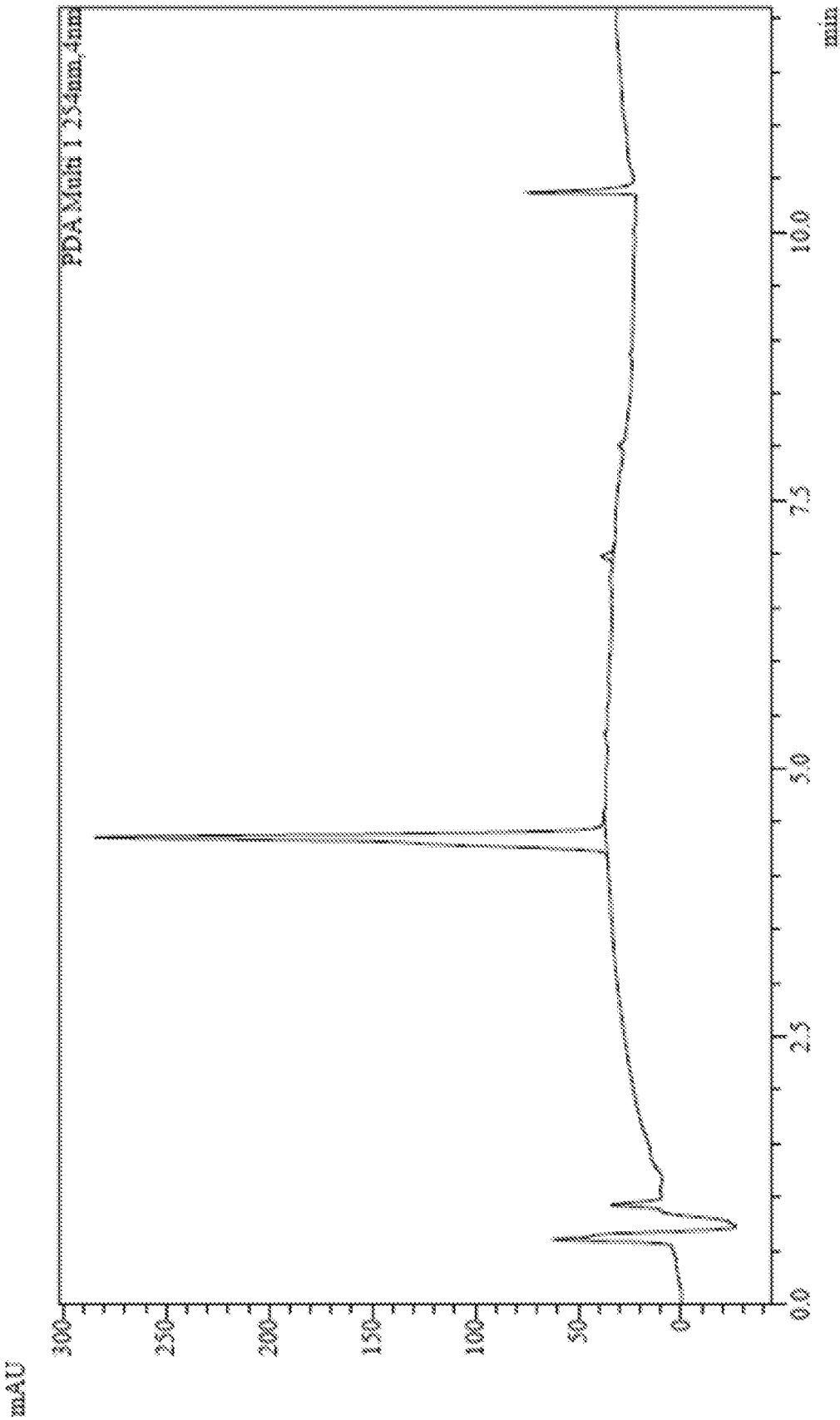


FIG. 54B

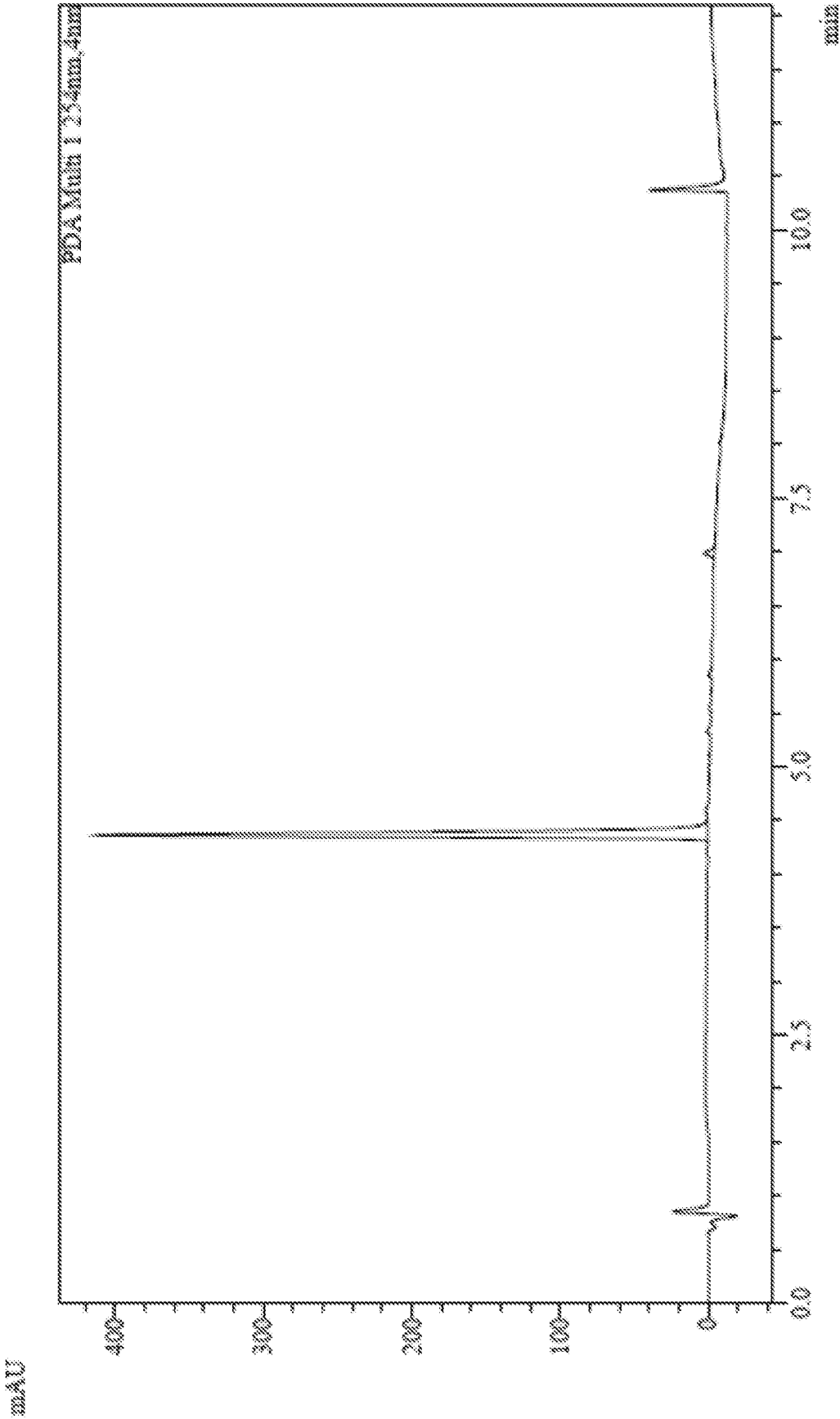


FIG. 54C

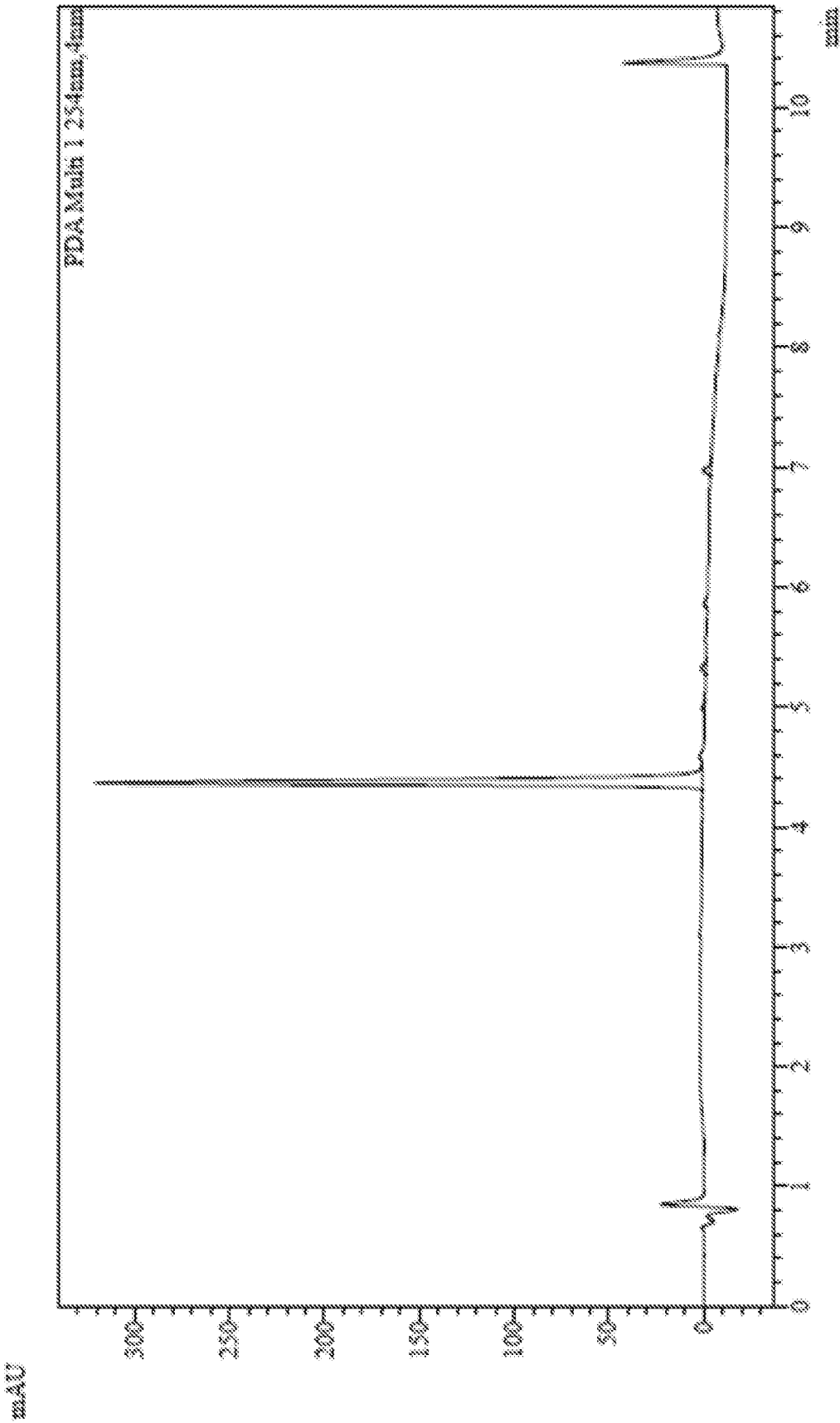


FIG. 55A

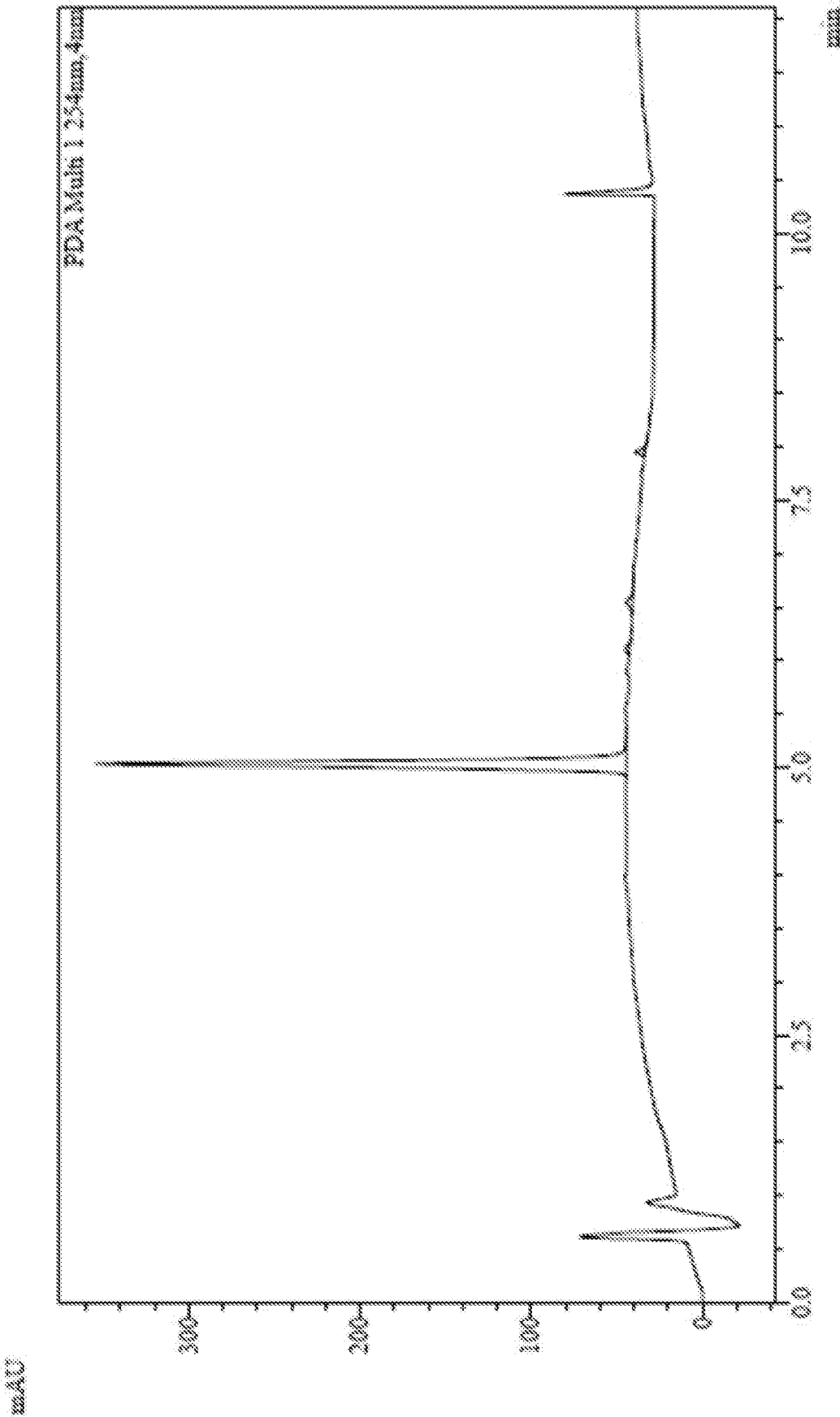


FIG. 55B

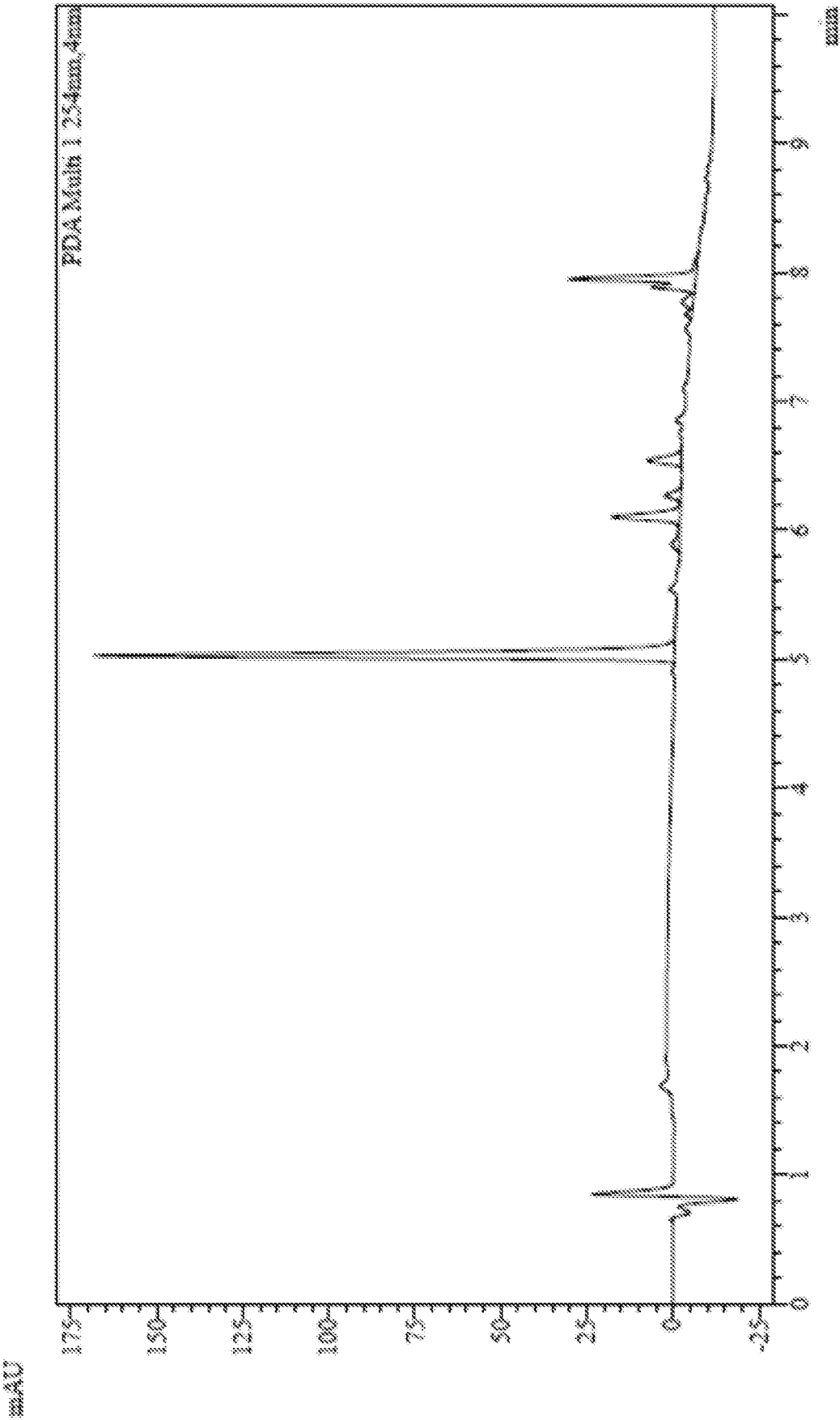


FIG. 55C

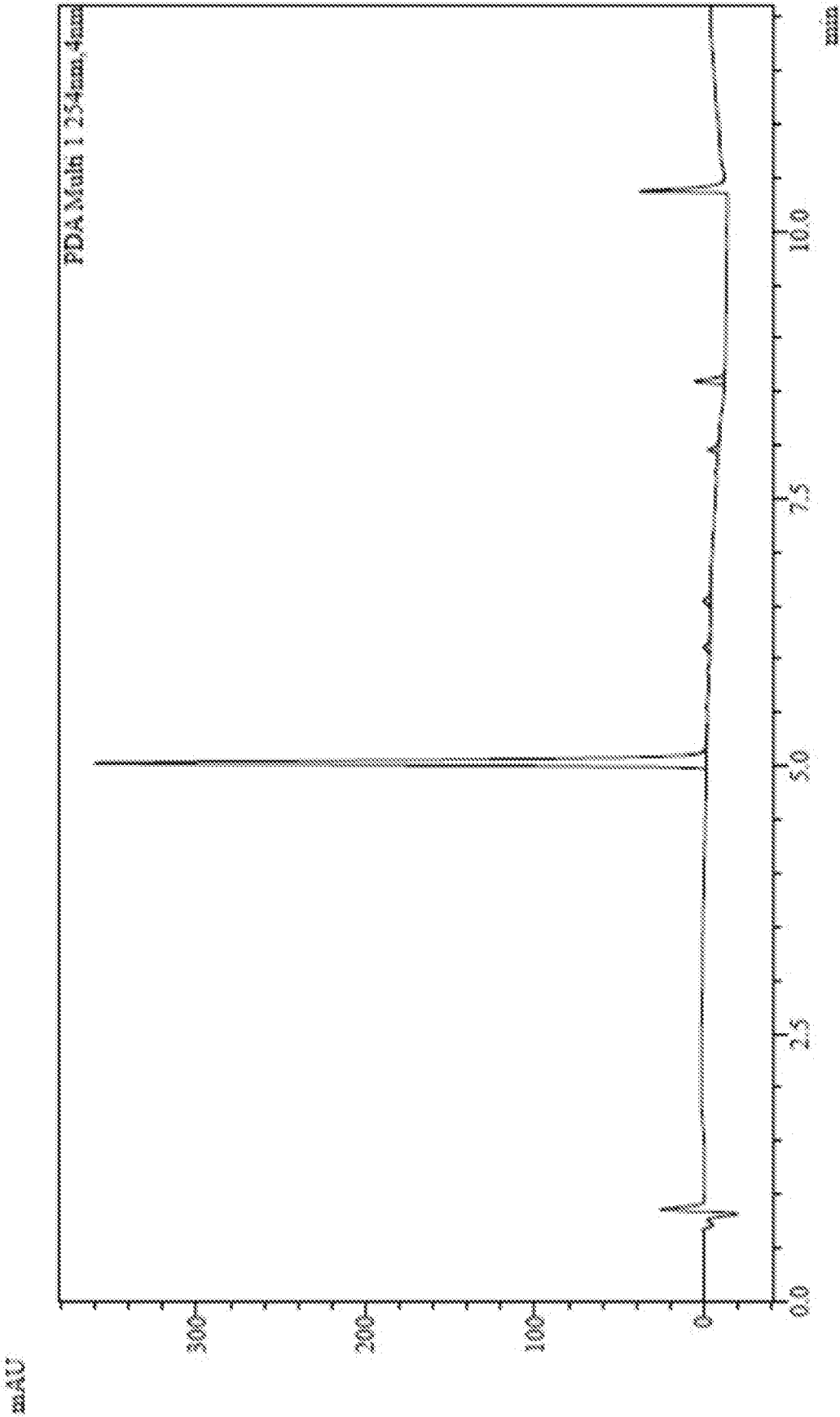


FIG. 56

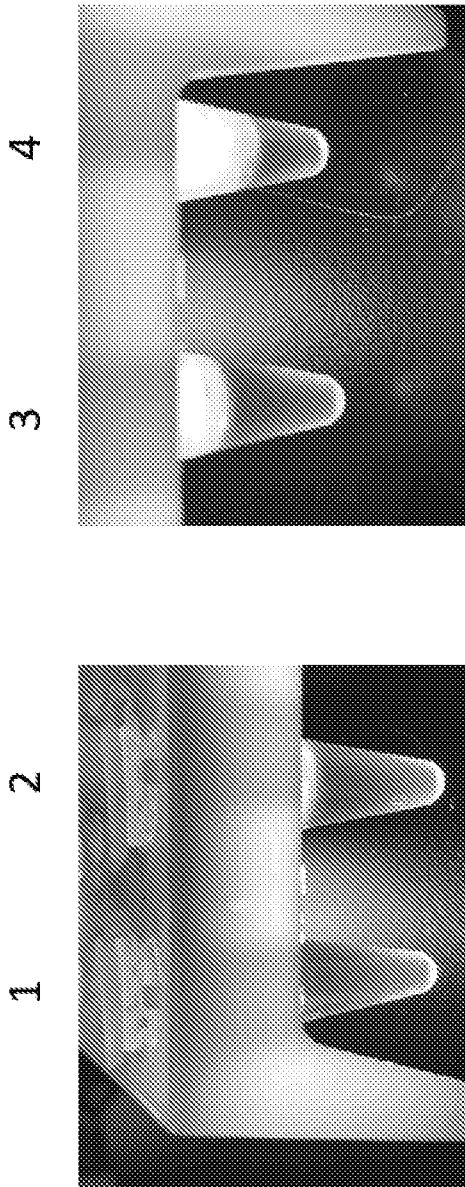


FIG. 57

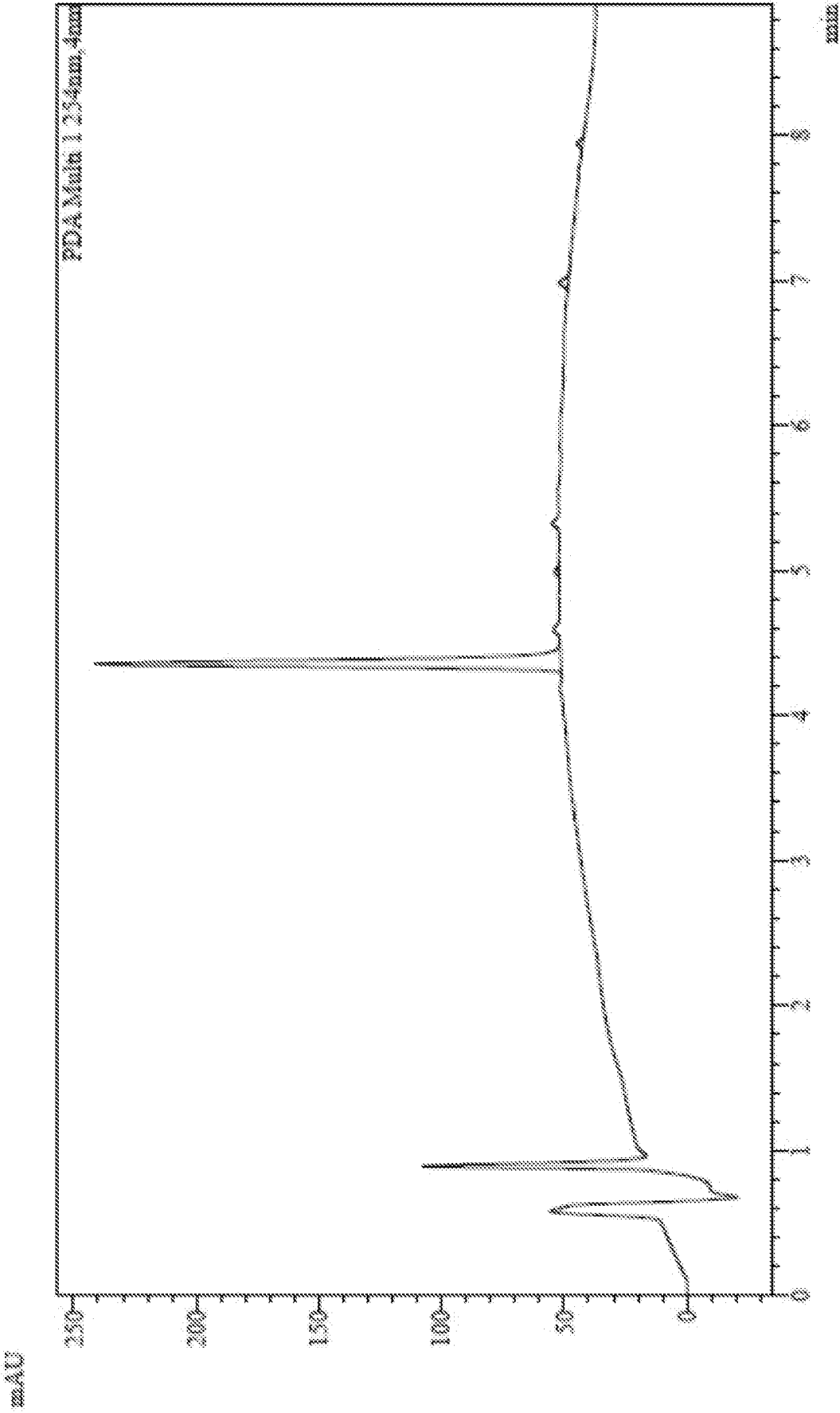
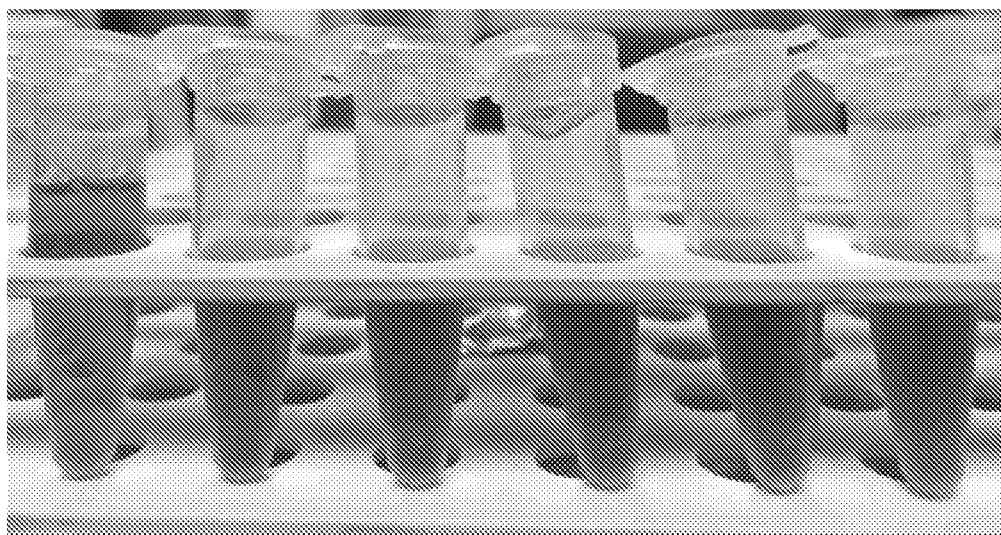


FIG. 58

A.

7x w/w 6x 5x 4x 3x 2x



B.

7x w/w 6x 5x 4x 3x 2x

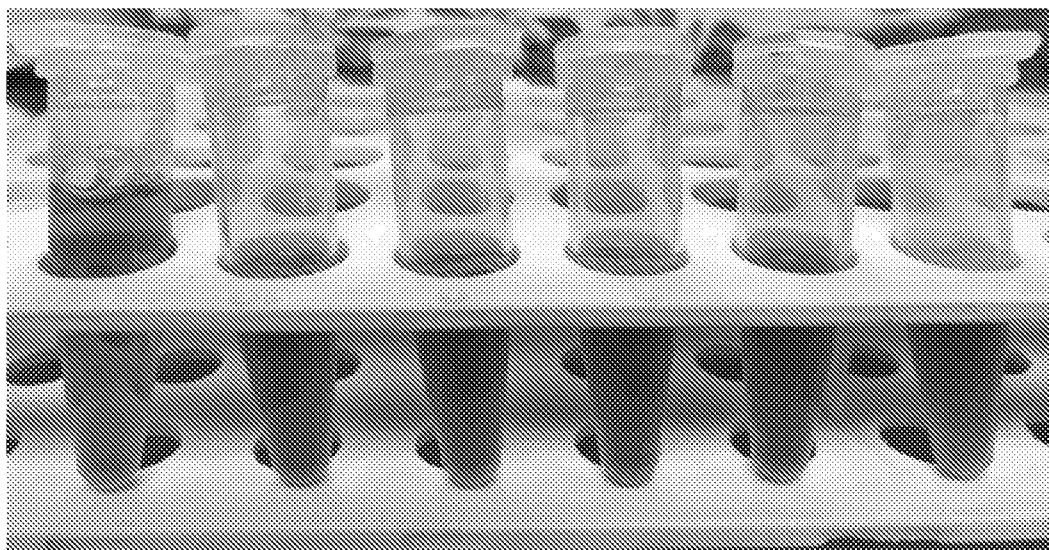
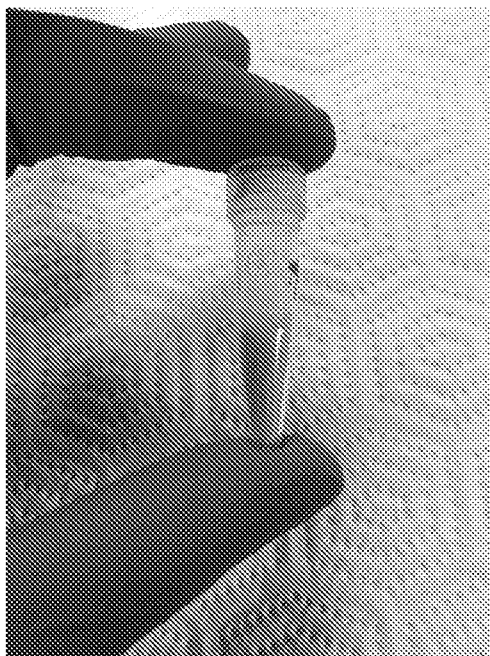


FIG. 59

A.



B.

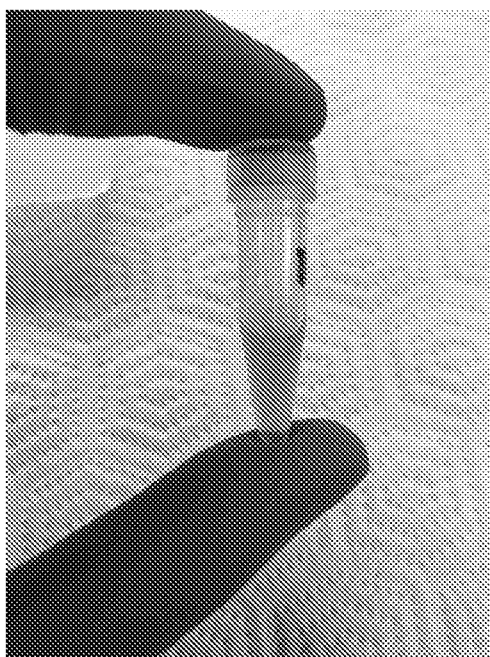
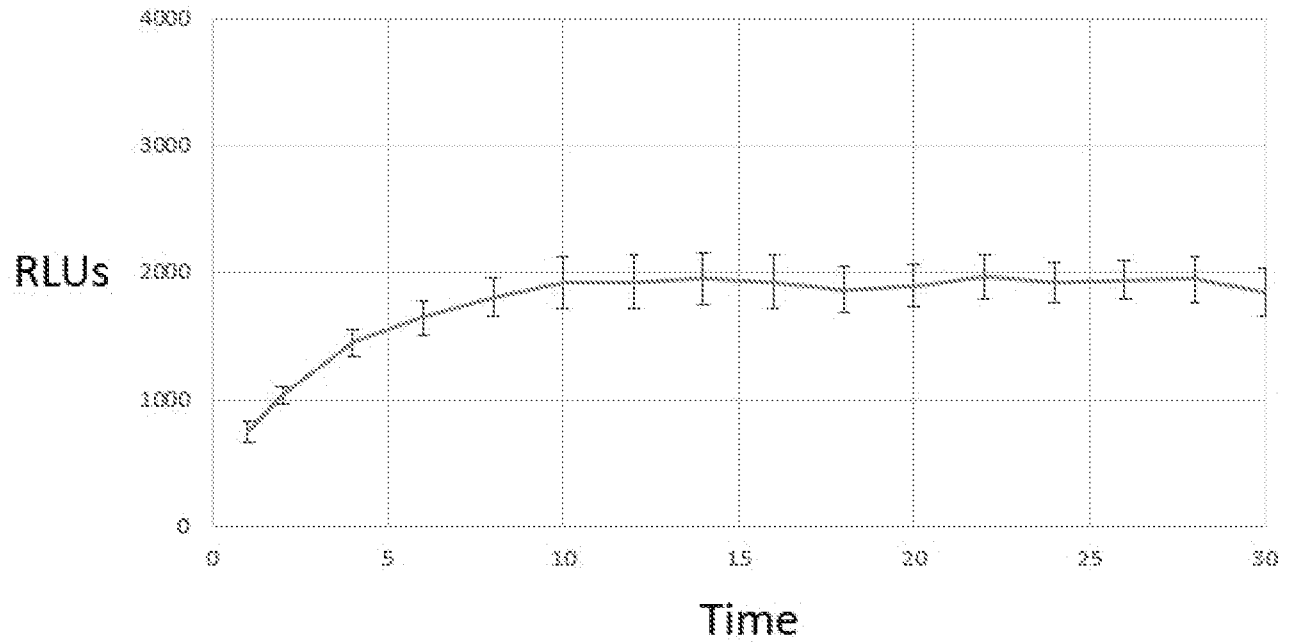


FIG. 60

A.



B.

min0-max3000

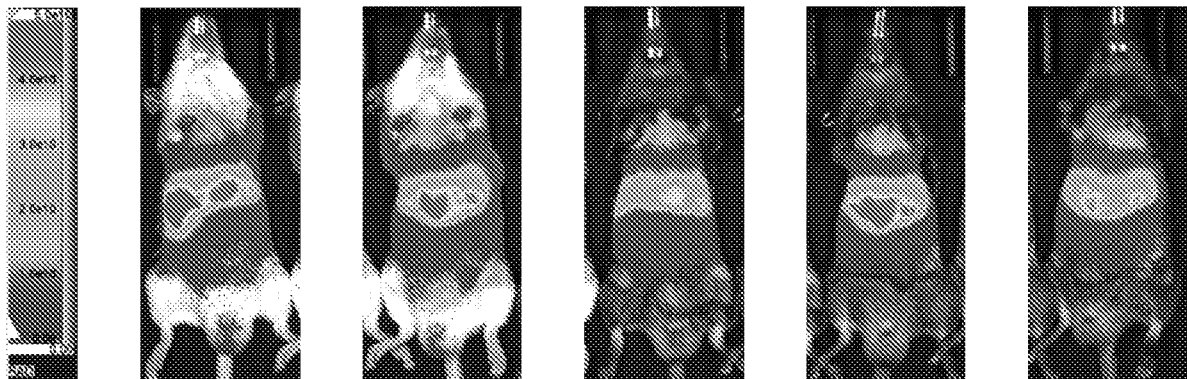
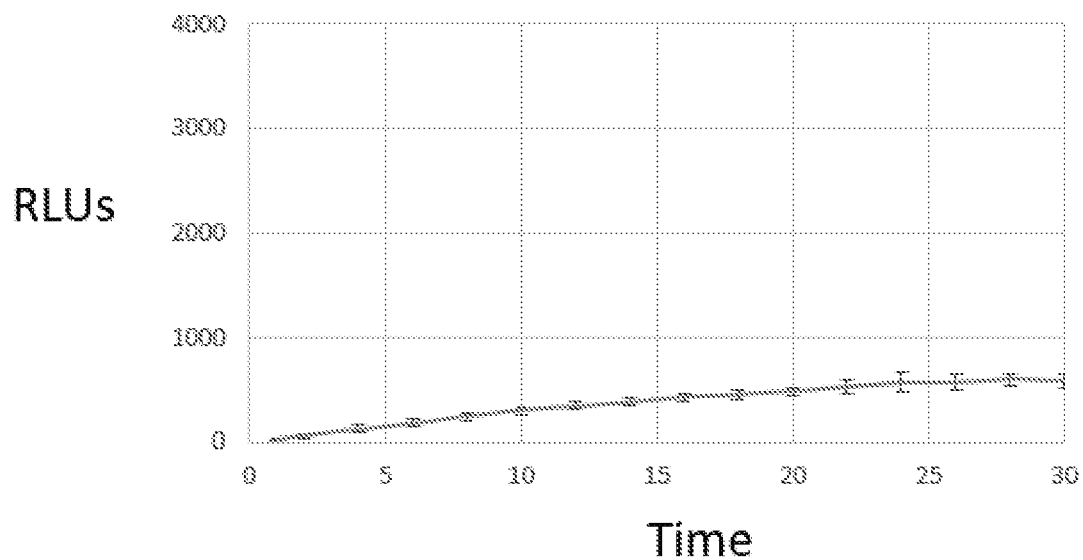


FIG. 61

A.



B.

min0-max3000

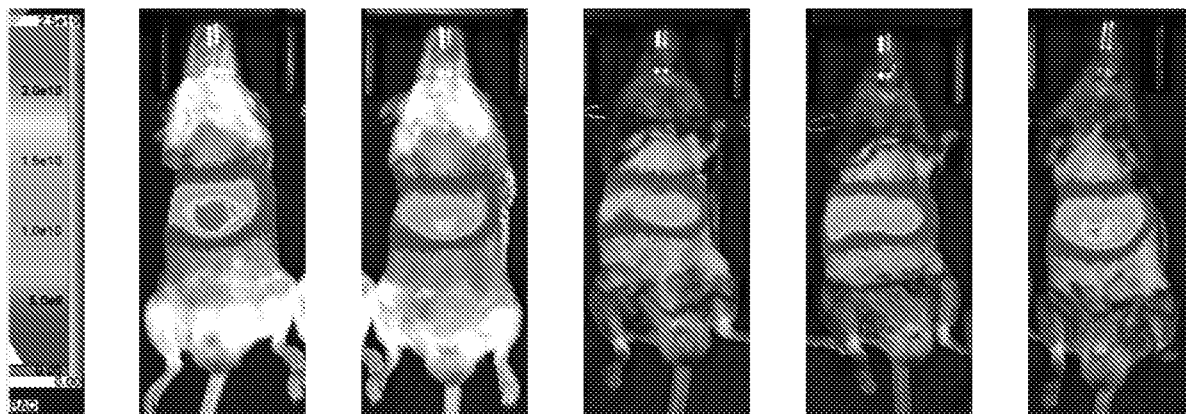
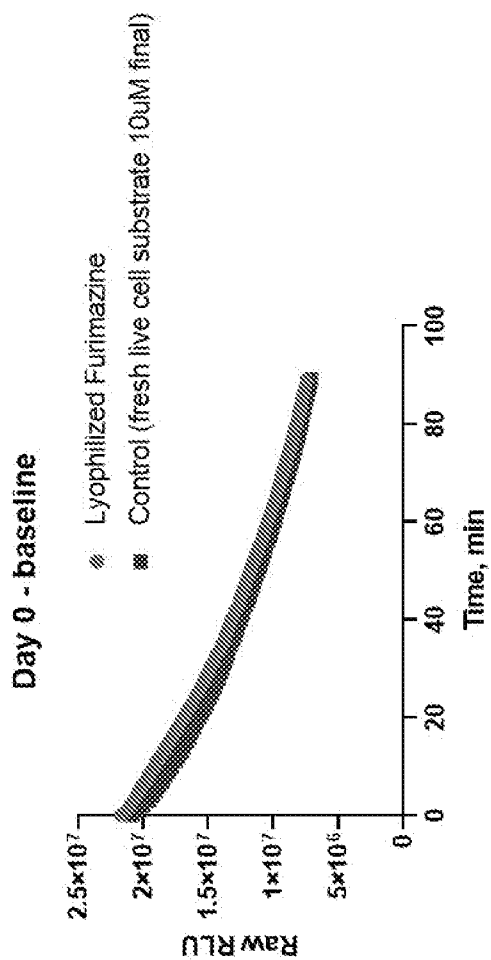


FIG. 62A-B

B



A



FIG. 63

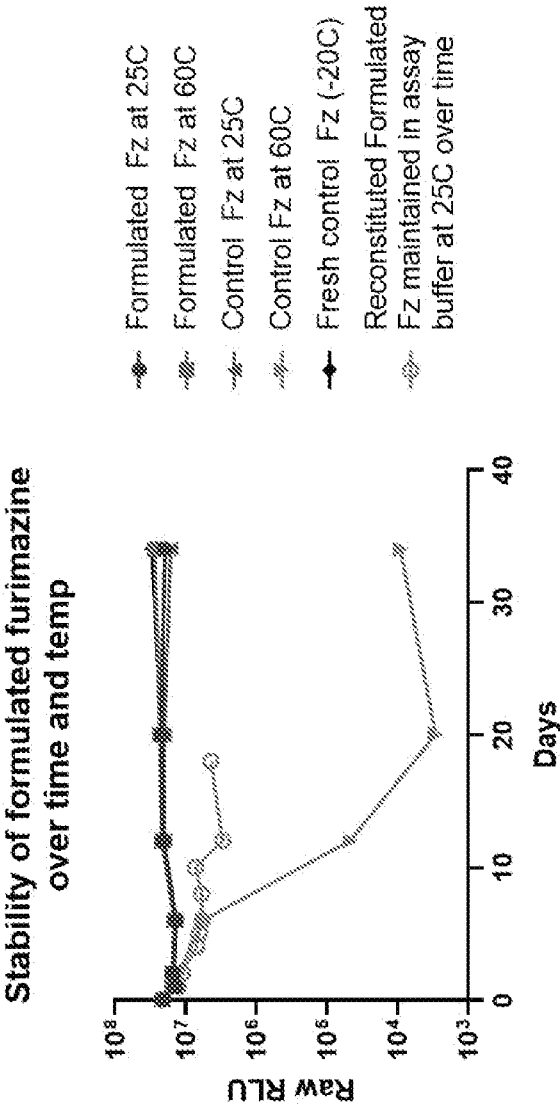


FIG. 64A-C

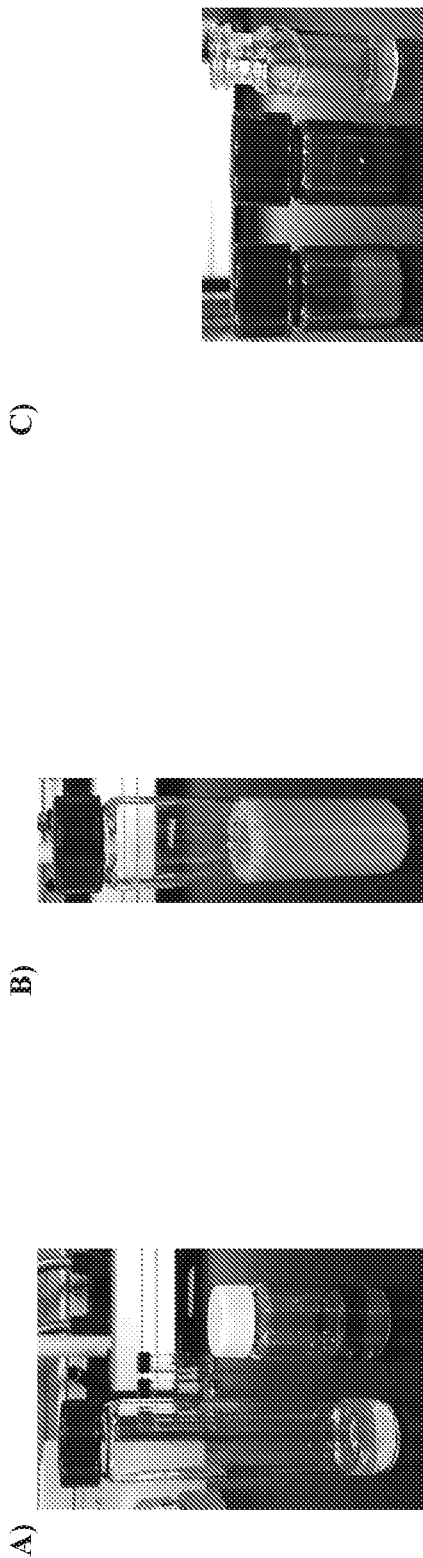


FIG. 65

