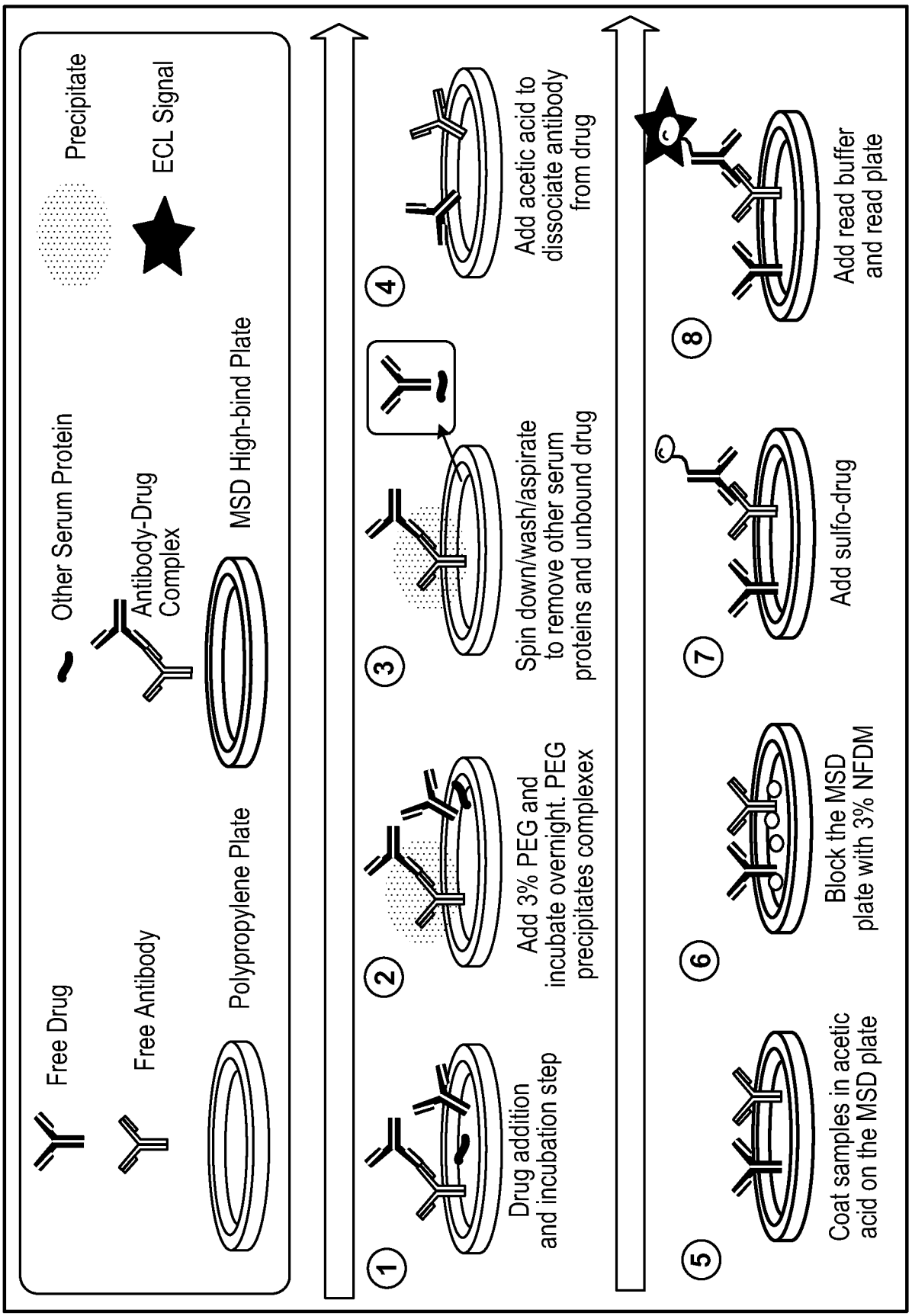

Free Drug
Free Antibody
Polypropylene Plate
Other Serum Protein
Antibody-Drug Complex
MSD High-bind Plate
Precipitate
ECL Signal
1
Drug addition and incubation step
2
Add 3% PEG and incubate overnight. PEG precipitates complexex
3
Spin down/wash/aspirate to remove other serum proteins and unbound drug
4
Add acetic acid to dissociate antibody from drug
5
Coat samples in acetic acid on the MSD plate
6
Block the MSD plate with 3% NFDM
7
Add sulfo-drug
8
Add read buffer and read plate

FIG. 1

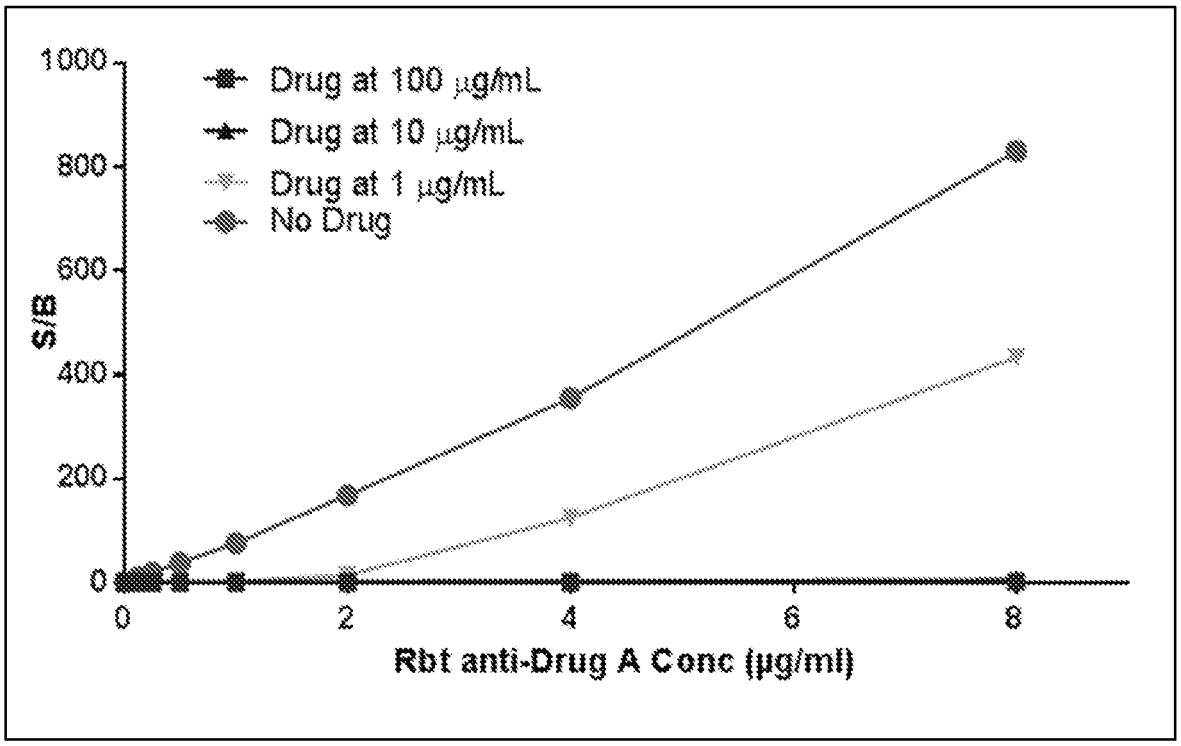

Drug at 100 µg/mL
Drug at 10 µg/mL
Drug at 1 µg/mL
No Drug
S/B
1000
800
600
400
200
0
0
2
4
6
8
Rbt anti-Drug A Conc (µg/ml)

FIG. 2A

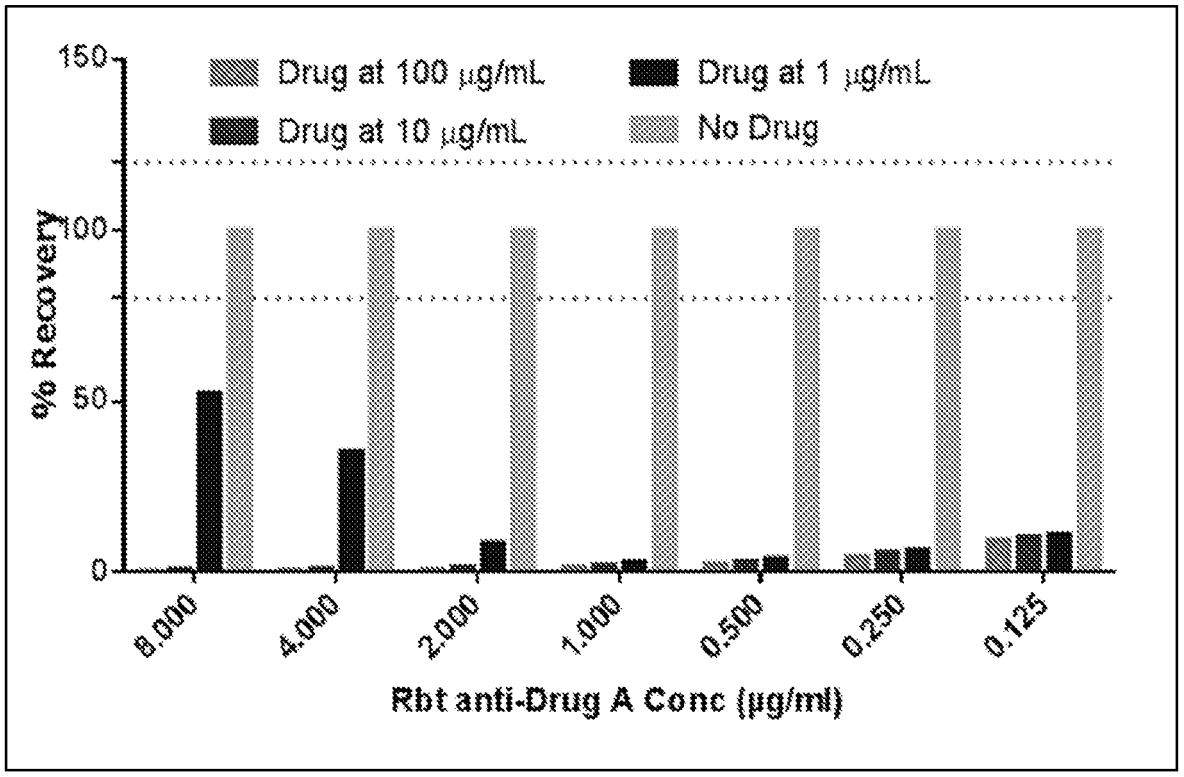

Drug at 100 µg/mL
Drug at 1 µg/mL
Drug at 10 µg/mL
No Drug
% Recovery
150
100
50
0
8.000
4.000
2.000
1.000
0.500
0.250
0.125
Rbt anti-Drug A Conc (µg/ml)

FIG. 2B

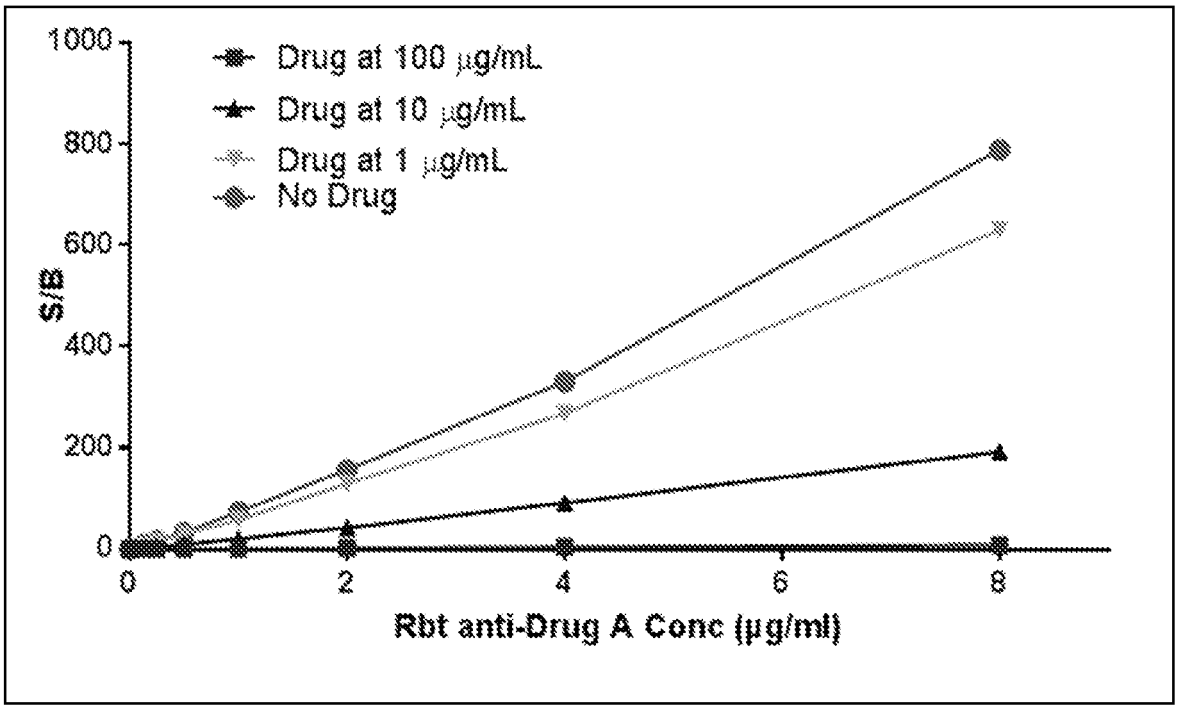

1000
800
600
400
200
0
S/B
Drug at 100 µg/mL
Drug at 10 µg/mL
Drug at 1 µg/mL
No Drug
0
2
4
6
8
Rbt anti-Drug A Conc (µg/ml)

FIG. 3A

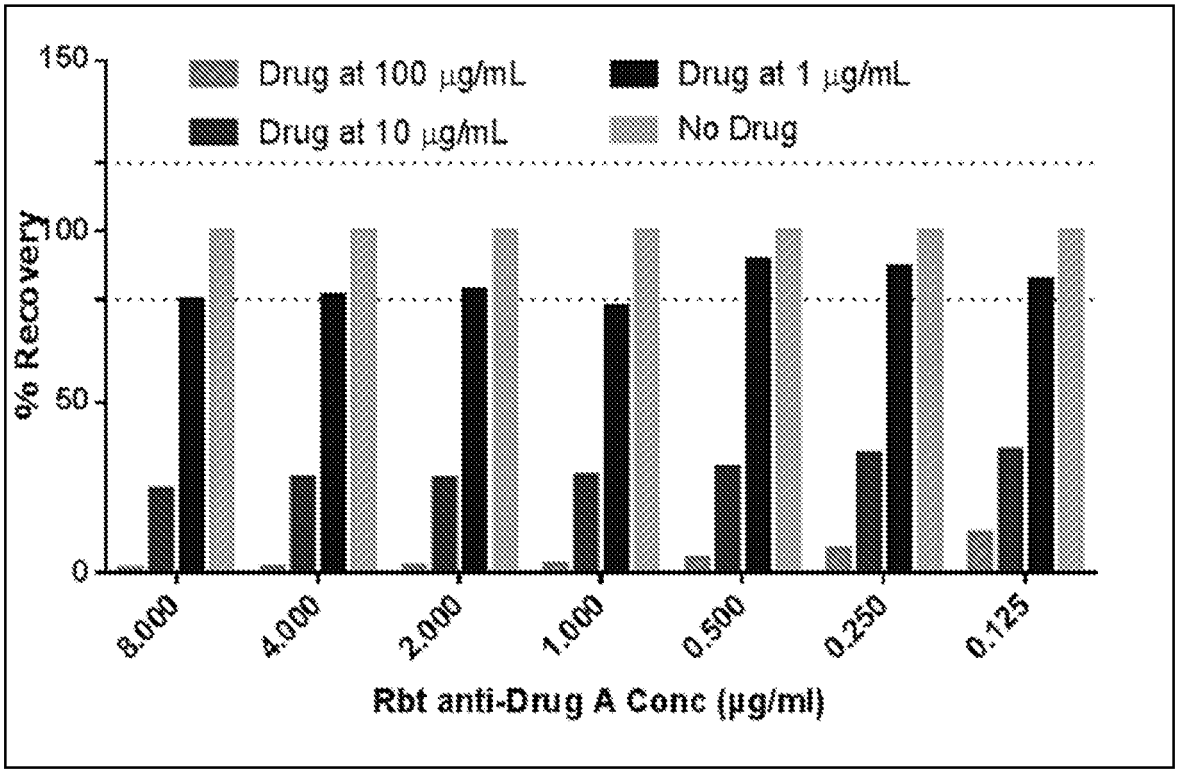

150
100
50
0
% Recovery
Drug at 100 µg/mL
Drug at 10 µg/mL
Drug at 1 µg/mL
No Drug
8.000
4.000
2.000
1.000
0.500
0.250
0.125
Rbt anti-Drug A Conc (µg/ml)

FIG. 3B

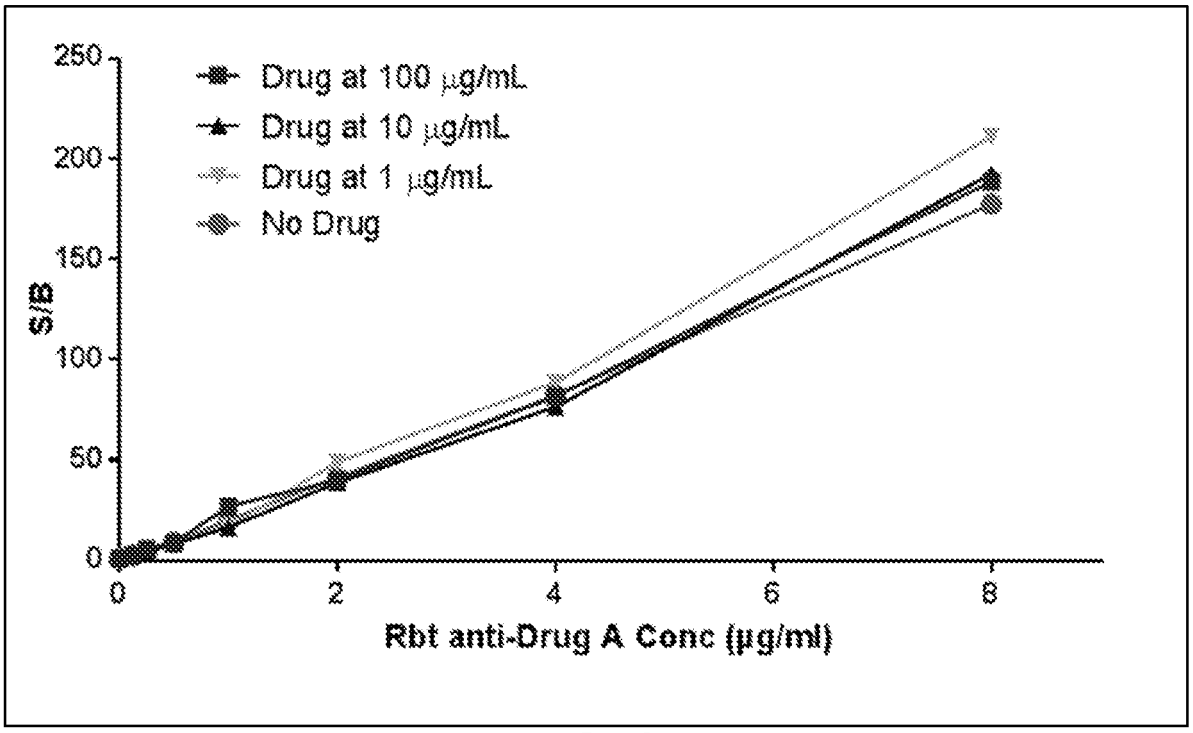

Drug at 100 µg/mL
Drug at 10 µg/mL
Drug at 1 µg/mL
No Drug
S/B
250
200
150
100
50
0
0
2
4
6
8
Rbt anti-Drug A Conc (µg/ml)

FIG. 4A

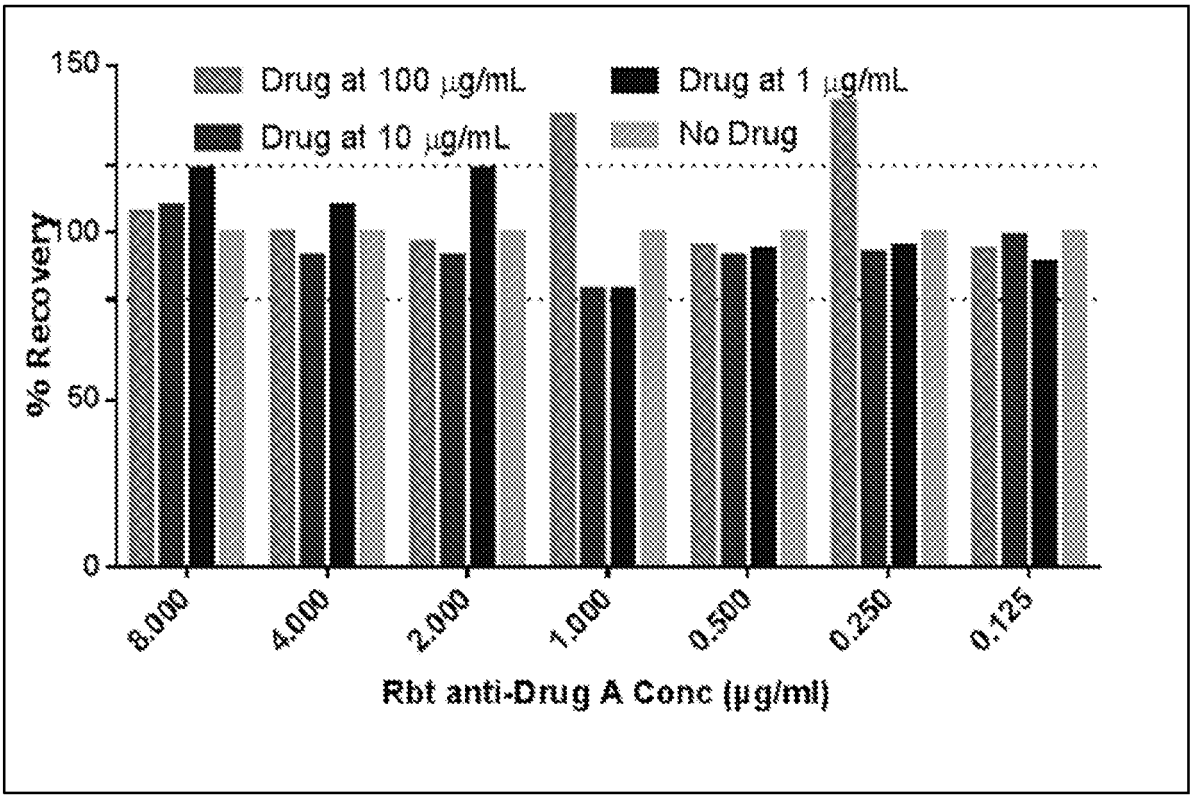

Drug at 100 µg/mL
Drug at 10 µg/mL
Drug at 1 µg/mL
No Drug
% Recovery
150
100
50
0
8.000
4.000
2.000
1.000
0.500
0.250
0.125
Rbt anti-Drug A Conc (µg/ml)

FIG. 4B

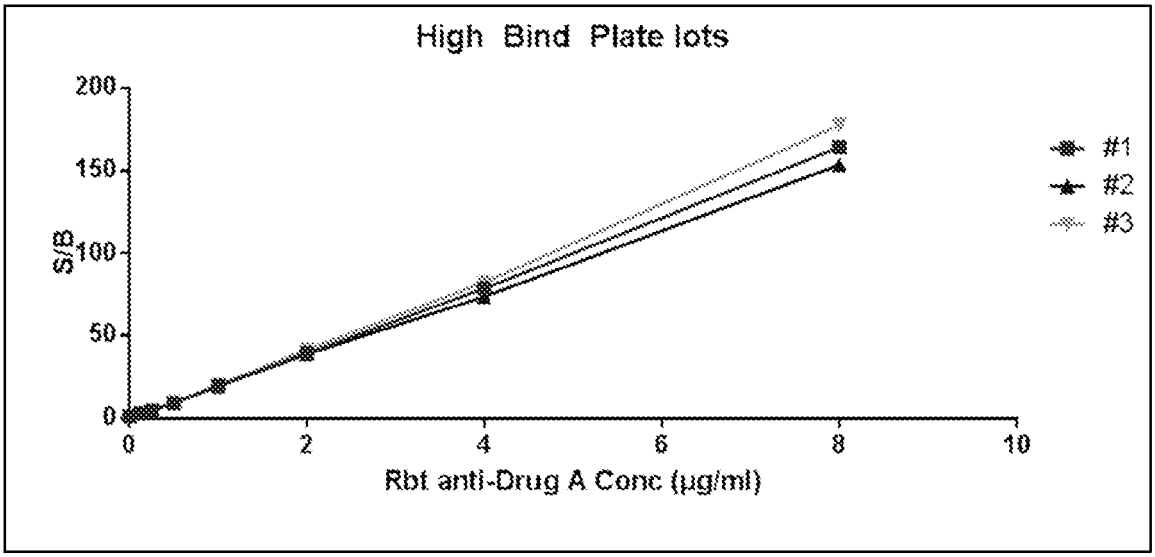

High Bind Plate lots
S/B
200
150
100
50
0
0
2
4
6
8
10
Rbt anti-Drug A Conc (µg/ml)
#1
#2
#3

FIG. 5

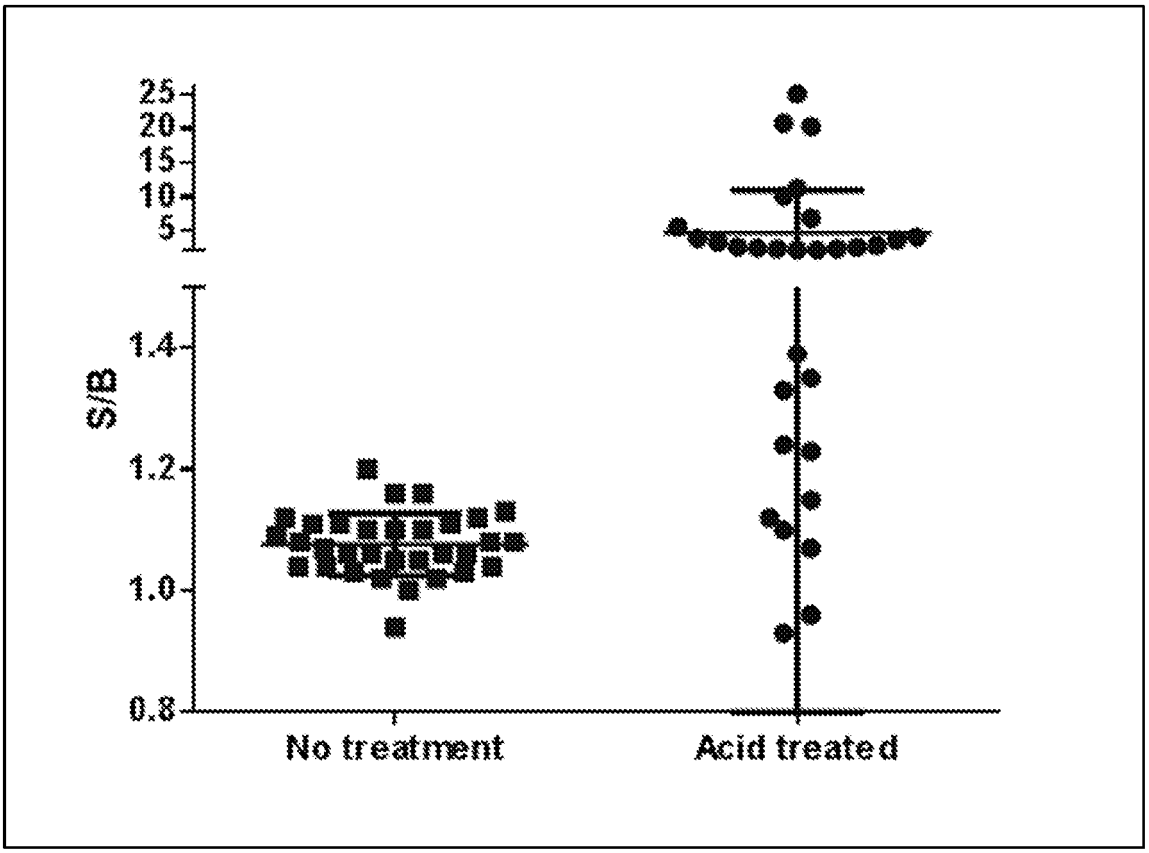

S/B
25
20
15
10
5
1.4
1.2
1.0
0.8
No treatment
Acid treated

FIG. 6

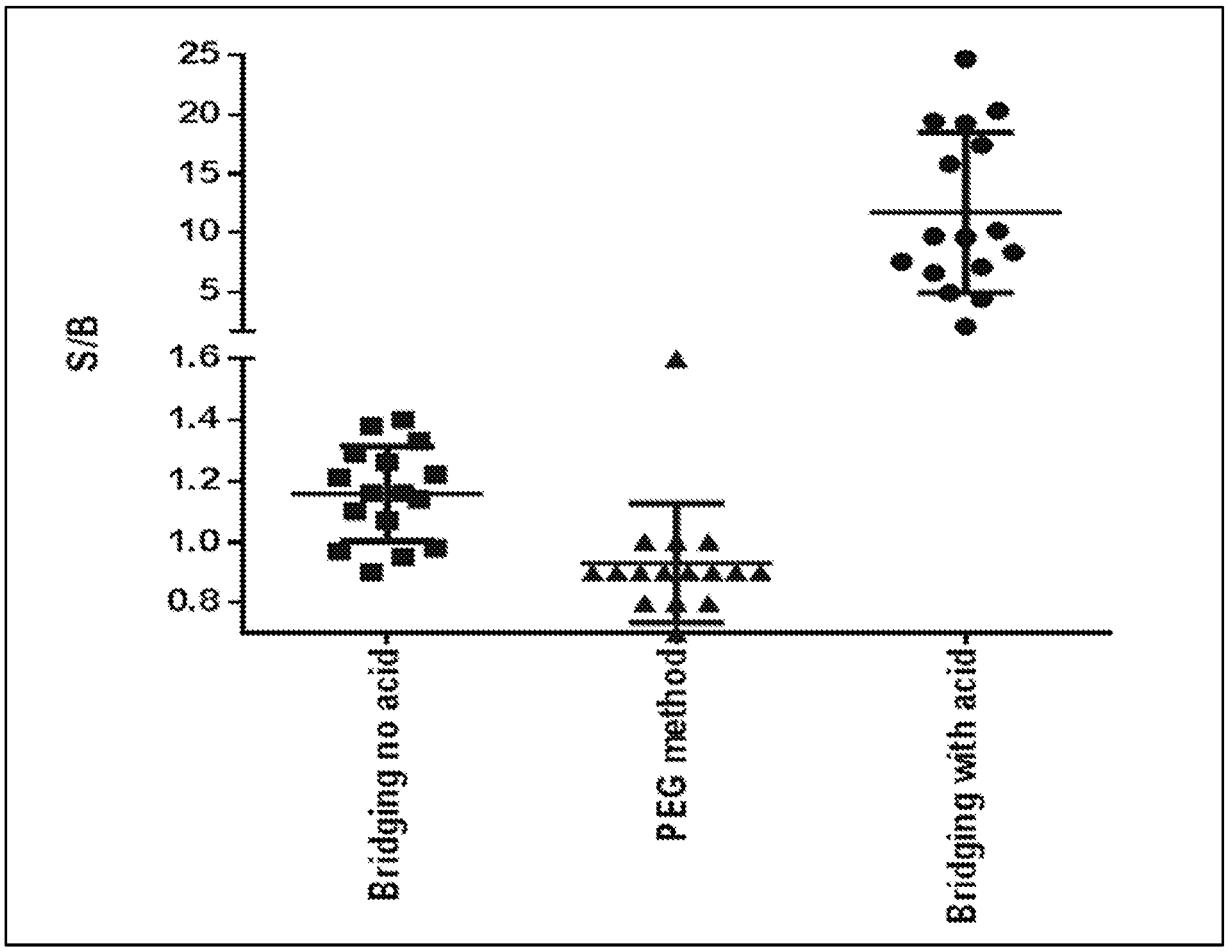

S/B
25
20
15
10
5
1.6
1.4
1.2
1.0
0.8
Bridging no acid
PEG method
Bridging with acid

FIG. 7

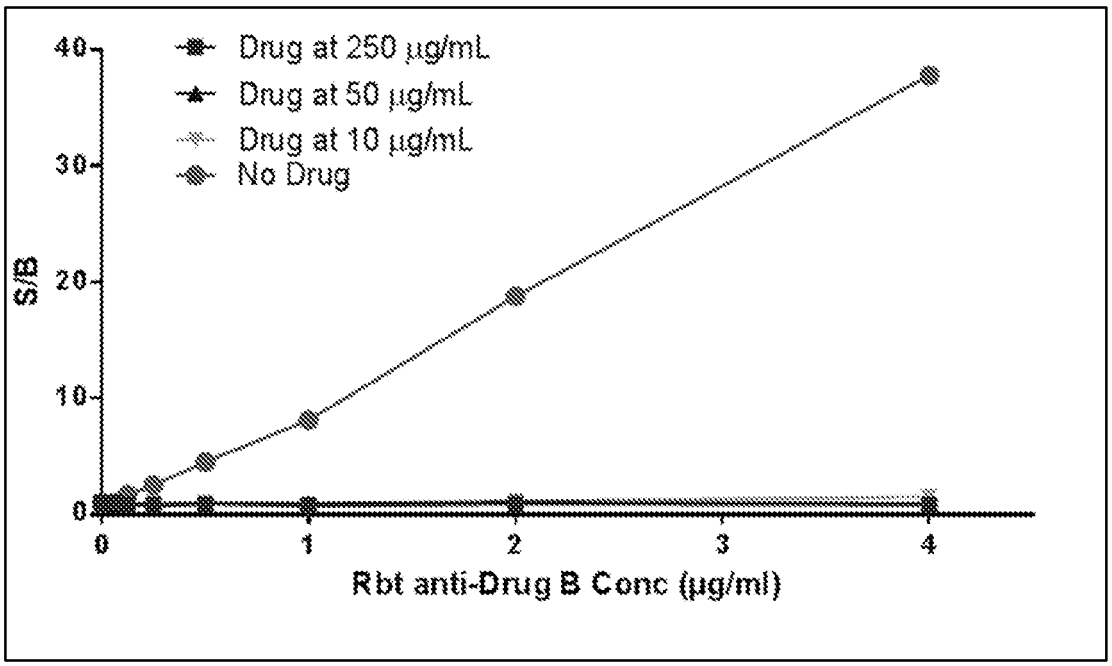

Drug at 250 µg/mL
Drug at 50 µg/mL
Drug at 10 µg/mL
No Drug
S/B
40
30
20
10
0
0
1
2
3
4
Rbt anti-Drug B Conc (µg/ml)

FIG. 8A

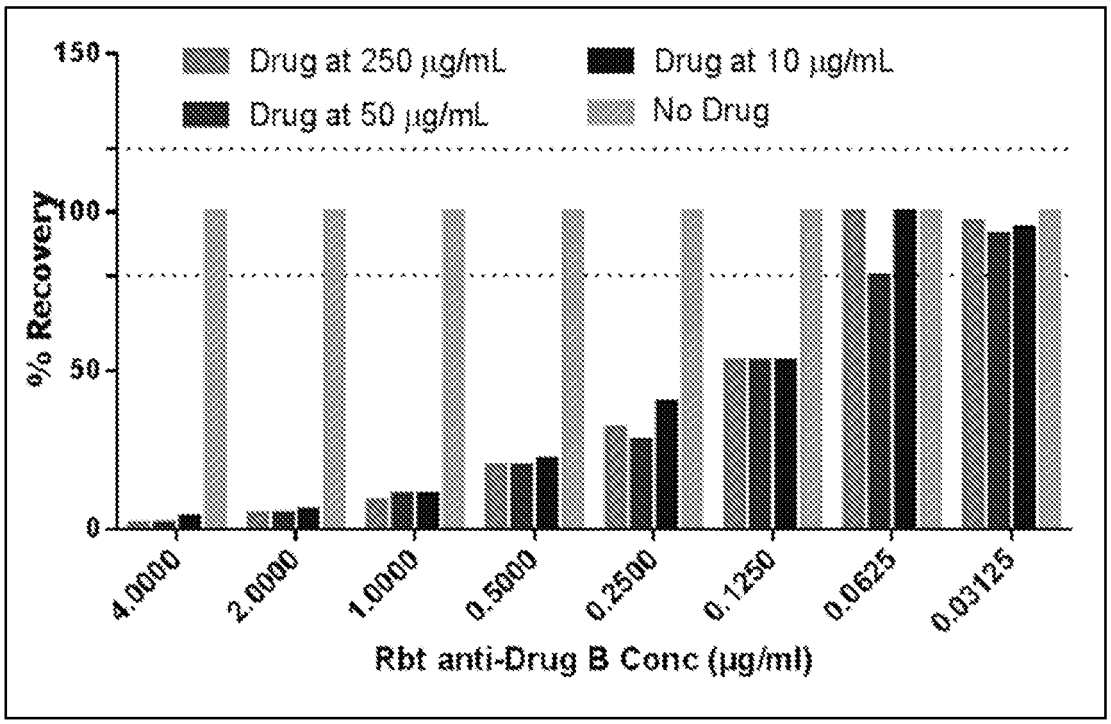

Drug at 250 µg/mL
Drug at 50 µg/mL
Drug at 10 µg/mL
No Drug
% Recovery
150
100
50
0
4.0000
2.0000
1.0000
0.5000
0.2500
0.1250
0.0625
0.03125
Rbt anti-Drug B Conc (µg/ml)

FIG. 8B

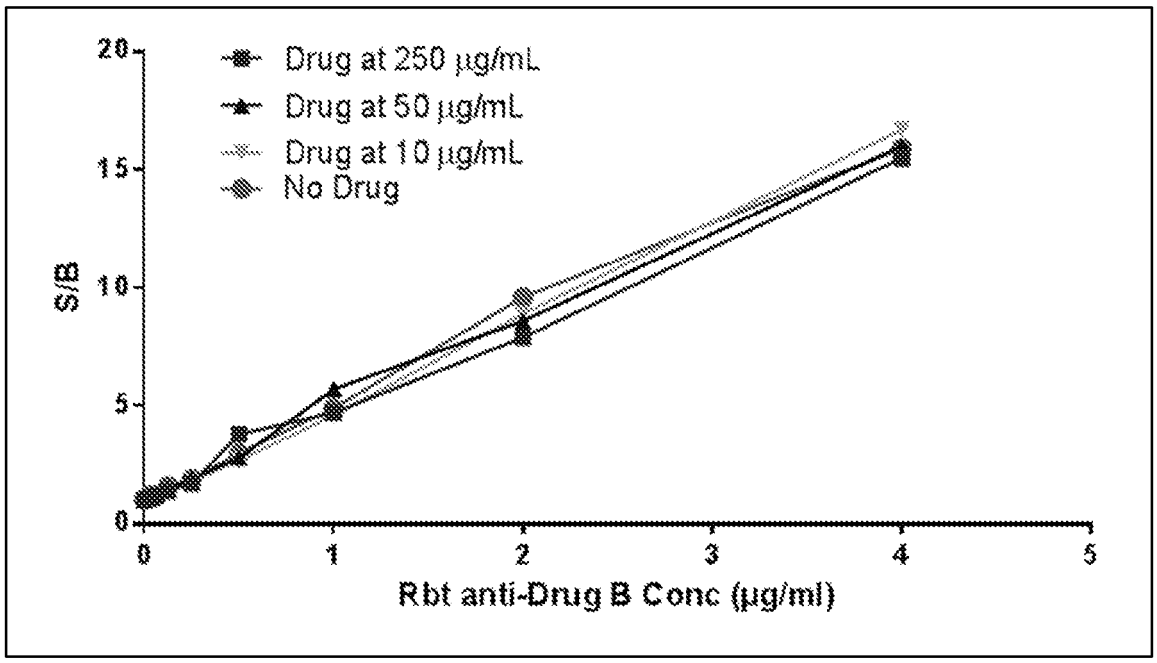

Drug at 250 µg/mL
Drug at 50 µg/mL
Drug at 10 µg/mL
No Drug
S/B
20
15
10
5
0
0
1
2
3
4
5
Rbt anti-Drug B Conc (µg/ml)

FIG. 9A

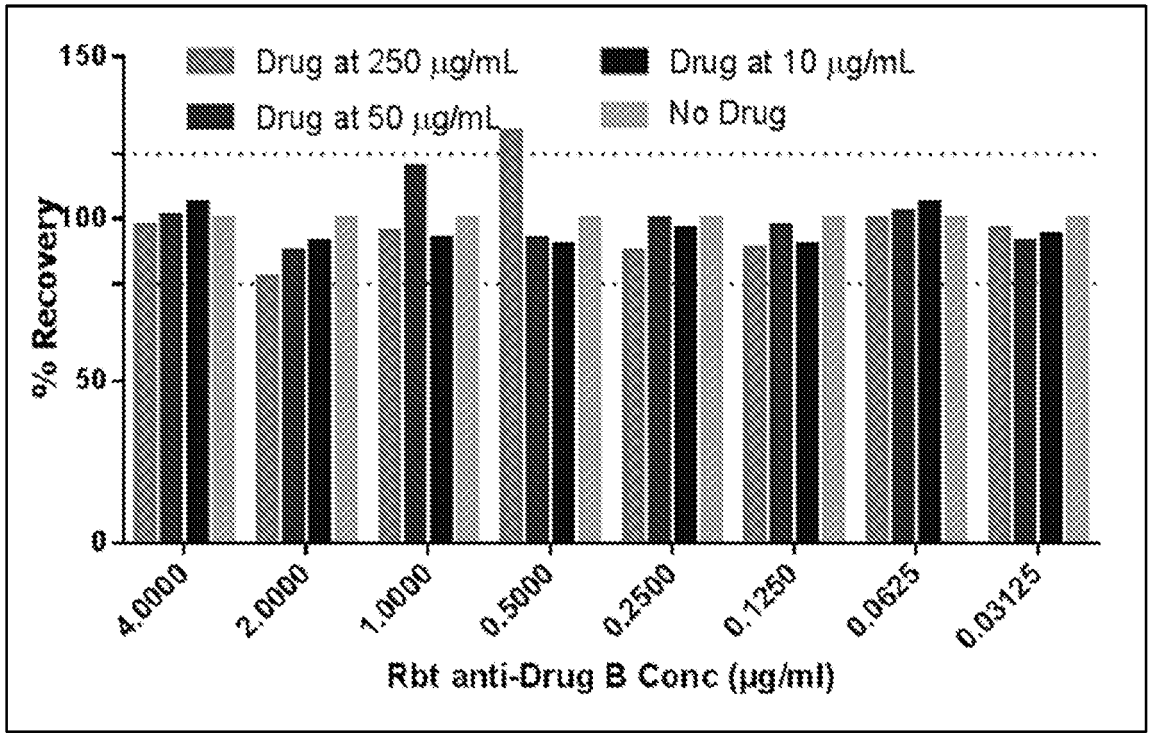

Drug at 250 µg/mL
Drug at 50 µg/mL
Drug at 10 µg/mL
No Drug
% Recovery
150
100
50
0
4.0000
2.0000
1.0000
0.5000
0.2500
0.1250
0.0625
0.03125
Rbt anti-Drug B Conc (µg/ml)

FIG. 9B

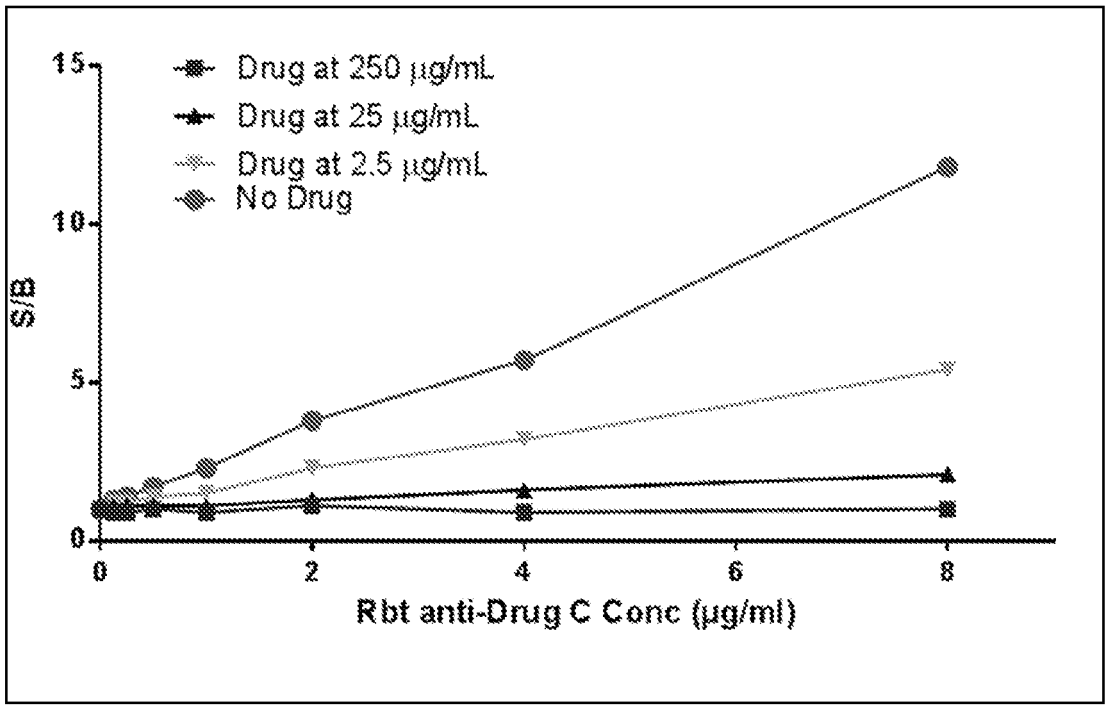


FIG. 10A

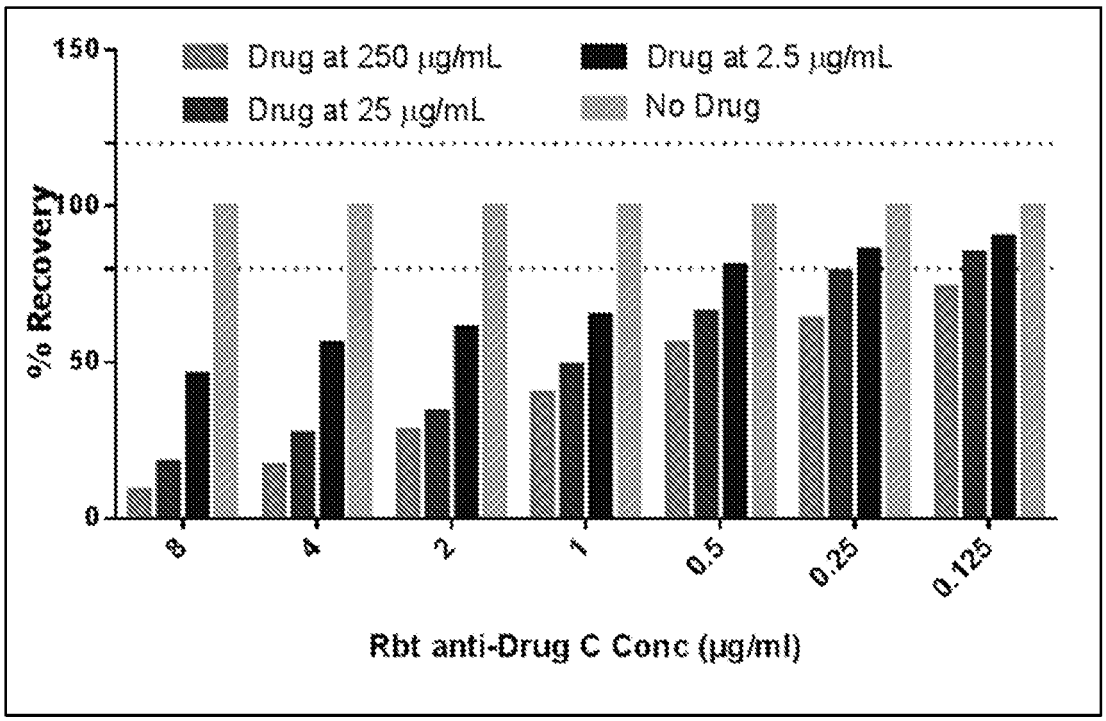


FIG. 10B

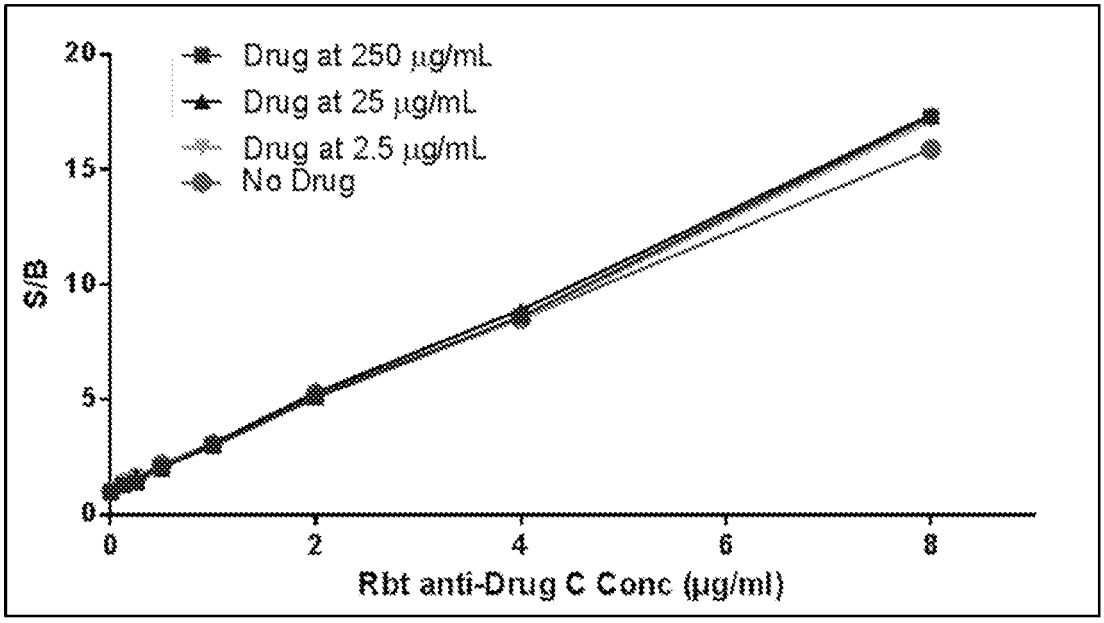


FIG. 11A

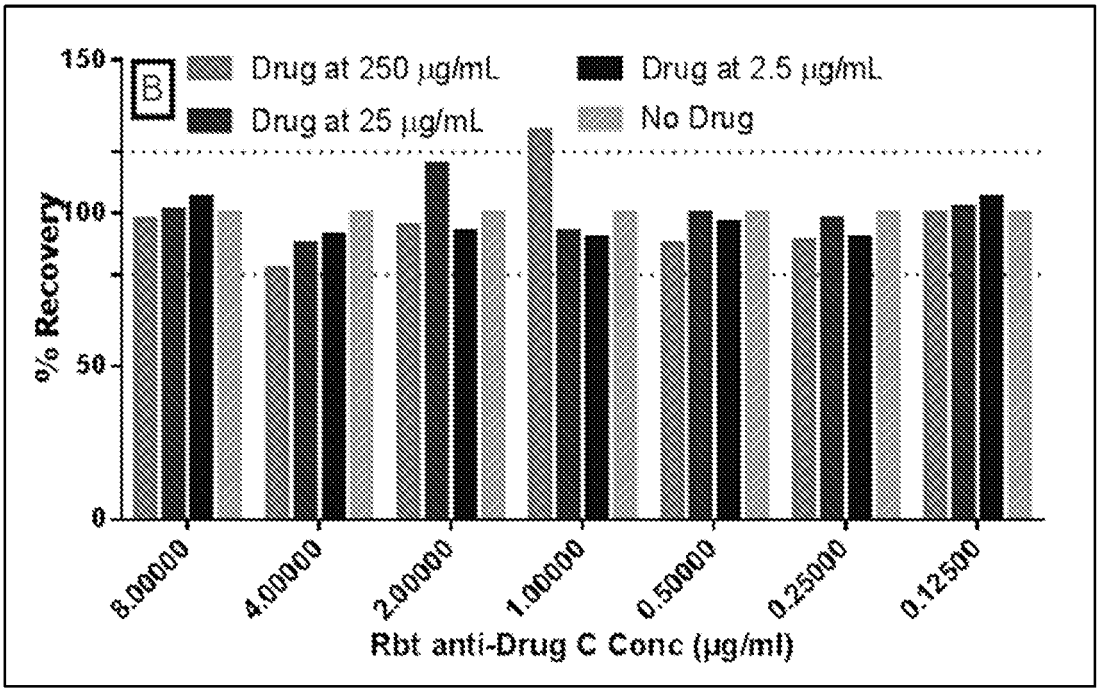


FIG. 11B