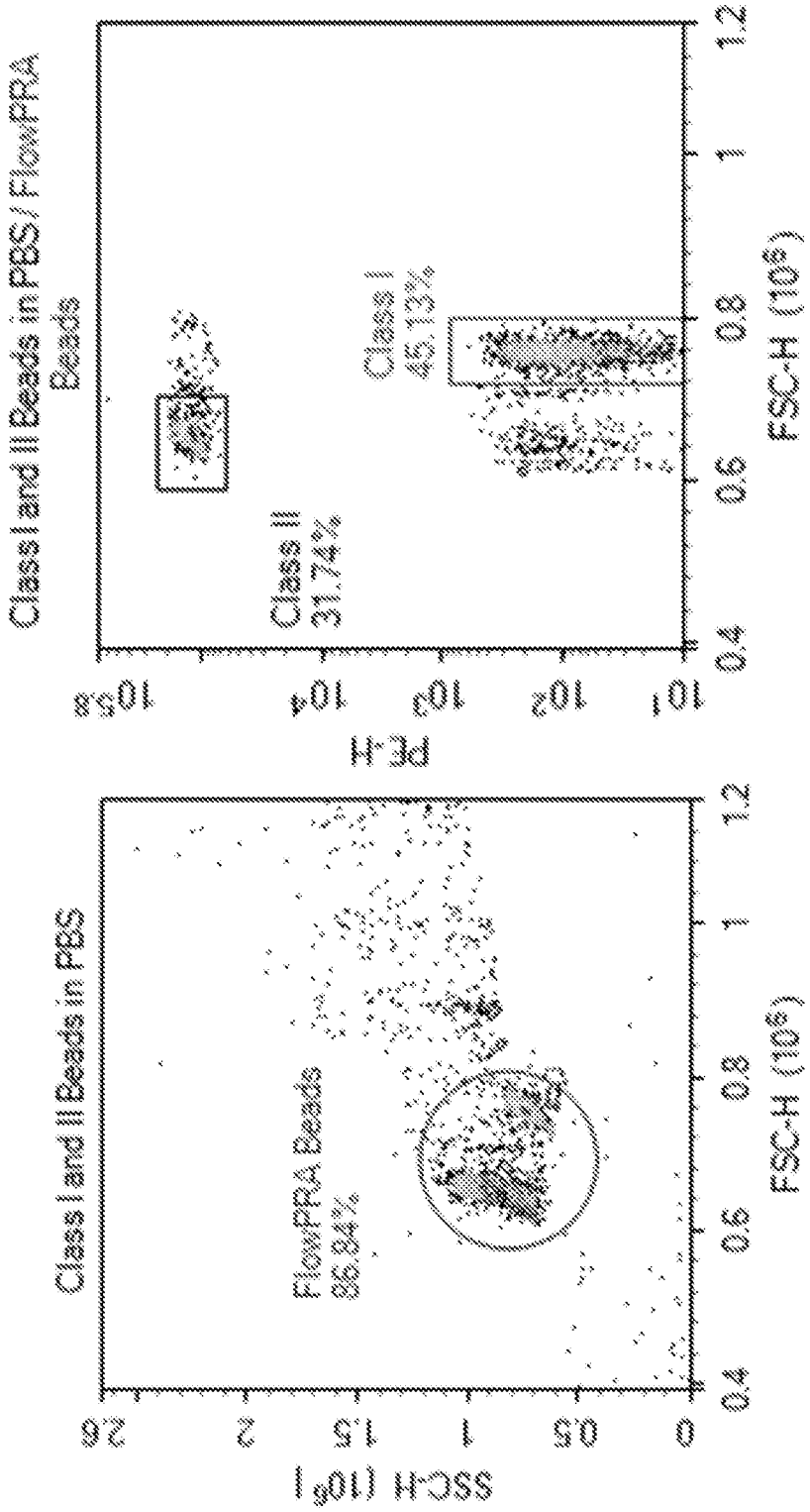

Class I and II Beads in PBS / FlowPRA Beads
Class I
45.13%
Class II
31.74%
PE-H
FSC-H (10⁶)
Class I and II Beads in PBS
FlowPRA Beads
86.84%
SSC-H (10⁶)
FSC-H (10⁶)

Fig. 1B

Fig. 1A

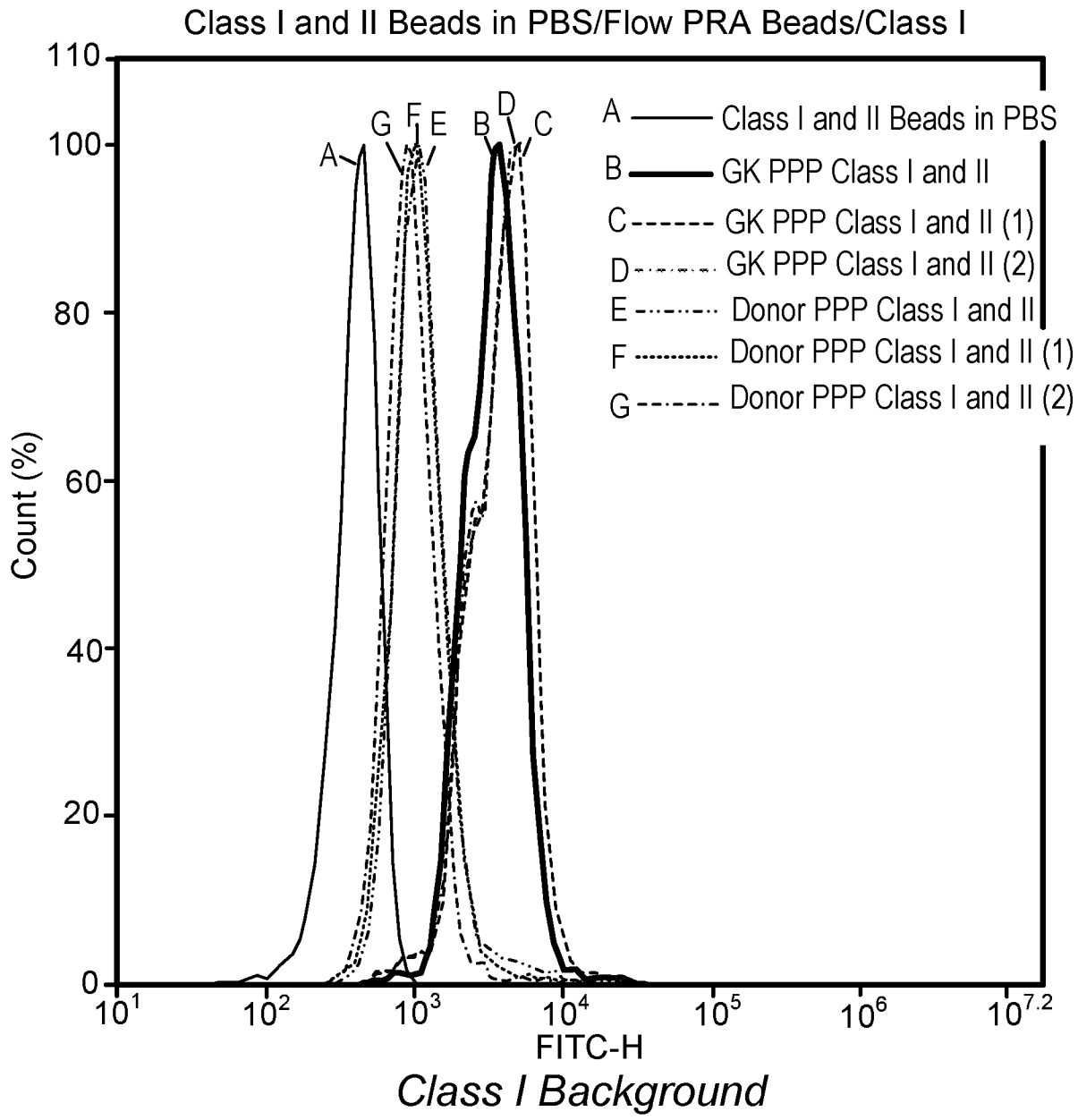

Class I and II Beads in PBS/Flow PRA Beads/Class I
110
100
80
60
40
20
0
Count (%)
A
G
F
E
B
D
C
A Class I and II Beads in PBS
B GK PPP Class I and II
C GK PPP Class I and II (1)
D GK PPP Class I and II (2)
E Donor PPP Class I and II
F Donor PPP Class I and II (1)
G Donor PPP Class I and II (2)
10^1
10^2
10^3
10^4
10^5
10^6
10^7.2
FITC-H
Class I Background

**Fig. 2A**

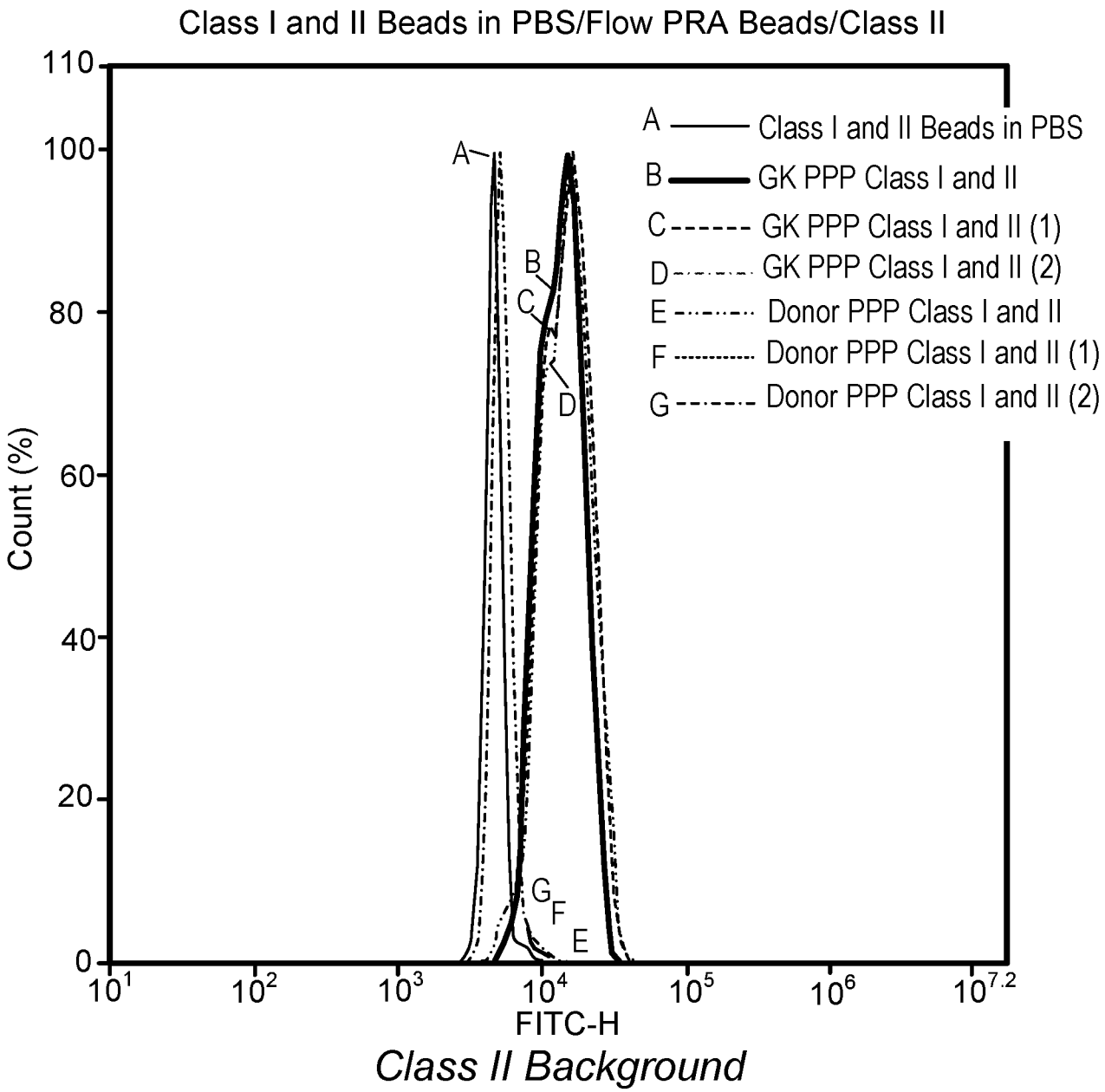

Class I and II Beads in PBS/Flow PRA Beads/Class II
A Class I and II Beads in PBS
B GK PPP Class I and II
C GK PPP Class I and II (1)
D GK PPP Class I and II (2)
E Donor PPP Class I and II
F Donor PPP Class I and II (1)
G Donor PPP Class I and II (2)
Count (%)
FITC-H
Class II Background

**Fig. 2B**

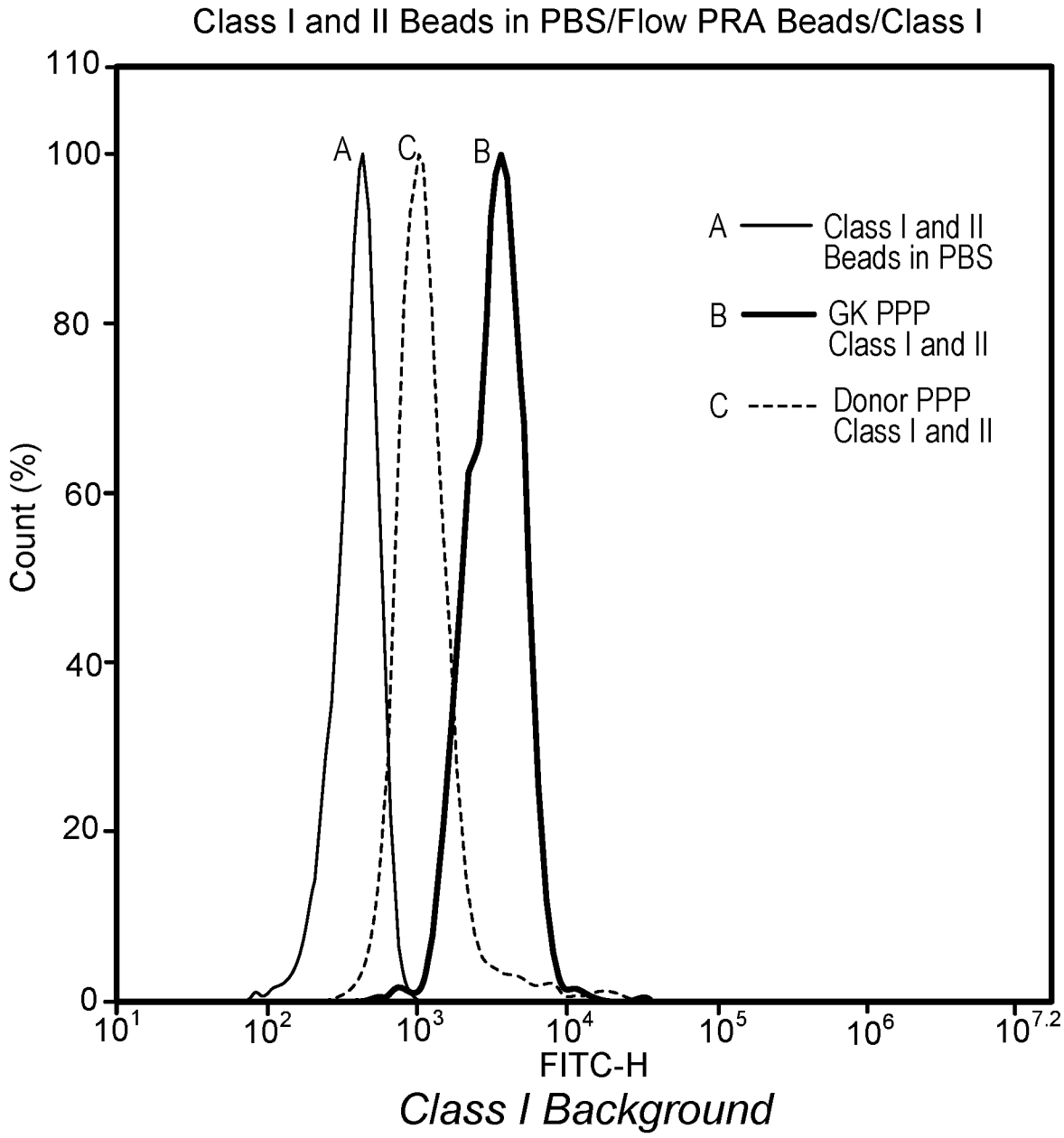
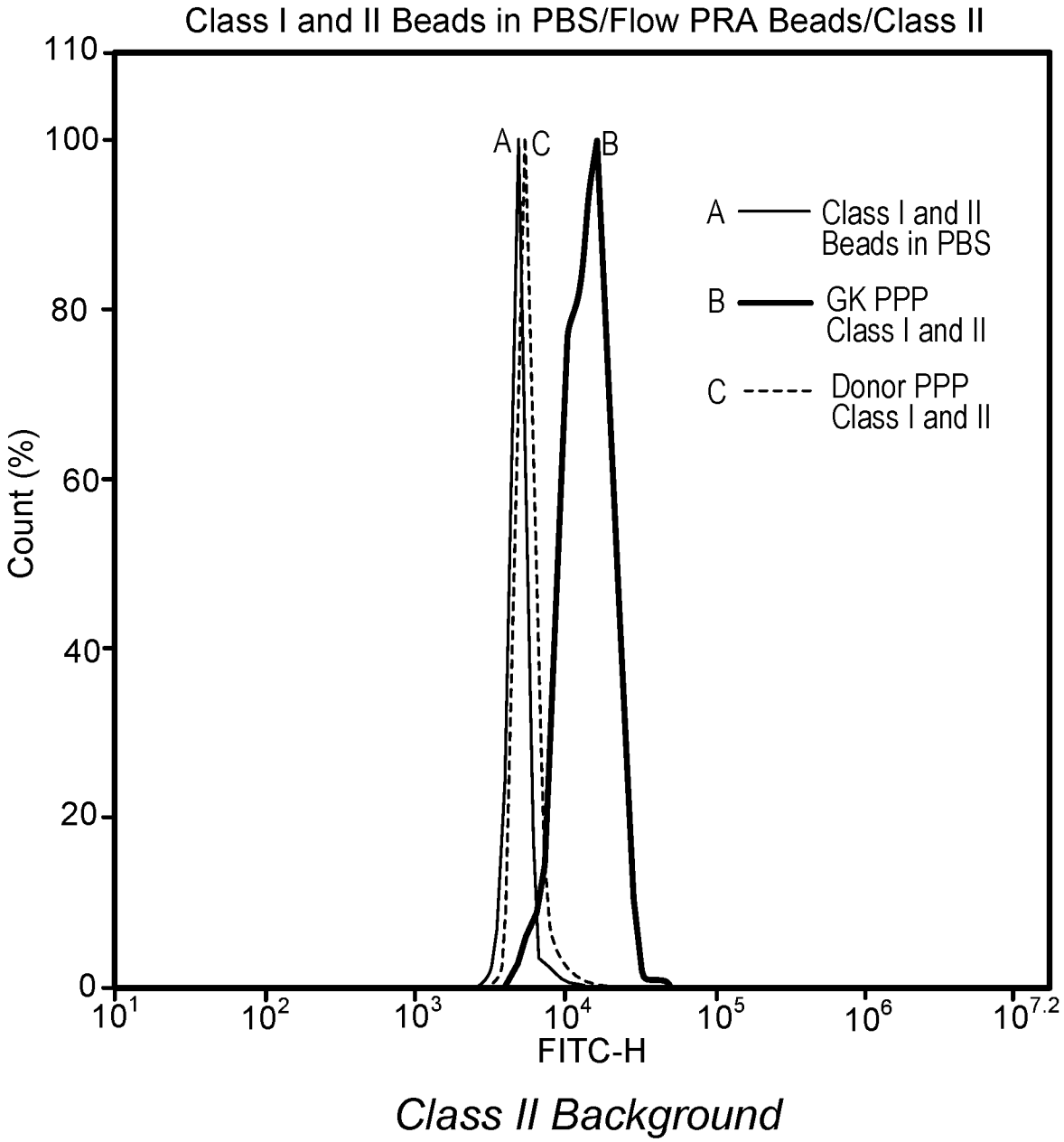

Class I and II Beads in PBS/Flow PRA Beads/Class I
110
100
80
60
40
20
0
Count (%)
A
C
B
A Class I and II Beads in PBS
B GK PPP Class I and II
C Donor PPP Class I and II
$10^1$
$10^2$
$10^3$
$10^4$
$10^5$
$10^6$
$10^{7.2}$
FITC-H
Class I Background

**Fig. 2C**




*Class II Background*

**Fig. 2D**

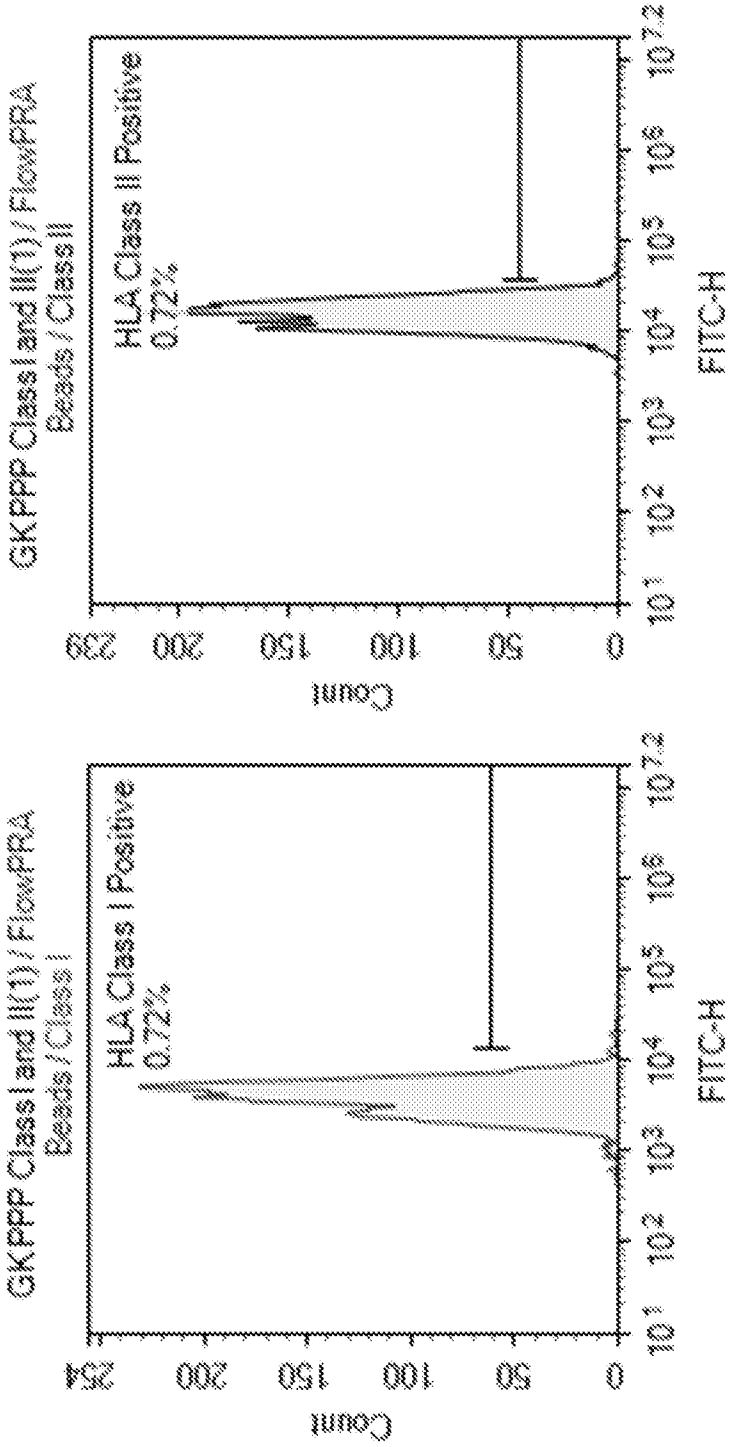

GKPPP Class I and II(1) / FlowPRA
Beads / Class I
HLA Class I Positive
0.72%
Count
FITC-H
Class I Gate
GKPPP Class I and II(1) / FlowPRA
Beads / Class II
HLA Class II Positive
0.72%
Count
FITC-H
Class II Gate

Fig. 3A

Fig. 3B

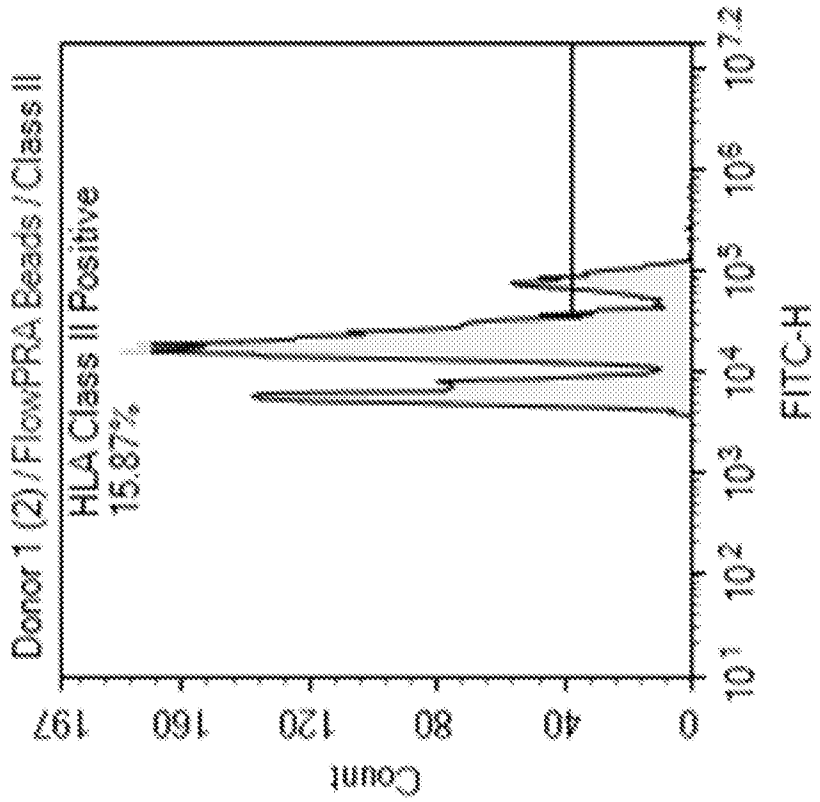

Donor 1 (2) / FlowPRA Beads / Class II
HLA Class II Positive
15.87%
Count
FITC-H
0
40
80
120
160
197
$10^1$
$10^2$
$10^3$
$10^4$
$10^5$
$10^6$
$10^{7.2}$

Fig. 4B

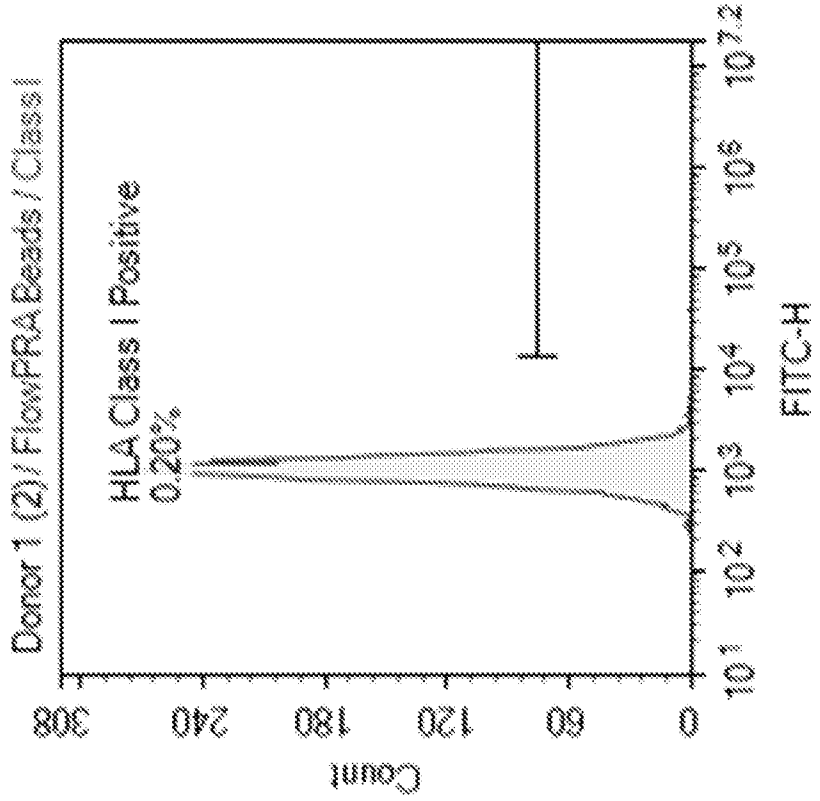

Donor 1 (2)/ FlowPRA Beads / Class I
HLA Class I Positive
0.20%
Count
FITC-H
0
60
120
180
240
308
$10^1$
$10^2$
$10^3$
$10^4$
$10^5$
$10^6$
$10^{7.2}$

Fig. 4A

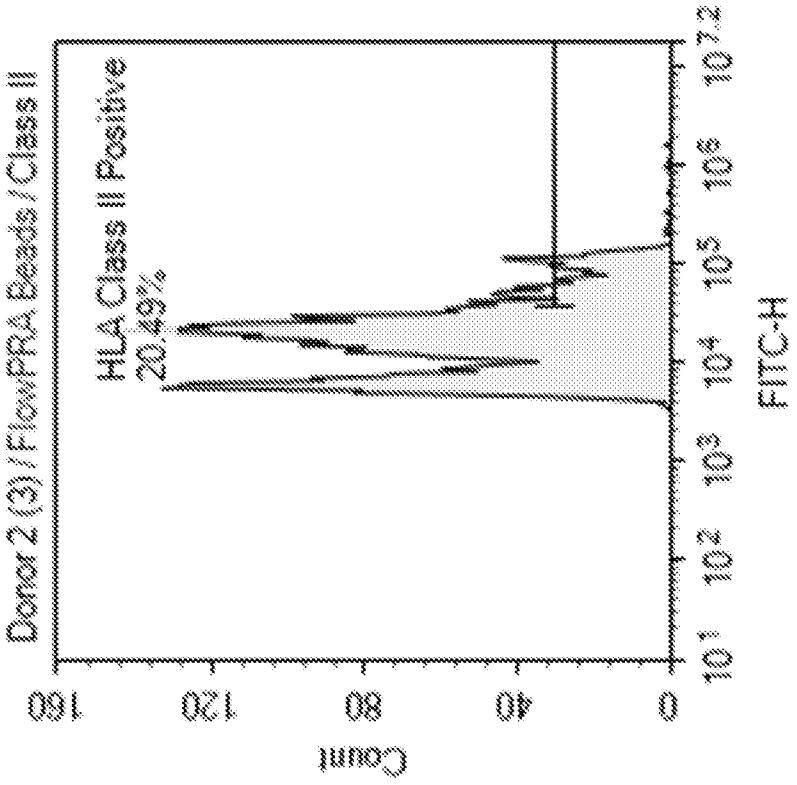

Donor 2 (3) / FlowPRA Beads / Class II
HLA Class II Positive
20.49%
Count
FITC-H
0
40
80
120
160
$10^1$
$10^2$
$10^3$
$10^4$
$10^5$
$10^6$
$10^{7.2}$

Fig. 5B

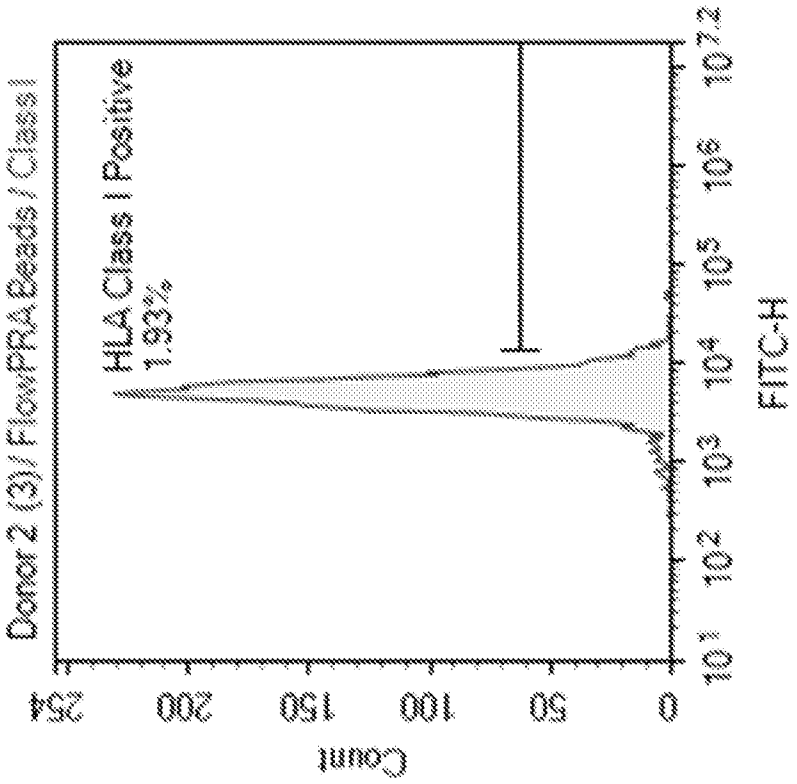

Donor 2 (3)/ FlowPRA Beads / Class I
HLA Class I Positive
1.93%
Count
FITC-H
0
50
100
150
200
254
$10^1$
$10^2$
$10^3$
$10^4$
$10^5$
$10^6$
$10^{7.2}$

Fig. 5A

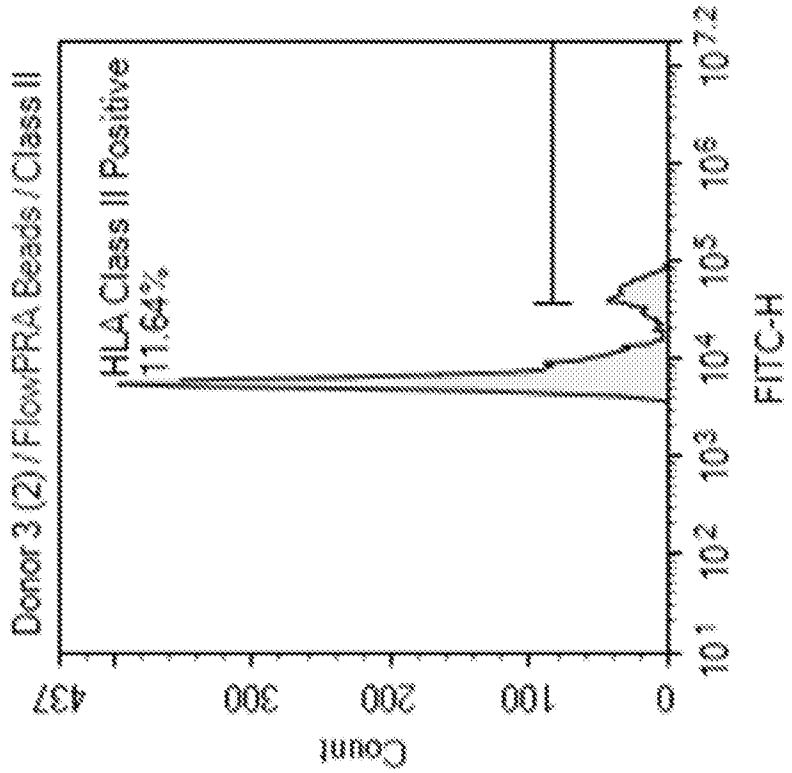

Donor 3 (2) / FlowPRA Beads / Class II
HLA Class II Positive
11.64%
Count
FITC-H
0
100
200
300
437
$10^1$
$10^2$
$10^3$
$10^4$
$10^5$
$10^6$
$10^{7.2}$

Fig. 6B

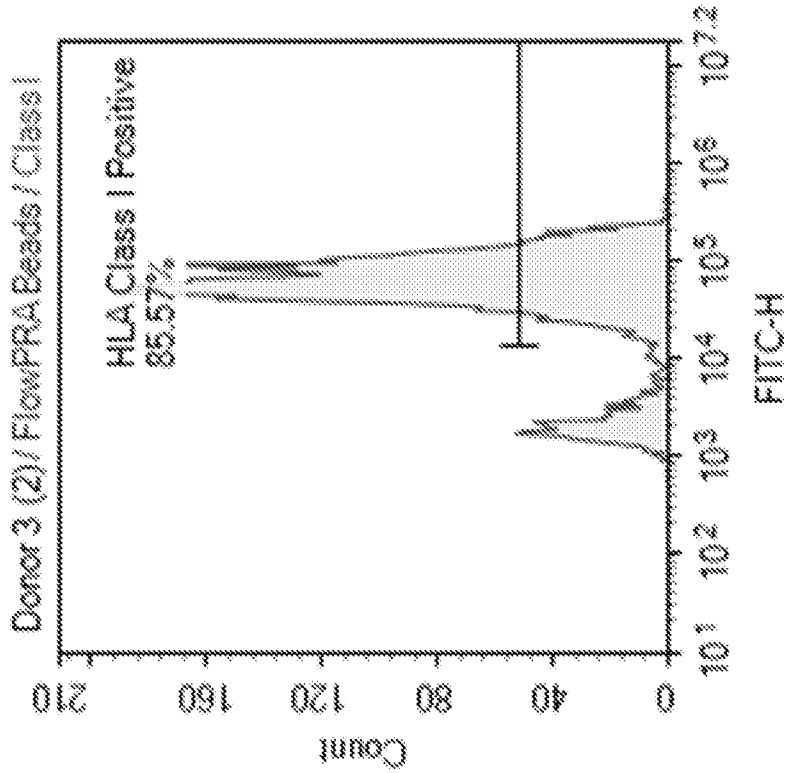

Donor 3 (2) / FlowPRA Beads / Class I
HLA Class I Positive
85.57%
Count
FITC-H
0
40
80
120
160
210
$10^1$
$10^2$
$10^3$
$10^4$
$10^5$
$10^6$
$10^{7.2}$

Fig. 6A

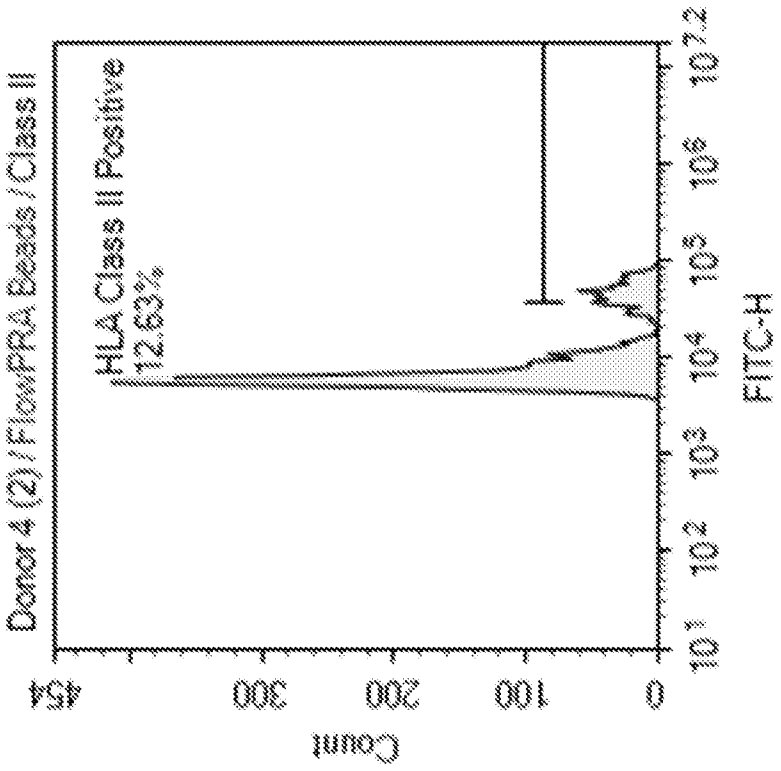

Donor 4 (2) / FlowPRA Beads / Class II
HLA Class II Positive
12.63%
Count
FITC-H
0
100
200
300
454
$10^1$
$10^2$
$10^3$
$10^4$
$10^5$
$10^6$
$10^{7.2}$

Fig. 7B

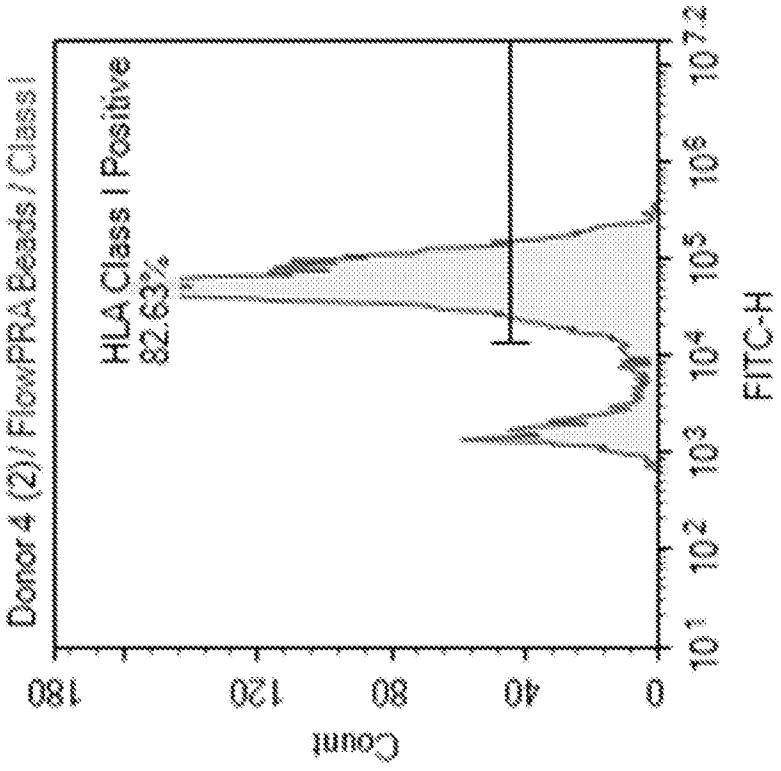

Donor 4 (2) / FlowPRA Beads / Class I
HLA Class I Positive
82.63%
Count
FITC-H
0
40
80
120
180
$10^1$
$10^2$
$10^3$
$10^4$
$10^5$
$10^6$
$10^{7.2}$

Fig. 7A

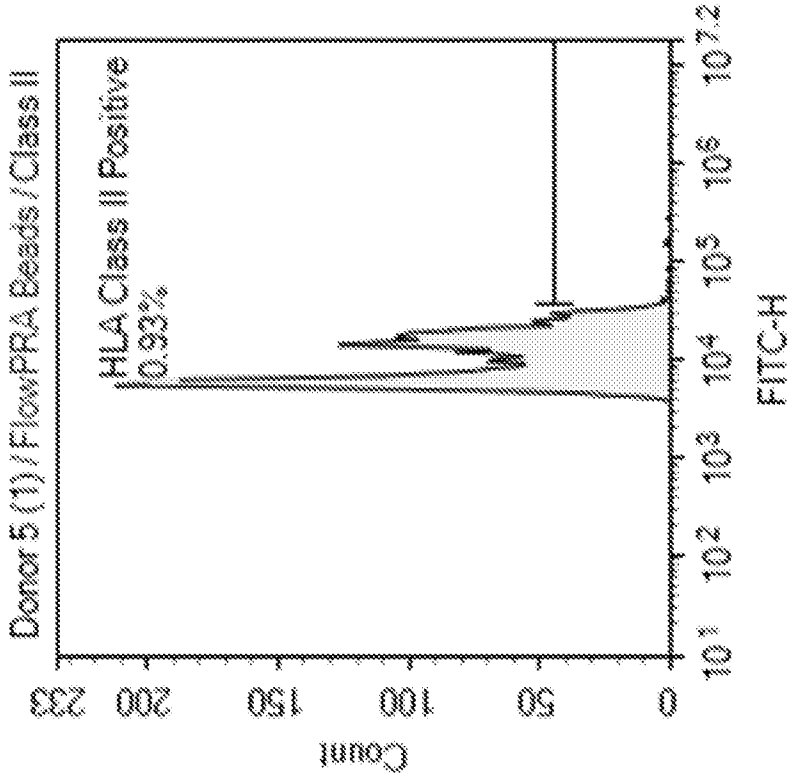

Donor 5 (1) / FlowPRA Beads / Class II
HLA Class II Positive
0.93%
Count
FITC-H
0
50
100
150
200
233
$10^1$
$10^2$
$10^3$
$10^4$
$10^5$
$10^6$
$10^7.2$

Fig. 8B

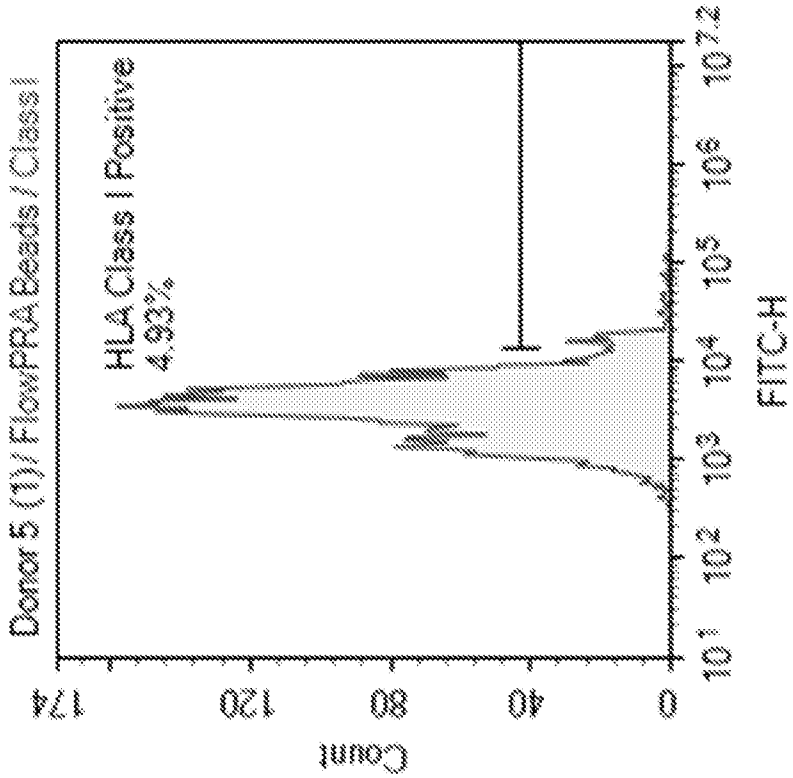

Donor 5 (1)/ FlowPRA Beads / Class I
HLA Class I Positive
4.93%
Count
FITC-H
0
40
80
120
174
$10^1$
$10^2$
$10^3$
$10^4$
$10^5$
$10^6$
$10^7.2$

Fig. 8A

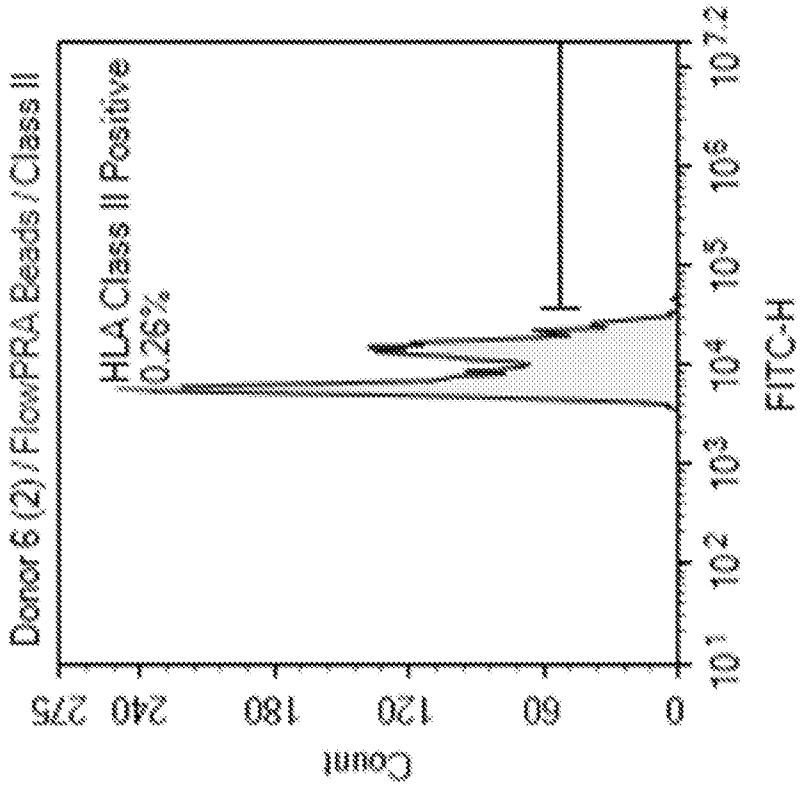

Donor 6 (2) / FlowPRA Beads / Class II
HLA Class II Positive
0.26%
Count
FITC-H
0
60
120
180
240
275
$10^1$
$10^2$
$10^3$
$10^4$
$10^5$
$10^6$
$10^{7.2}$

Fig. 9B

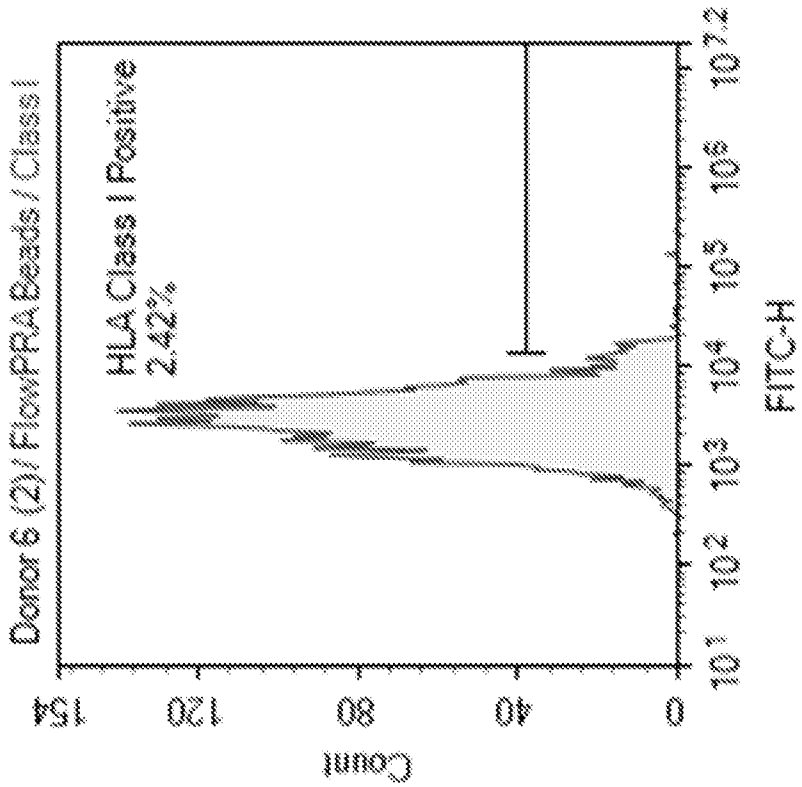

Donor 6 (2)/ FlowPRA Beads / Class I
HLA Class I Positive
2.42%
Count
FITC-H
0
40
80
120
154
$10^1$
$10^2$
$10^3$
$10^4$
$10^5$
$10^6$
$10^{7.2}$

Fig. 9A

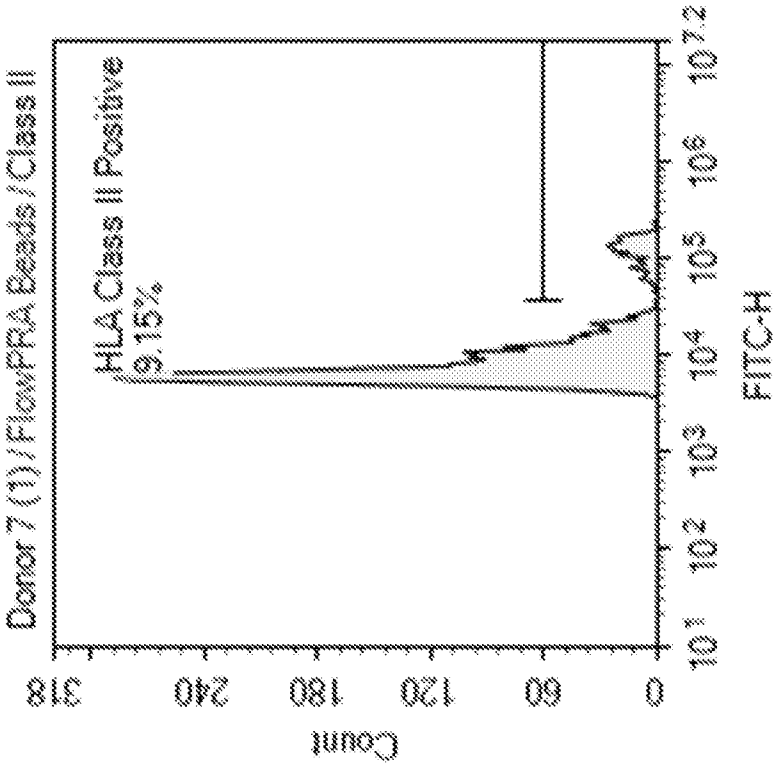



Fig. 10B

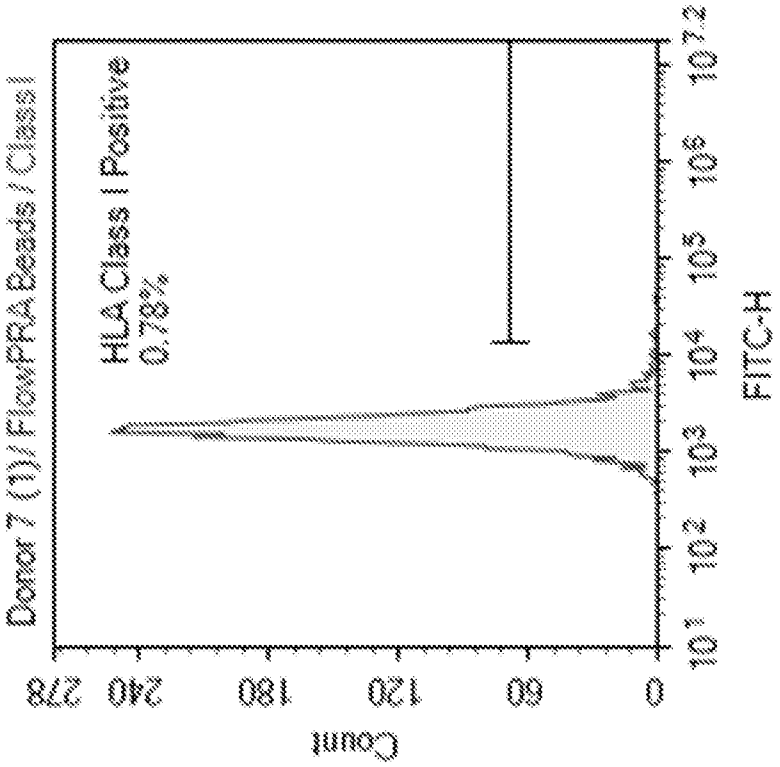



Fig. 10A

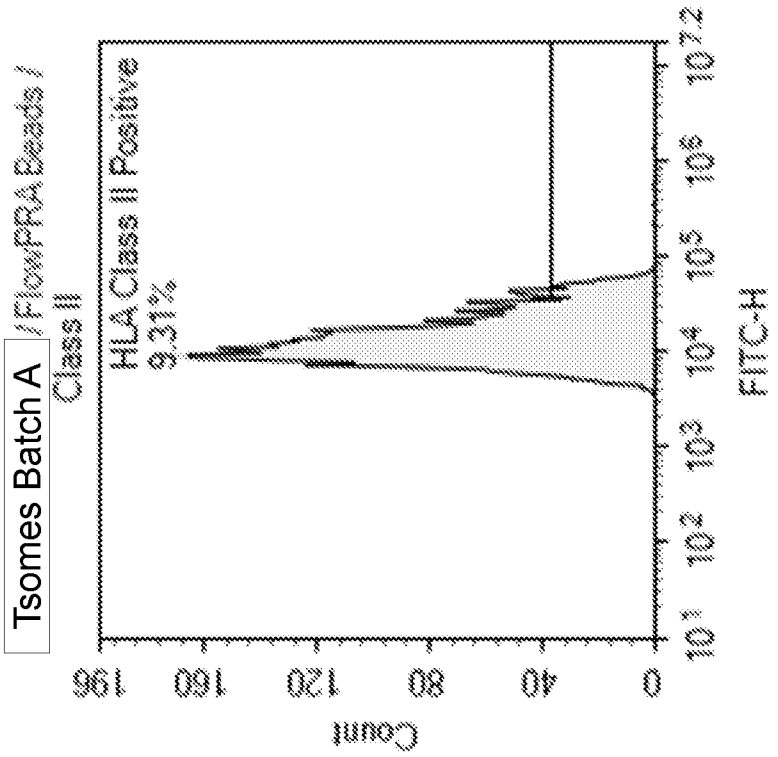



Fig. 11B

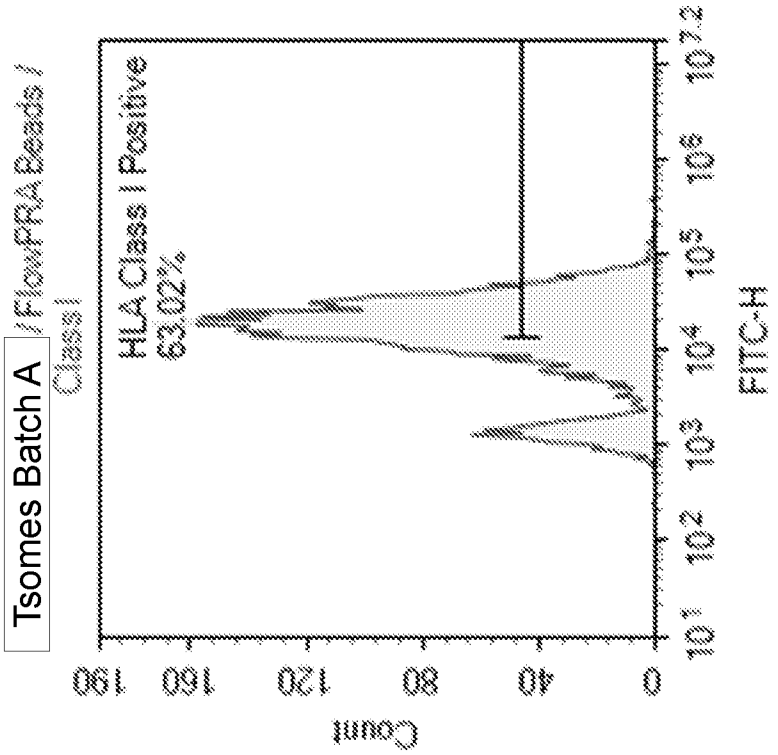



Fig. 11A

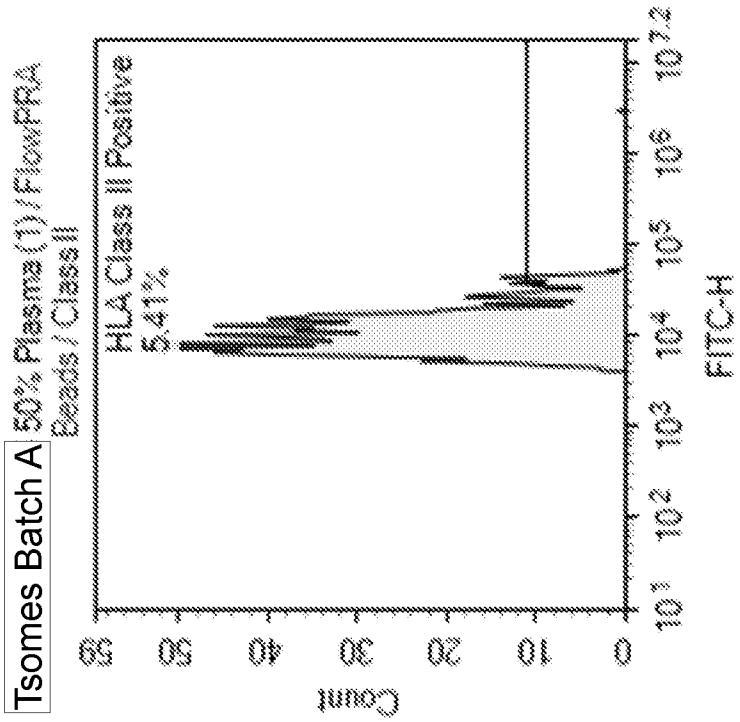



Fig. 12B

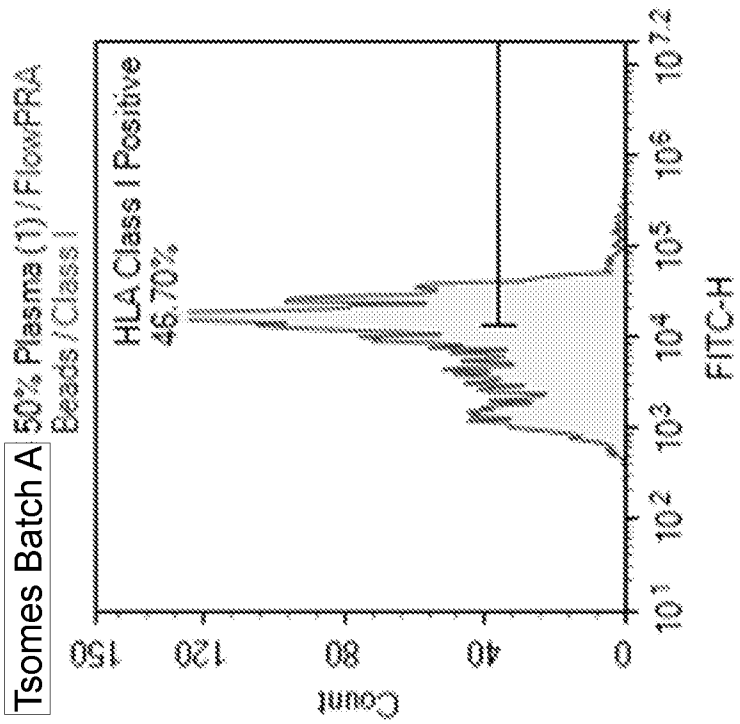



Fig. 12A

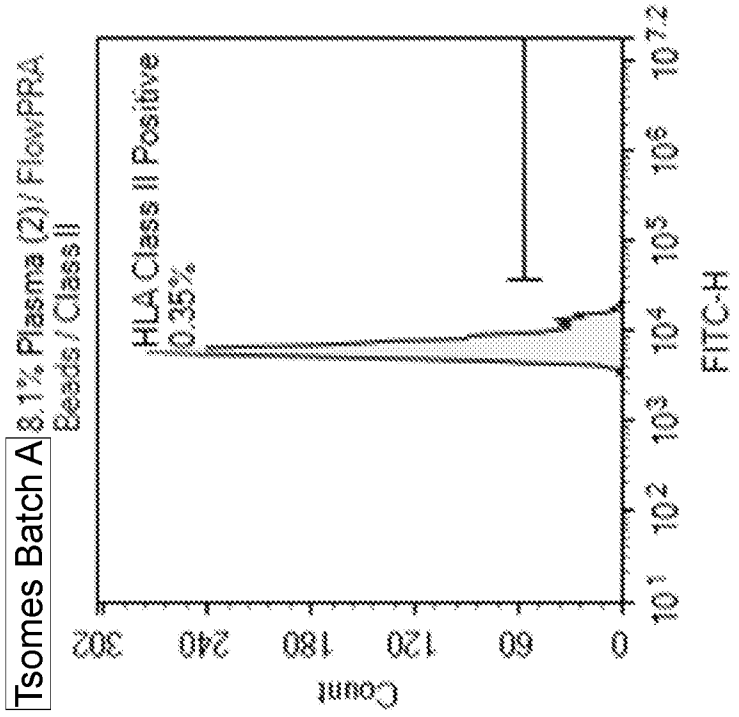



Fig. 13B

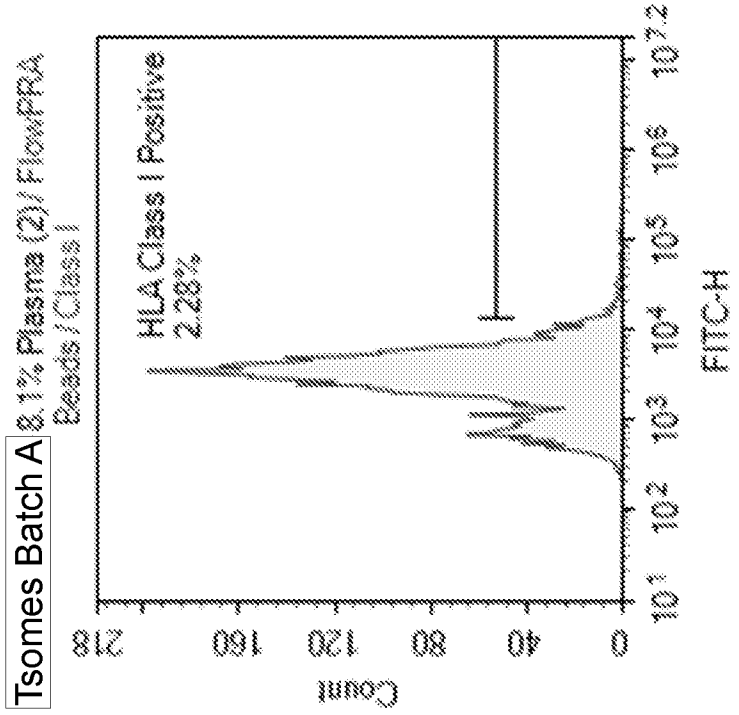



Fig. 13A

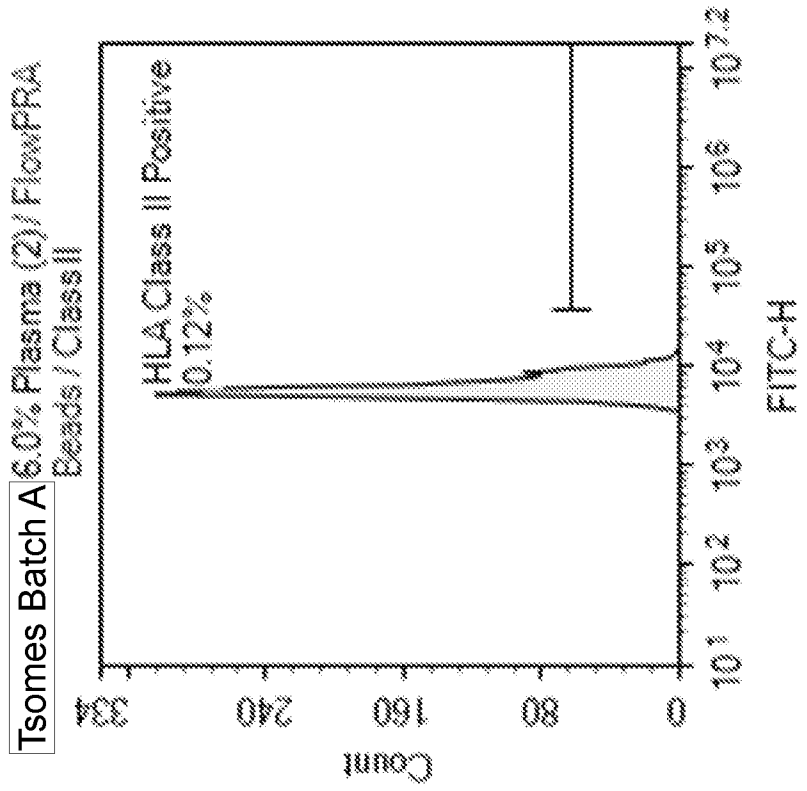



Fig. 14B

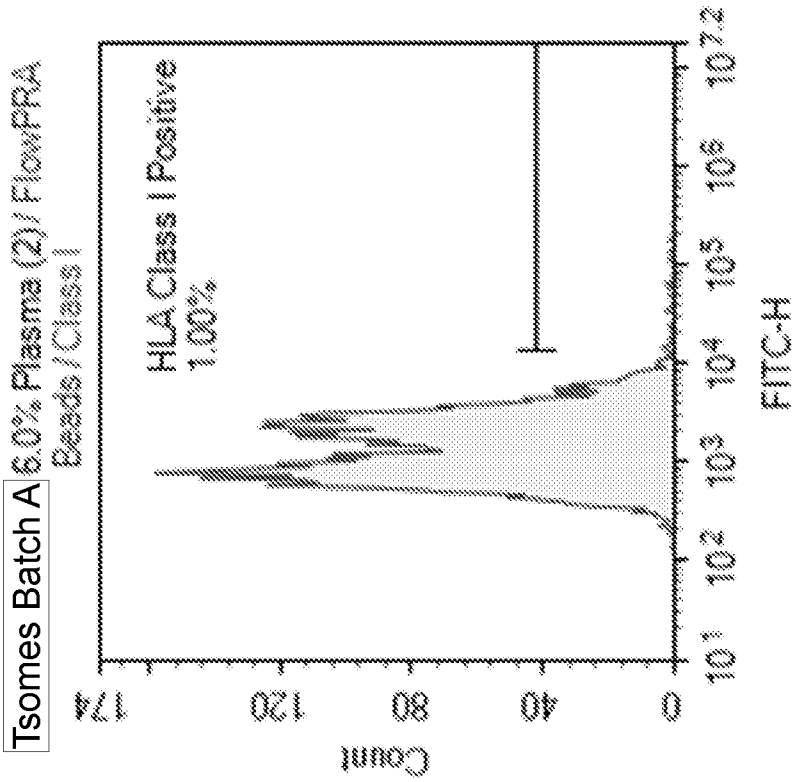



Fig. 14A

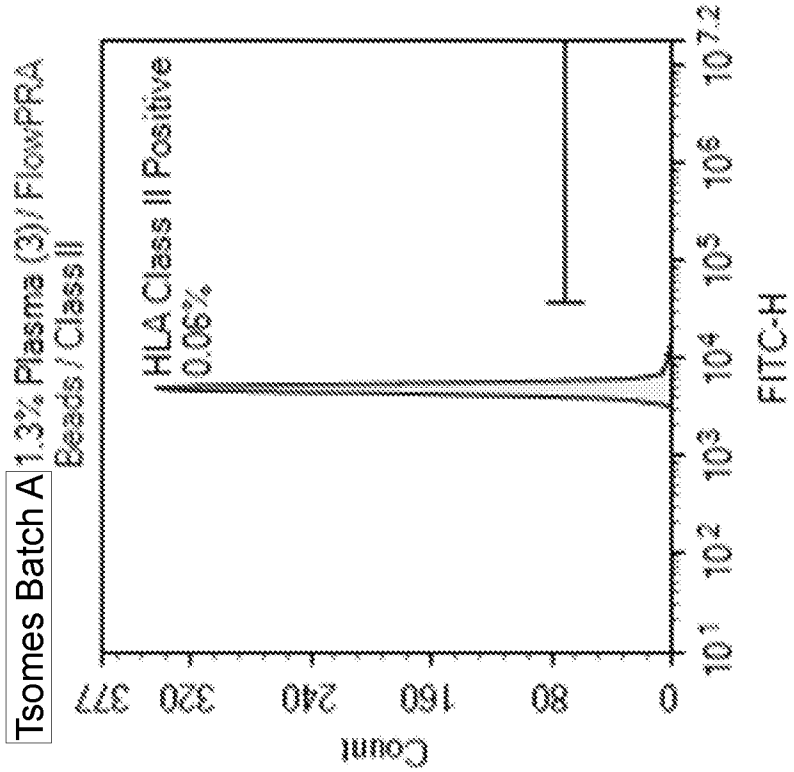



Fig. 15B

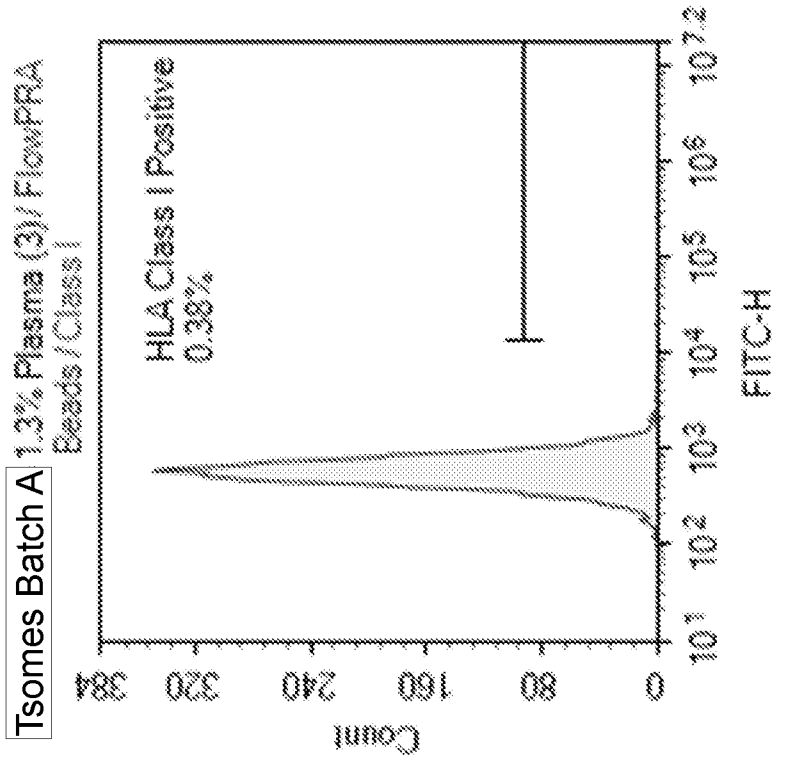



Fig. 15A

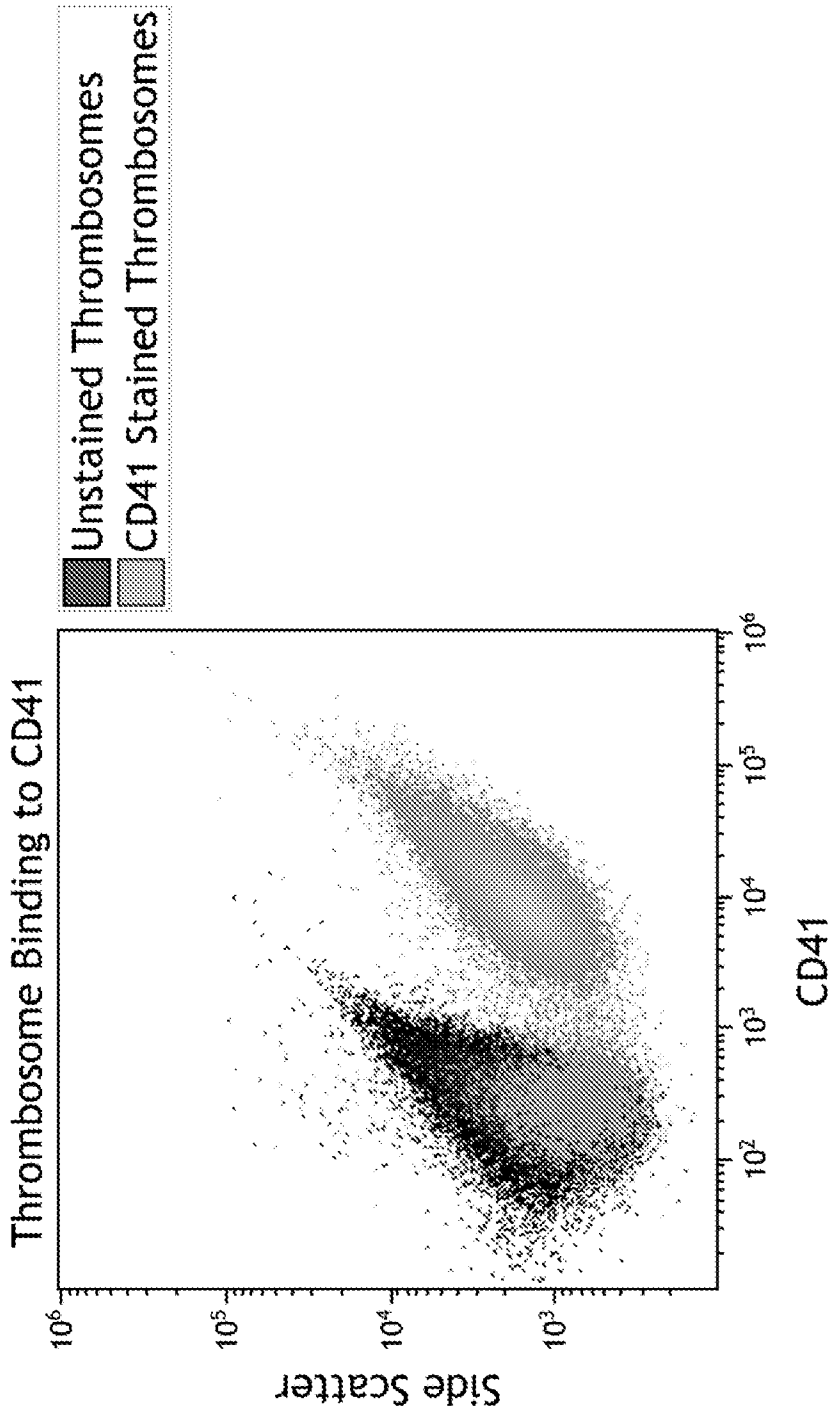

Unstained Thrombosomes
CD41 Stained Thrombosomes
Thrombosome Binding to CD41
Side Scatter
CD41
$10^2$
$10^3$
$10^4$
$10^5$
$10^6$

Fig. 16

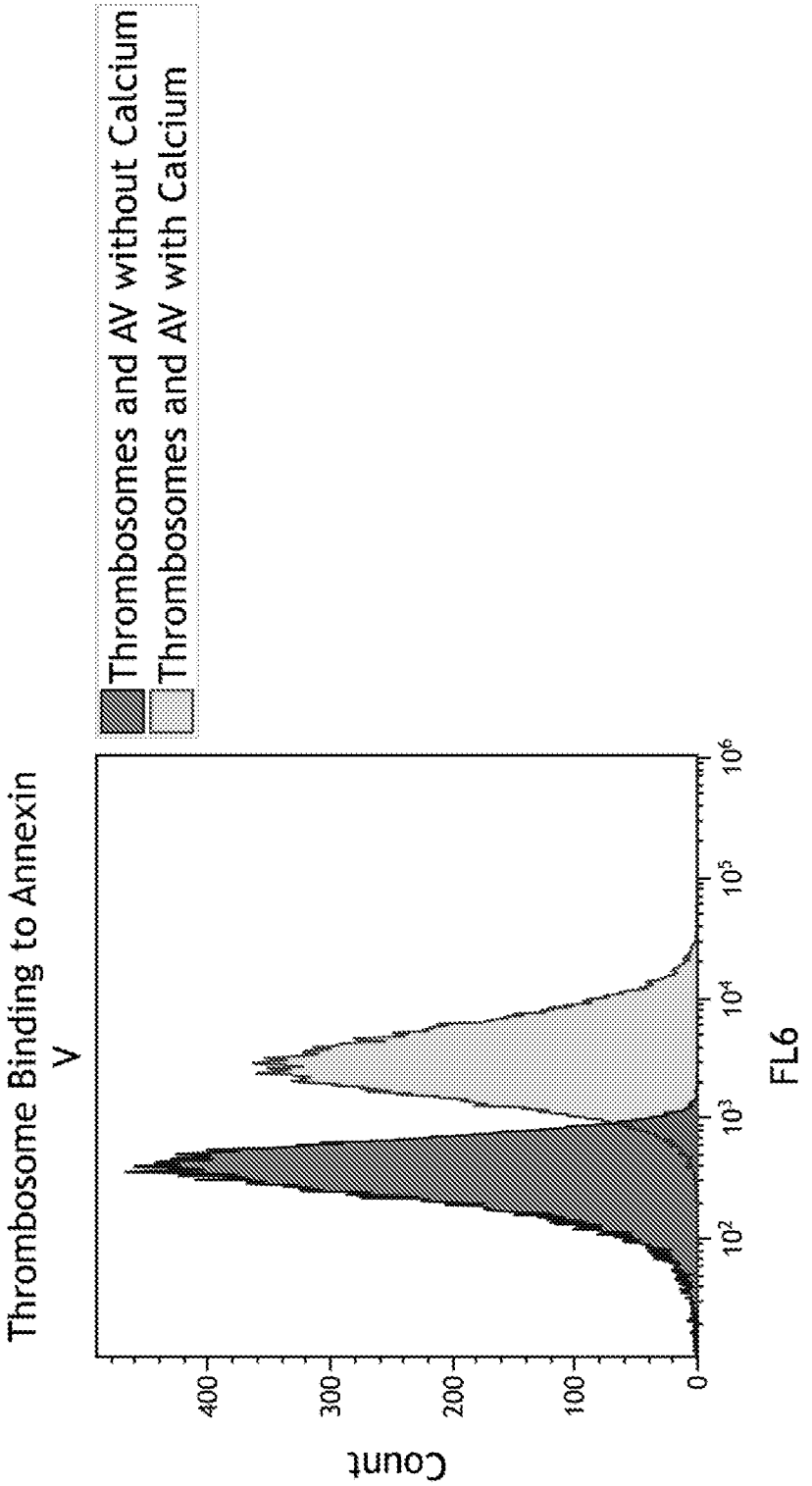

Thrombosomes and AV without Calcium
Thrombosomes and AV with Calcium
Thrombosome Binding to Annexin V
Count
400
300
200
100
0
$10^2$
$10^3$
$10^4$
$10^5$
$10^6$
FL6

Fig. 17

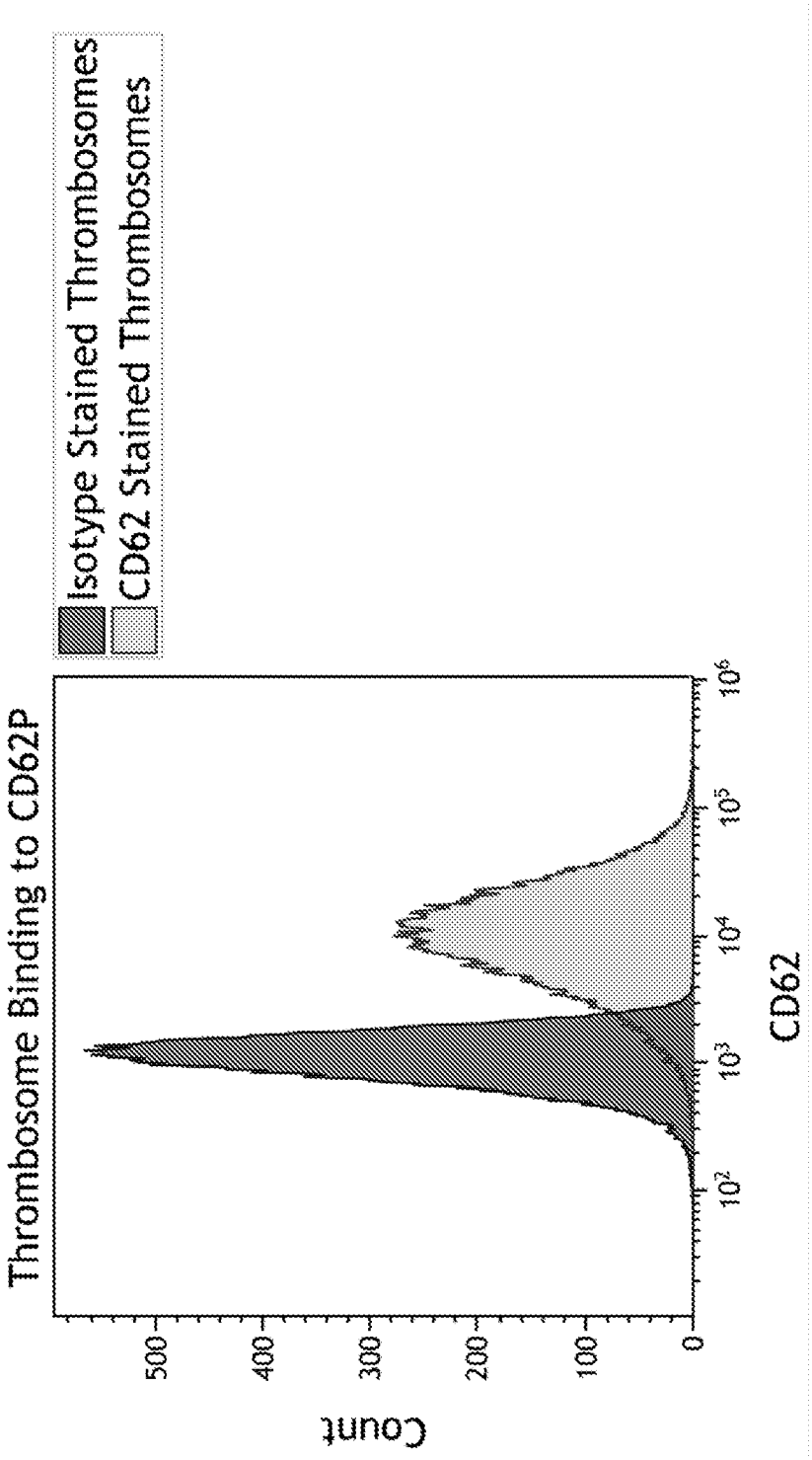

Thrombosome Binding to CD62P
Isotype Stained Thrombosomes
CD62 Stained Thrombosomes
Count
0
100
200
300
400
500
$10^2$
$10^3$
$10^4$
$10^5$
$10^6$
CD62

Fig. 18

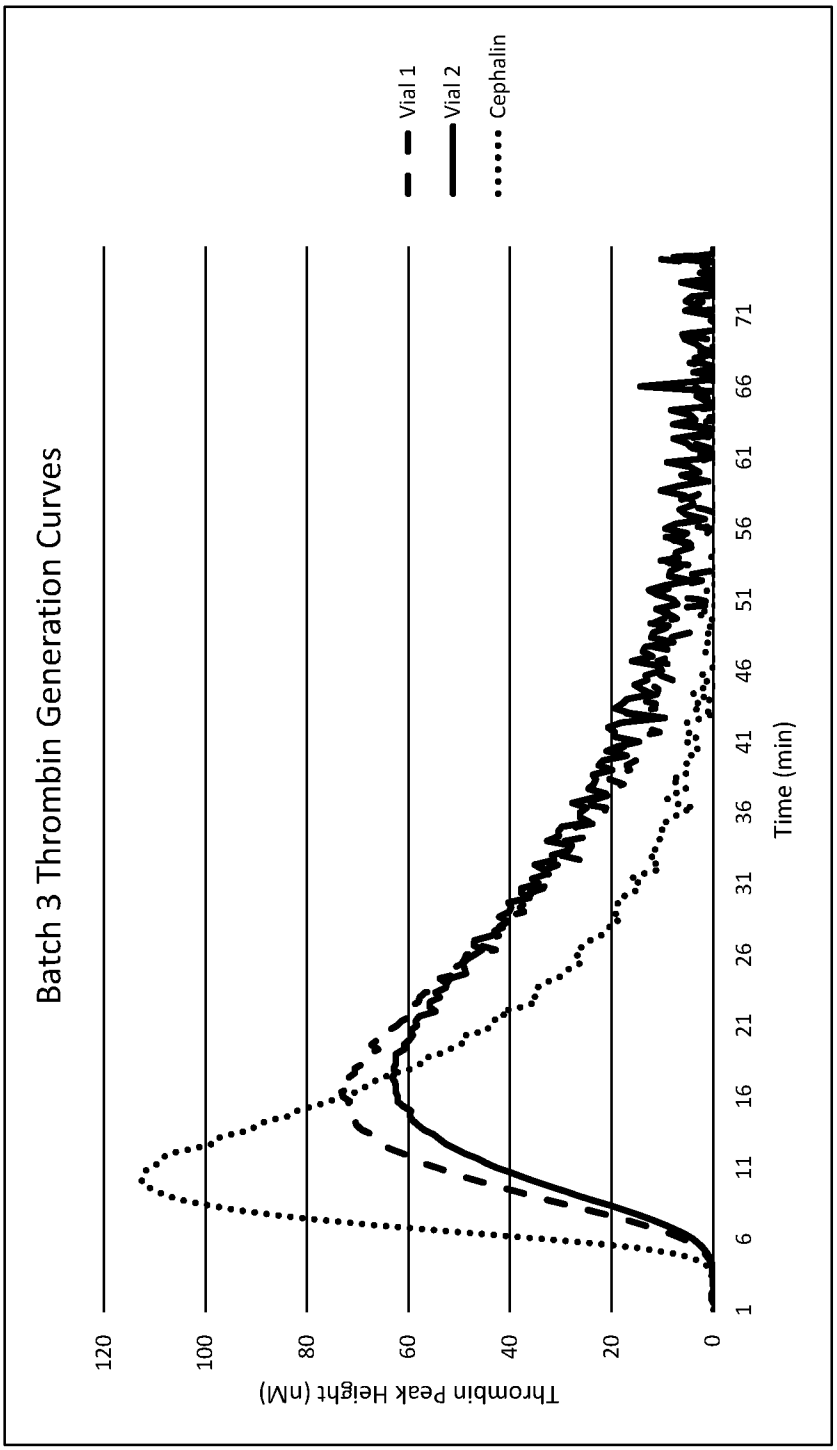
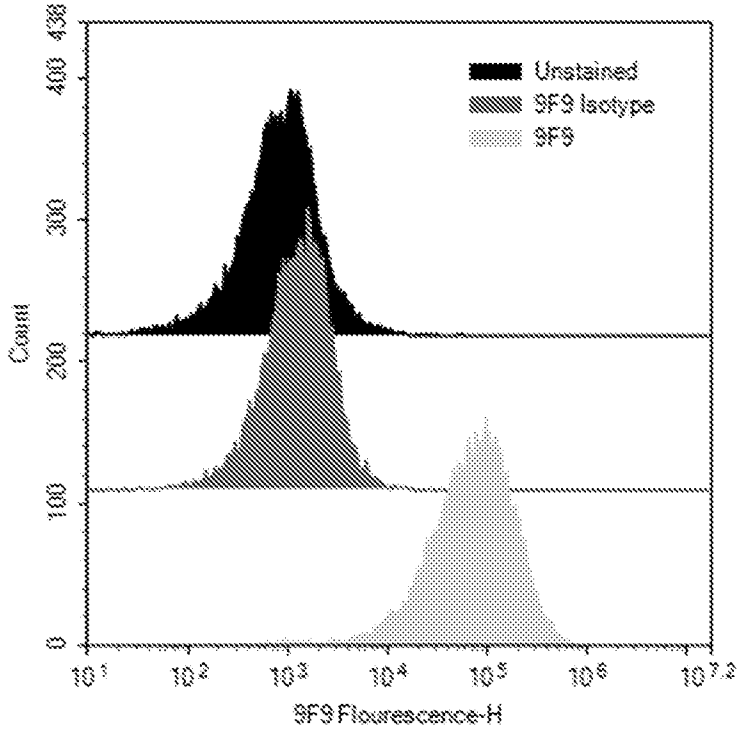
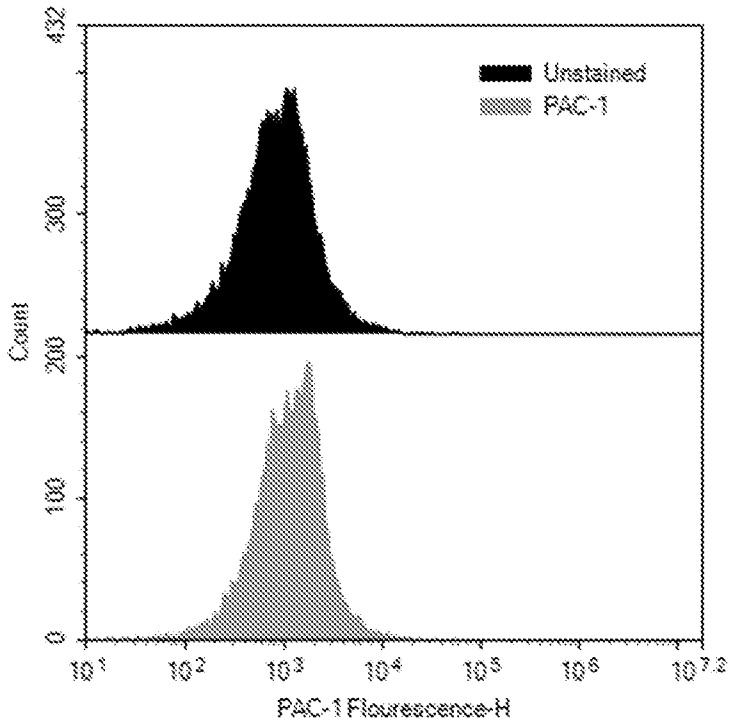

Batch 3 Thrombin Generation Curves
Vial 1
Vial 2
Cephalin
Thrombin Peak Height (nM)
120
100
80
60
40
20
0
1
6
11
16
21
26
31
36
41
46
51
56
61
66
71
Time (min)

Fig. 19




| Batch I (TFF) MFI | | | |
|---|---|---|---|
| Rep | Unstained | 9F9 Isotype | 9F9 Test |
| 1 | 1200 | 1691 | 105960 |
| 2 | 1190 | 2090 | 102894 |
| Average | 1195 | 1891 | 104427 |

**Fig. 20**




| Batch I (TFF) PAC-1 MFI | | |
|---|---|---|
| Rep | Unstained | PAC-1 Test |
| 1 | 1200 | 1792 |
| 2 | 1190 | 1329 |
| Average | 1195 | 1561 |

**Fig. 21**

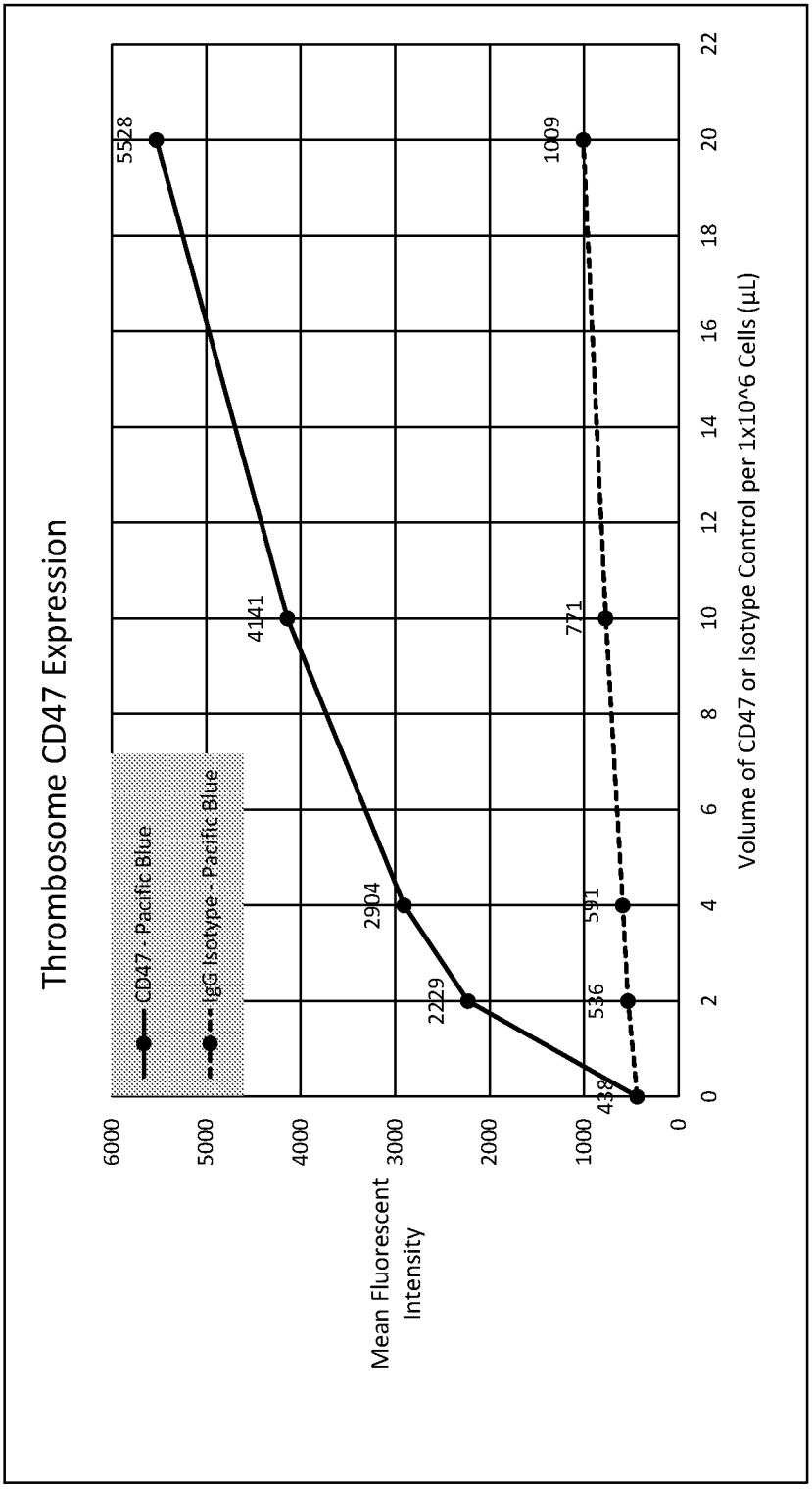
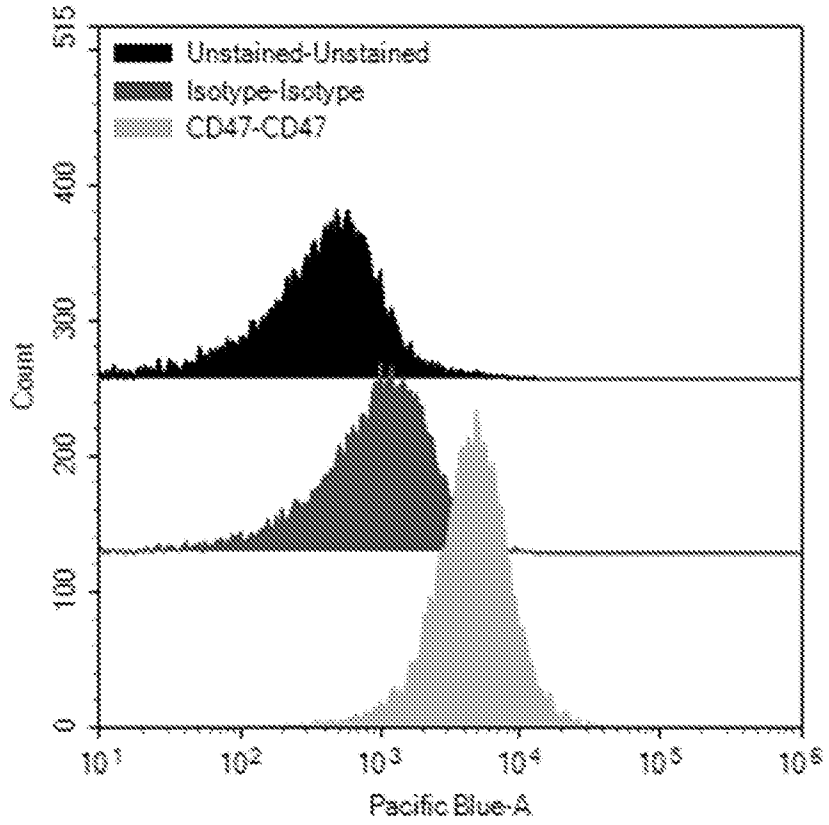

Thrombosome CD47 Expression
CD47 - Pacific Blue
IgG Isotype - Pacific Blue
Mean Fluorescent Intensity
Volume of CD47 or Isotype Control per 1x10^6 Cells (µL)
6000
5000
4000
3000
2000
1000
0
0
2
4
6
8
10
12
14
16
18
20
22
5528
4141
2904
2229
1009
771
591
536
438

Fig. 22A




| # | Sample | Gate | Mean X |
|---|---|---|---|
| 1 | Unstained | Thrombosomes | 441 |
| 2 | Isotype | Thrombosomes* | 991 |
| 3 | CD47 | Thrombosomes* | 5,440 |

**Fig. 22B**

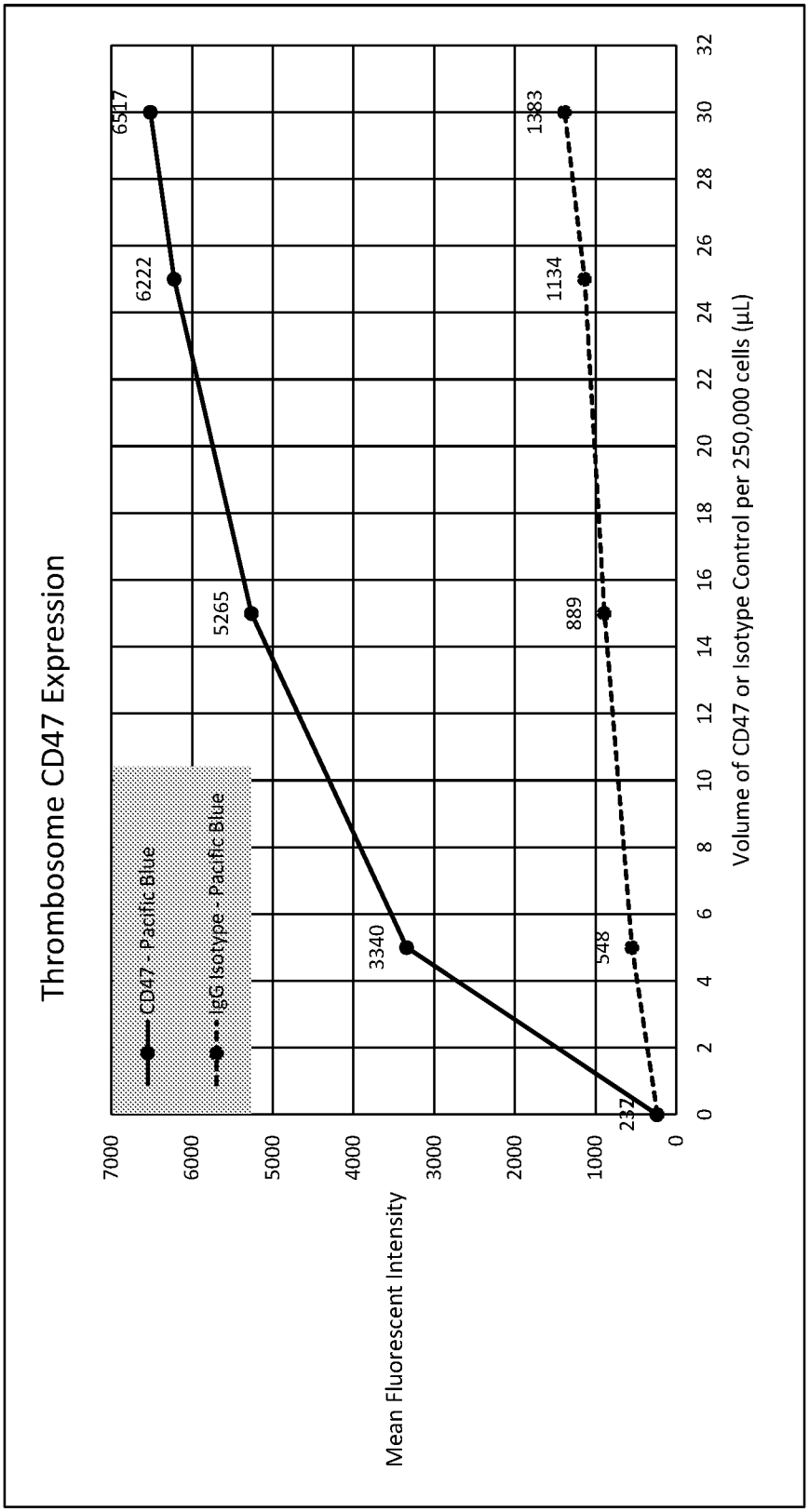
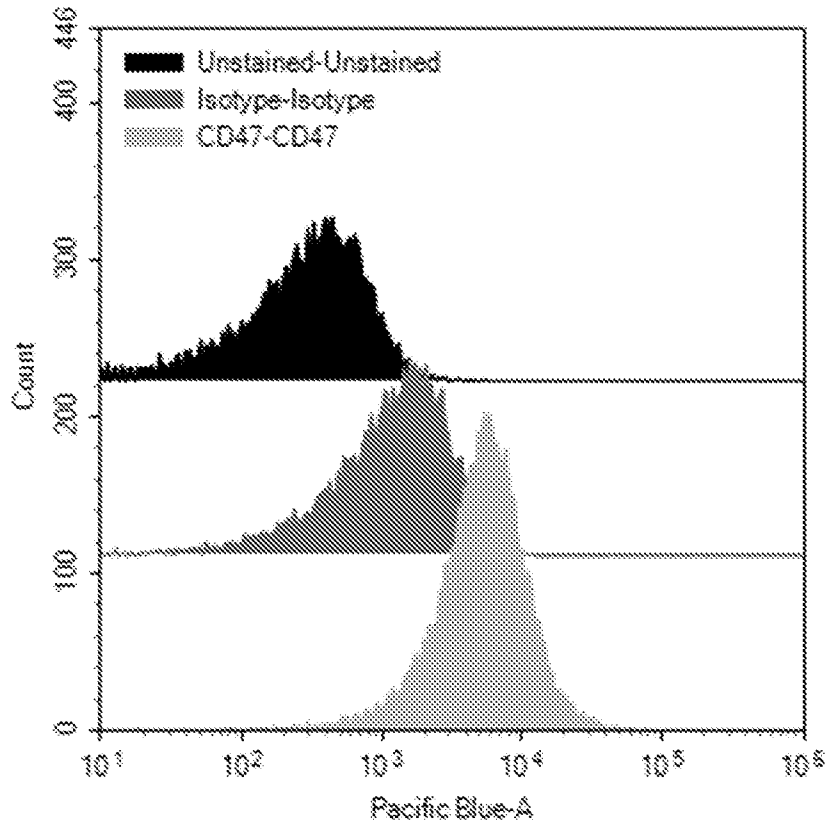

Thrombosome CD47 Expression
CD47 - Pacific Blue
IgG Isotype - Pacific Blue
Mean Fluorescent Intensity
Volume of CD47 or Isotype Control per 250,000 cells (µL)
7000
6000
5000
4000
3000
2000
1000
0
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
6517
6222
5265
3340
237
1383
1134
889
548

Fig. 22C




| # | Sample | Gate | Mean X |
|---|---|---|---|
| 1 | Unstained | Thrombosomes | 239 |
| 2 | Isotype | Thrombosomes* | 1,418 |
| 3 | CD47 | Thrombosomes* | 6,508 |

**Fig. 22D**

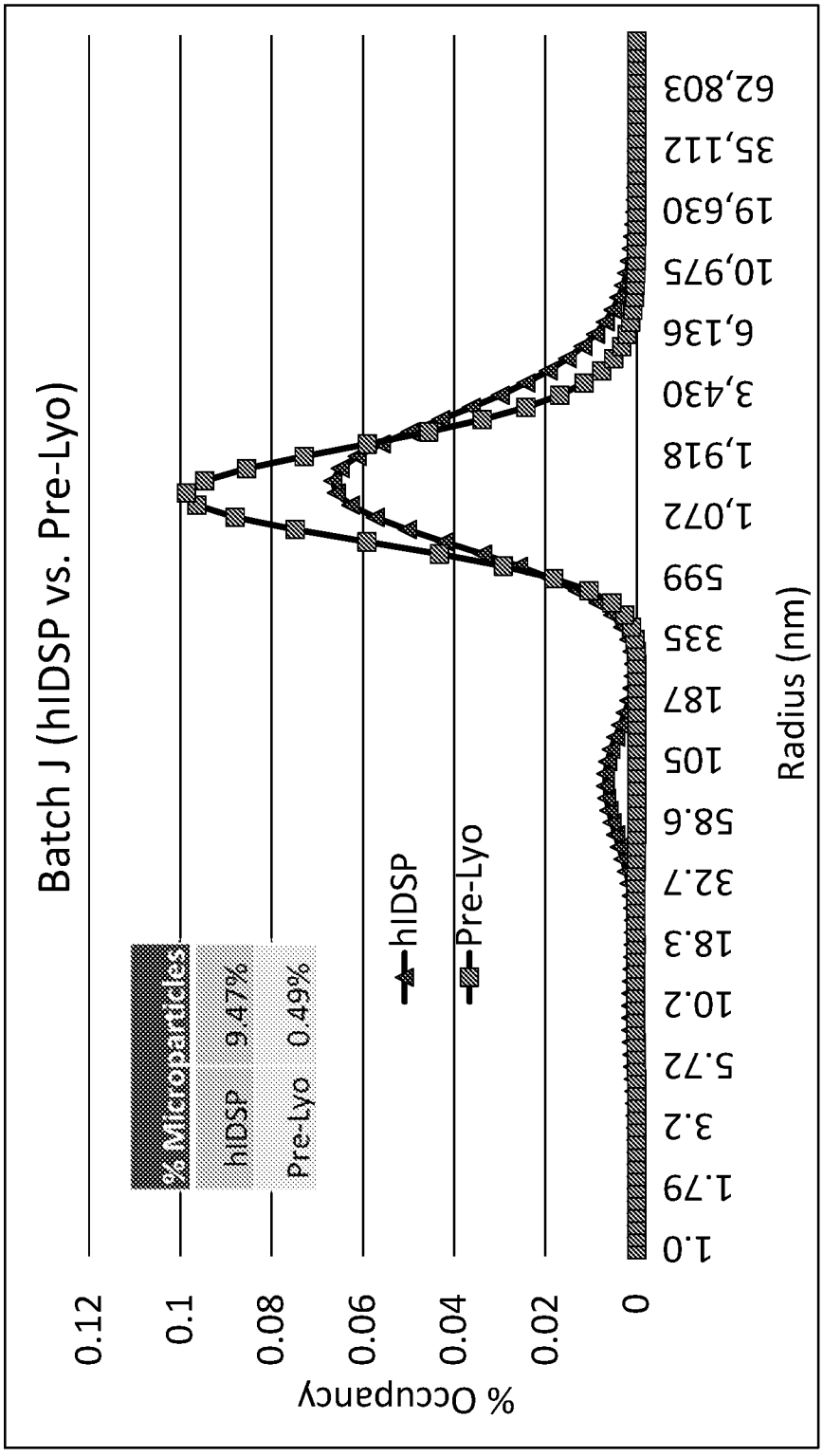

Batch J (hIDSP vs. Pre-Lyo)
% Occupancy
0.12
0.1
0.08
0.06
0.04
0.02
0
Radius (nm)
1.0
1.79
3.2
5.72
10.2
18.3
32.7
58.6
105
187
335
599
1,072
1,918
3,430
6,136
10,975
19,630
35,112
62,803
hIDSP
Pre-Lyo
% Micropaticles
hIDSP 9.47%
Pre-Lyo 0.49%

Fig. 23A

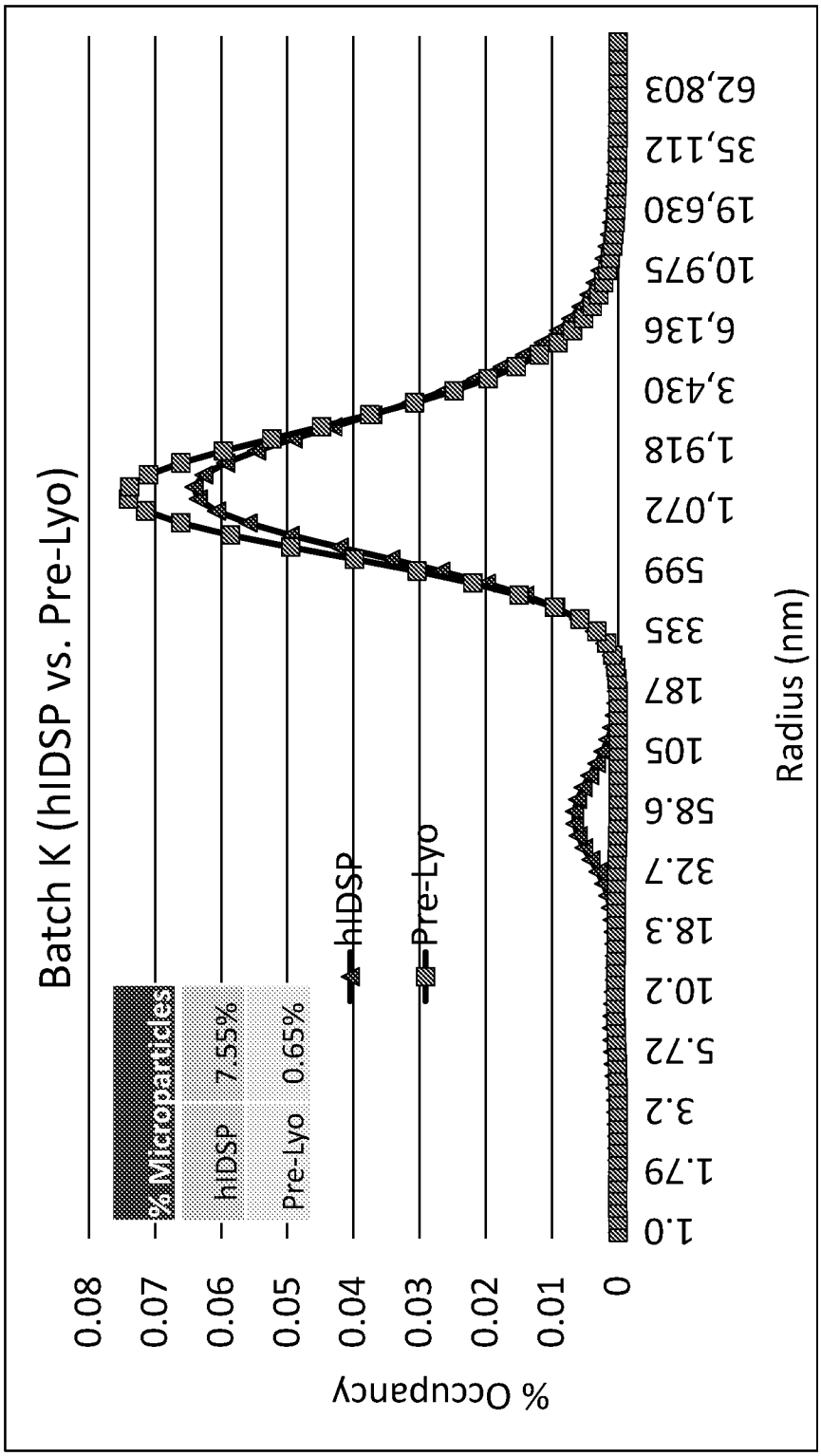

Batch K (hIDSP vs. Pre-Lyo)
% Occupancy
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Radius (nm)
1.0
1.79
3.2
5.72
10.2
18.3
32.7
58.6
105
187
335
599
1,072
1,918
3,430
6,136
10,975
19,630
35,112
62,803
hIDSP
Pre-Lyo
% Microparticles
hIDSP 7.55%
Pre-Lyo 0.65%

**Fig. 23B**

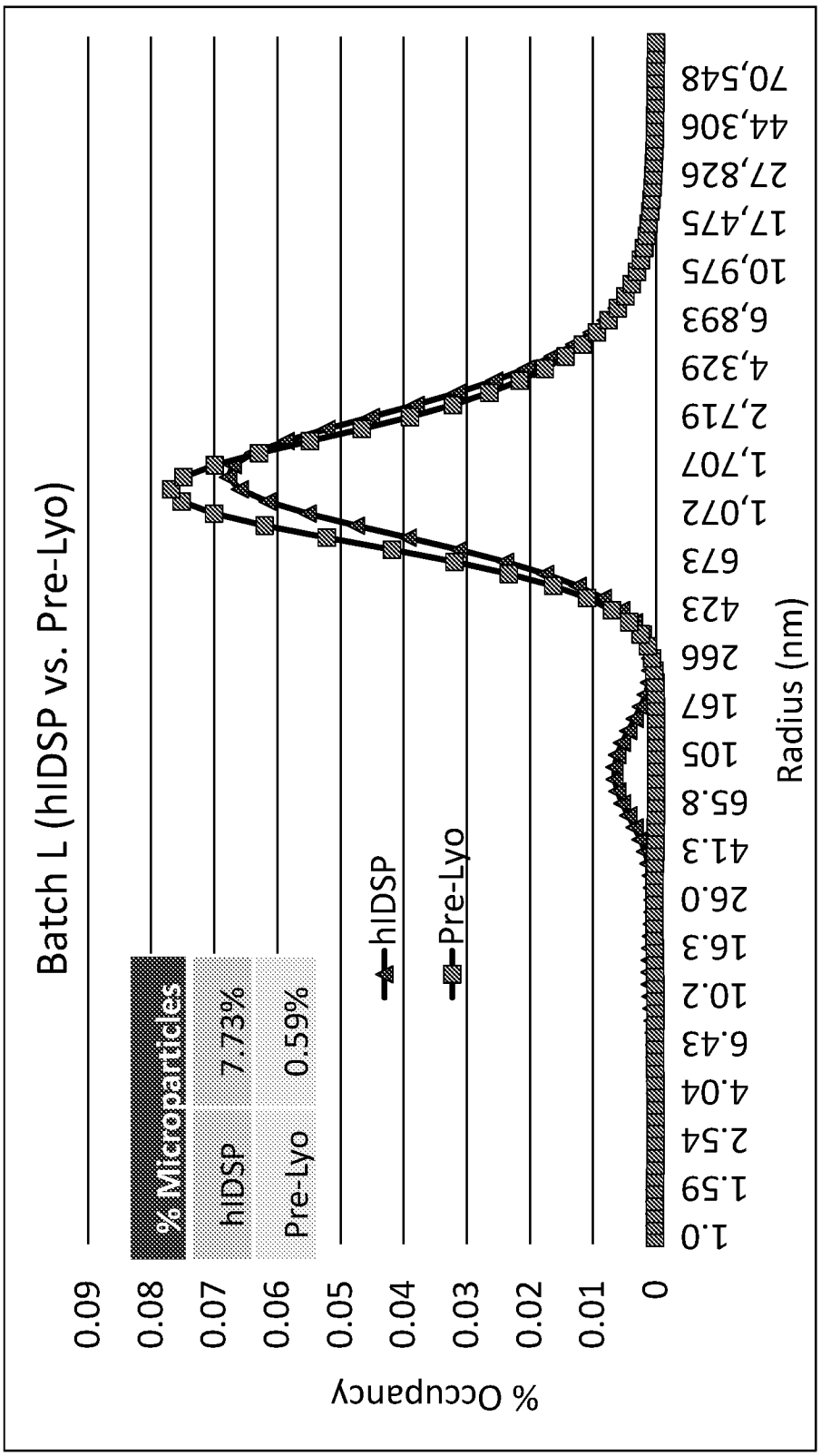

Batch L (hIDSP vs. Pre-Lyo)
% Occupancy
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
% Micoparticles
hIDSP 7.73%
Pre-Lyo 0.59%
hIDSP
Pre-Lyo
Radius (nm)
1.0
1.59
2.54
4.04
6.43
10.2
16.3
26.0
41.3
65.8
105
167
266
423
673
1,072
1,707
2,719
4,329
6,893
10,975
17,475
27,826
44,306
70,548

Fig. 23C

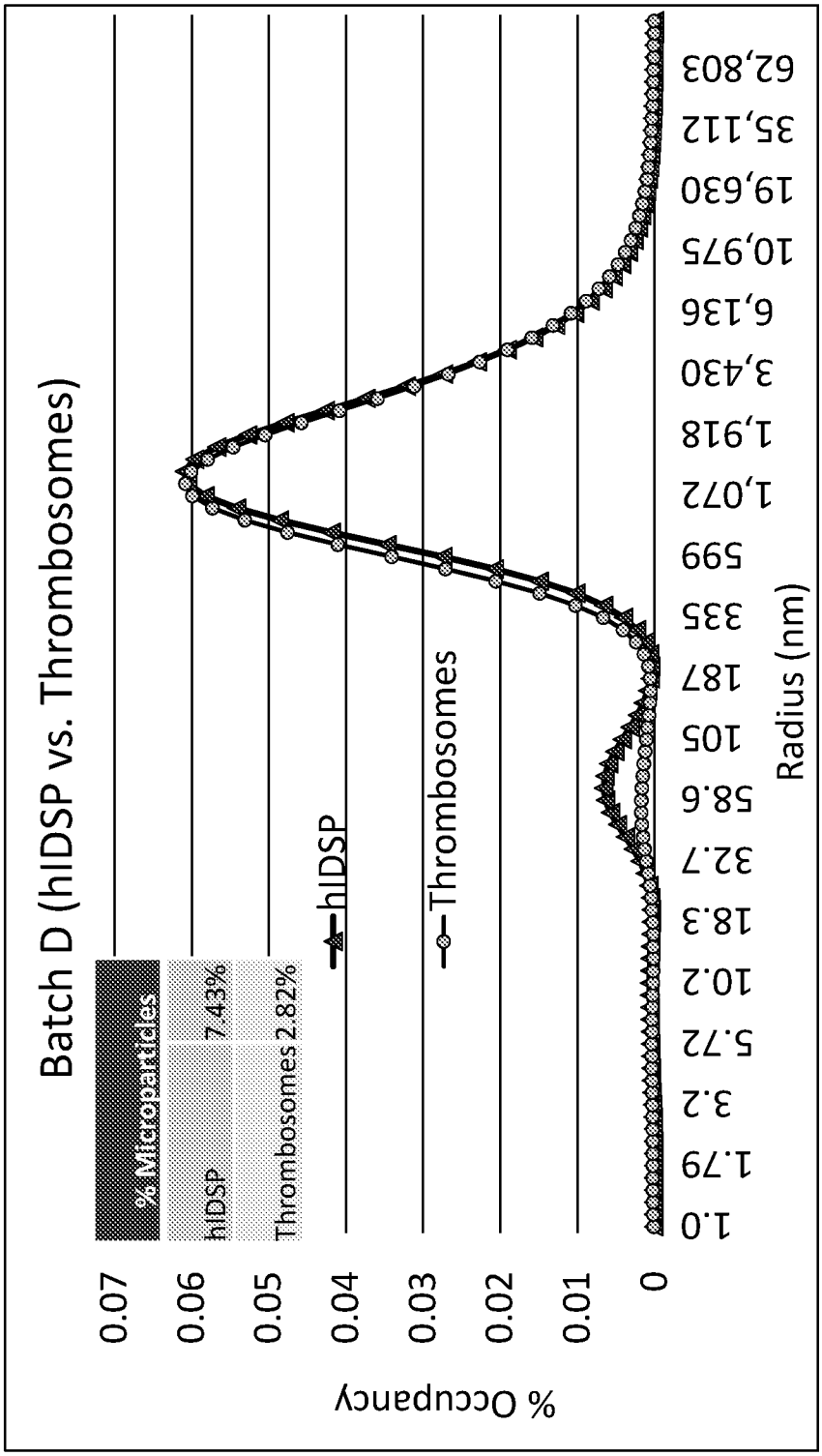

Batch D (hIDSP vs. Thrombosomes)
% Occupancy
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
% Microparticles
hIDSP 7.43%
Thrombosomes 2.82%
hIDSP
Thrombosomes
1.0
1.79
3.2
5.72
10.2
18.3
32.7
58.6
105
187
335
599
1,072
1,918
3,430
6,136
10,975
19,630
35,112
62,803
Radius (nm)

Fig. 24A

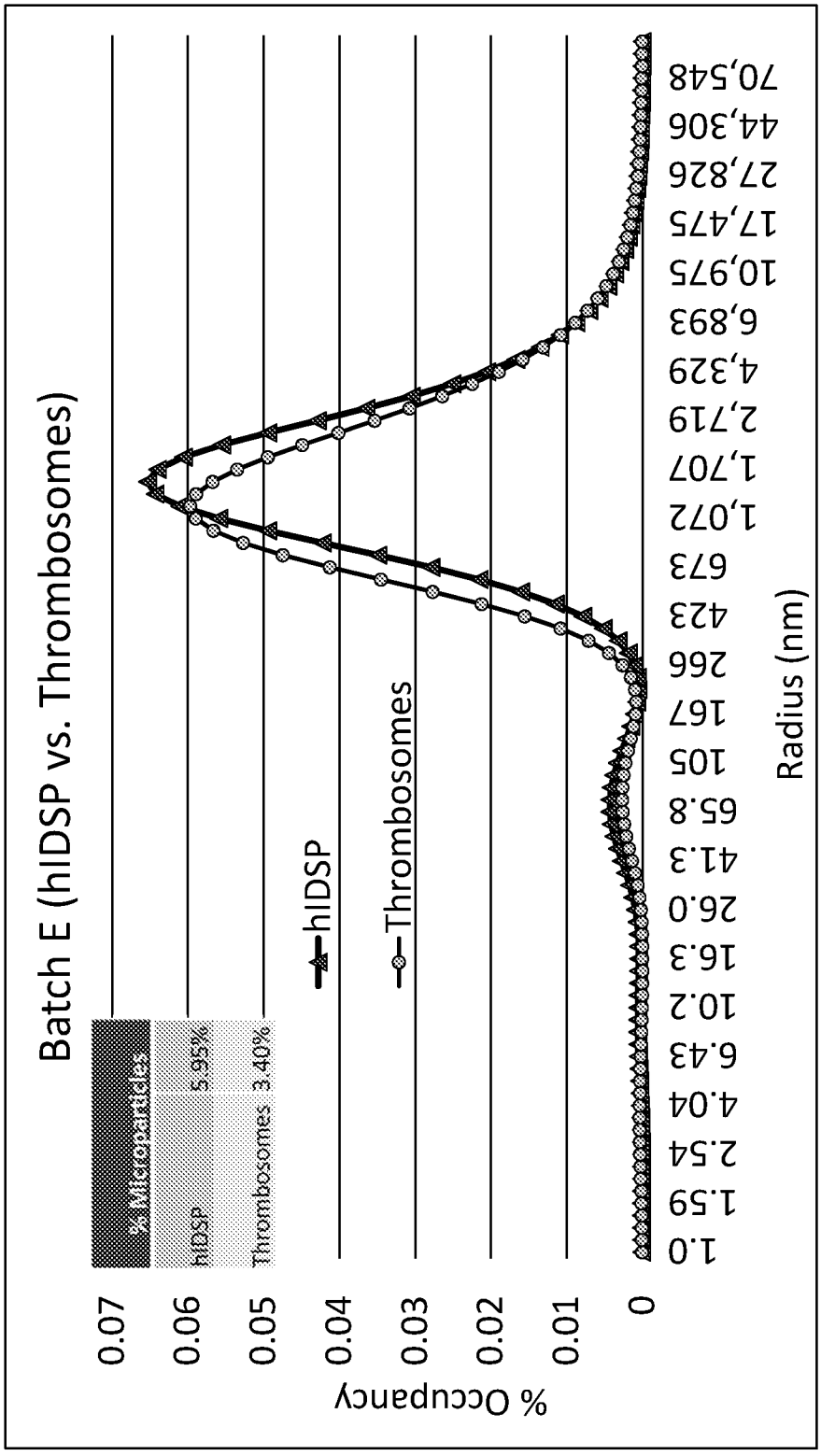

Batch E (hIDSP vs. Thrombosomes)
% Occupancy
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Radius (nm)
1.0
1.59
2.54
4.04
6.43
10.2
16.3
26.0
41.3
65.8
105
167
266
423
673
1,072
1,707
2,719
4,329
6,893
10,975
17,475
27,826
44,306
70,548
hIDSP
Thrombosomes
Pd Microparticles
hIDSP 5.95%
Thrombosomes 3.40%

**Fig. 24B**

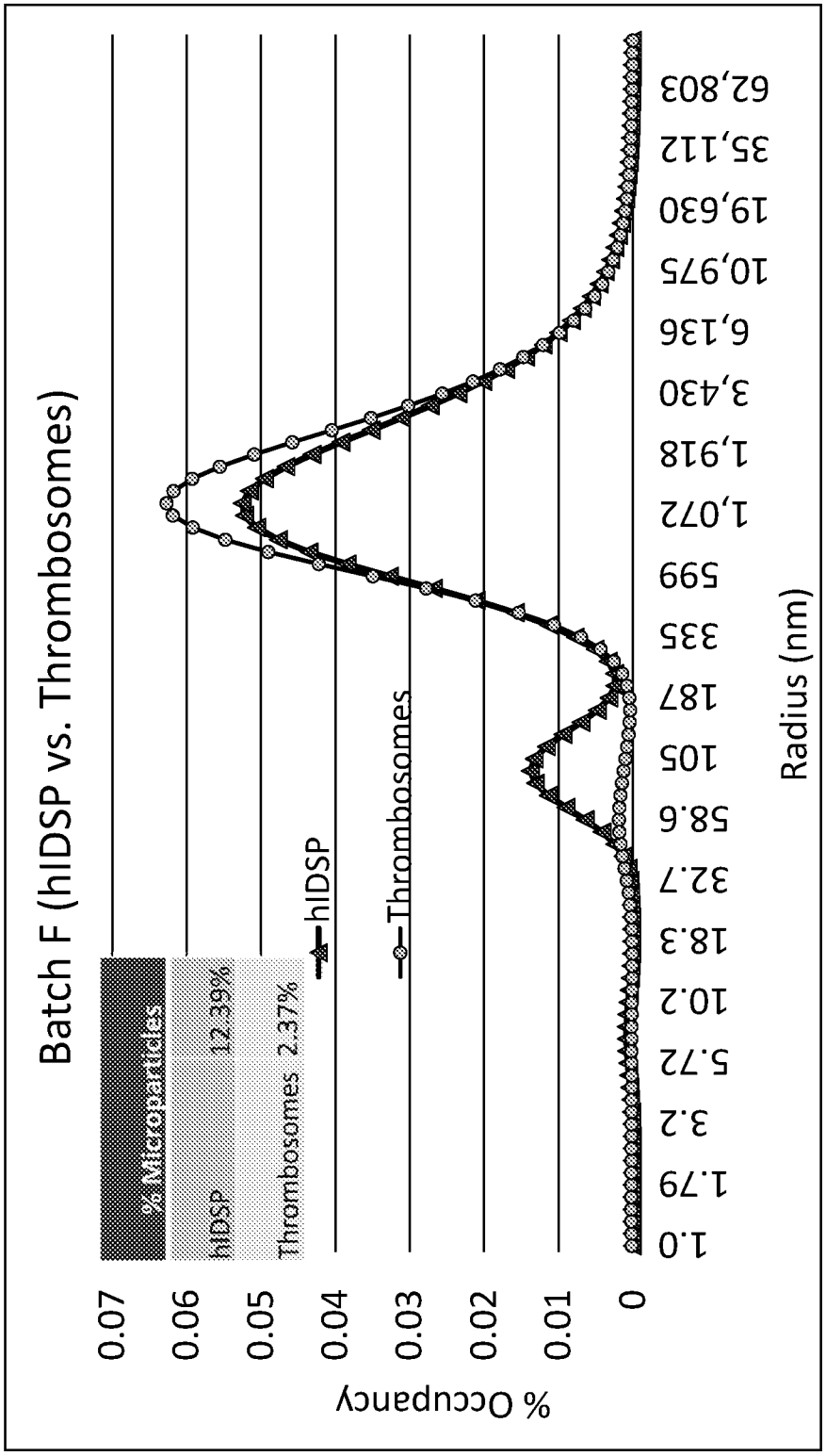

Batch F (hIDSP vs. Thrombosomes)
% Microparticles
hIDSP 12.39%
Thrombosomes 2.37%
hIDSP
Thrombosomes
% Occupancy
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Radius (nm)
1.0
1.79
3.2
5.72
10.2
18.3
32.7
58.6
105
187
335
599
1,072
1,918
3,430
6,136
10,975
19,630
35,112
62,803

Fig. 24C

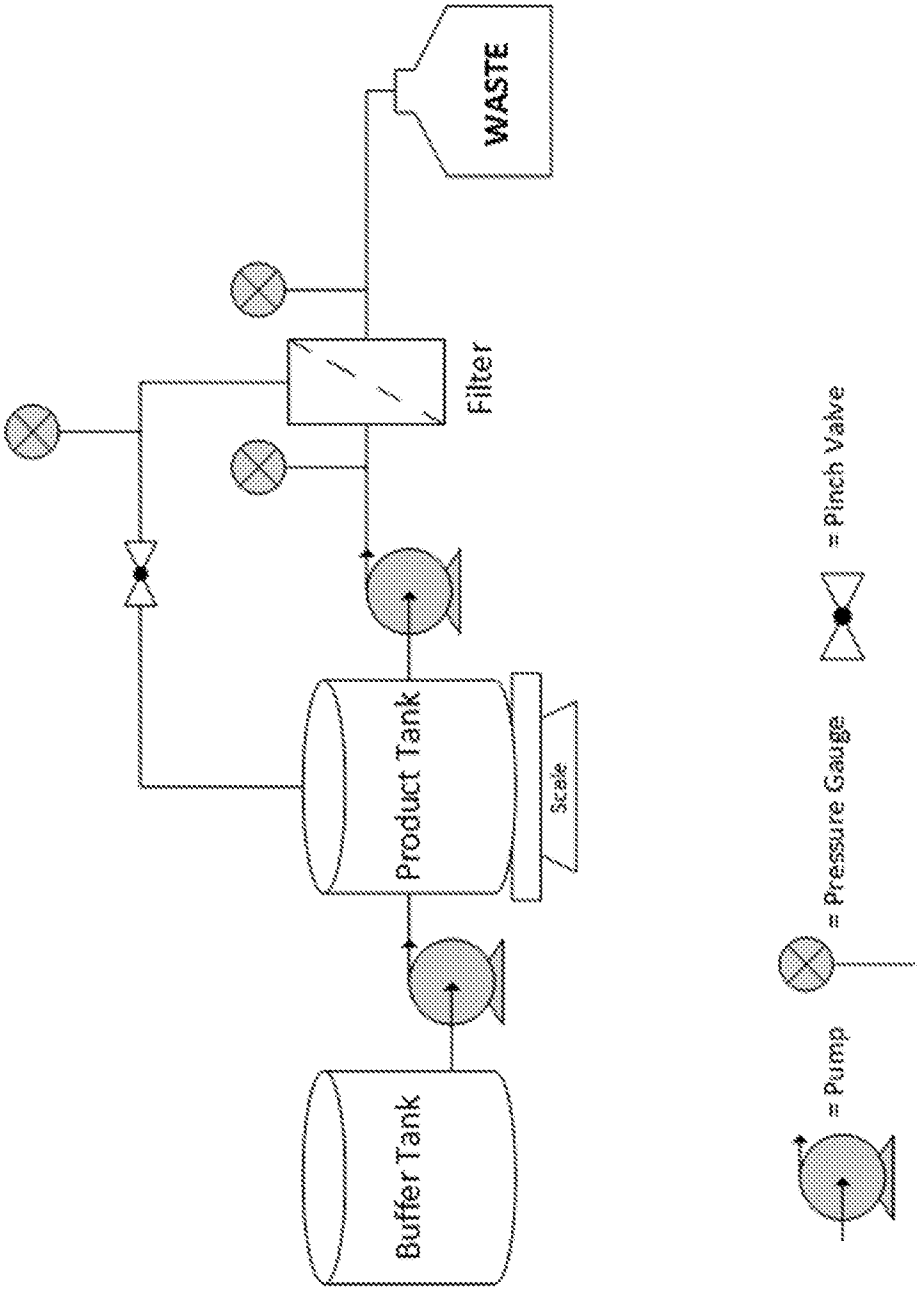

WASTE
Filter
Product Tank
Scale
Buffer Tank
= Pinch Valve
= Pressure Gauge
= Pump

Fig. 25A

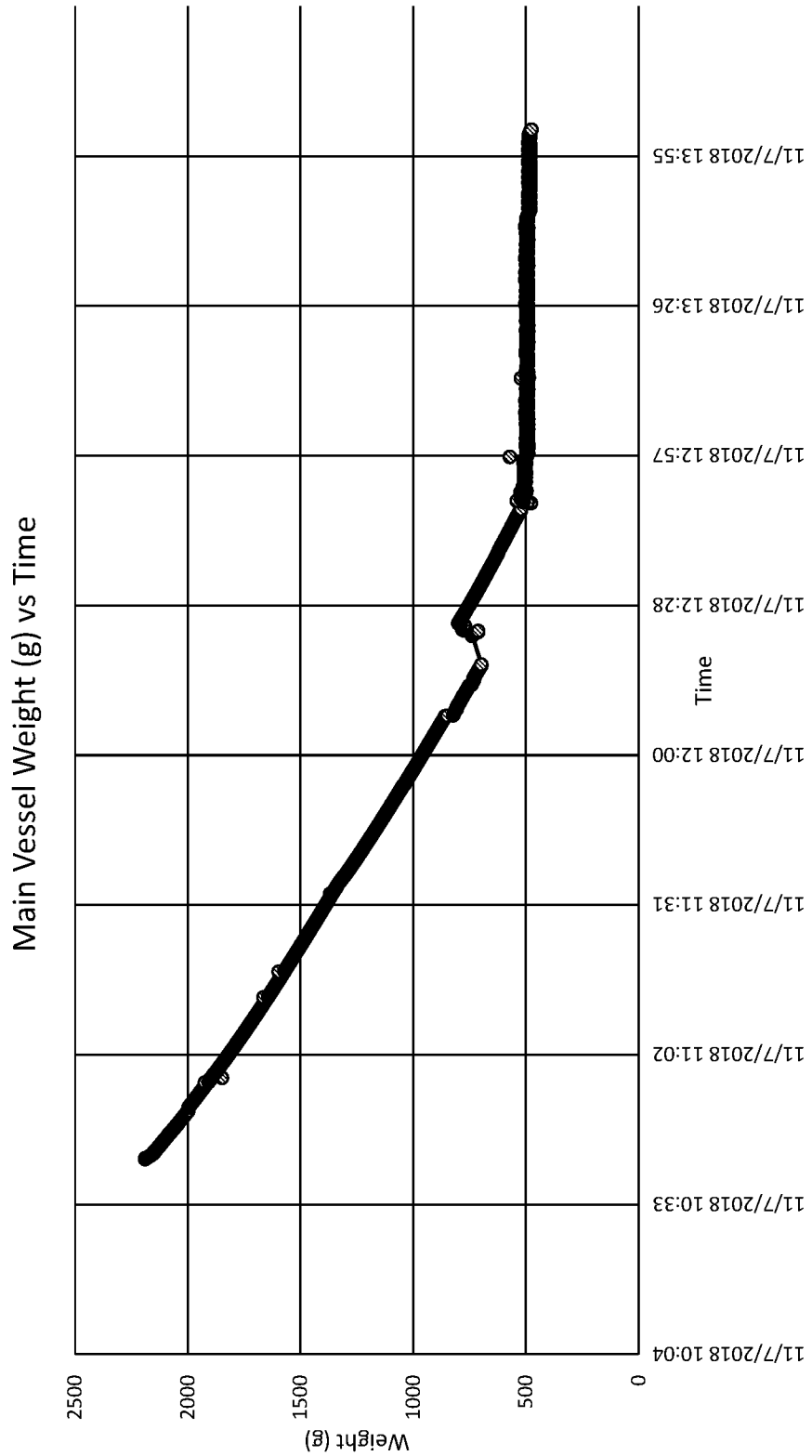

Main Vessel Weight (g) vs Time
Weight (g)
2500
2000
1500
1000
500
0
Time
11/7/2018 10:04
11/7/2018 10:33
11/7/2018 11:02
11/7/2018 11:31
11/7/2018 12:00
11/7/2018 12:28
11/7/2018 12:57
11/7/2018 13:26
11/7/2018 13:55

Fig. 25B

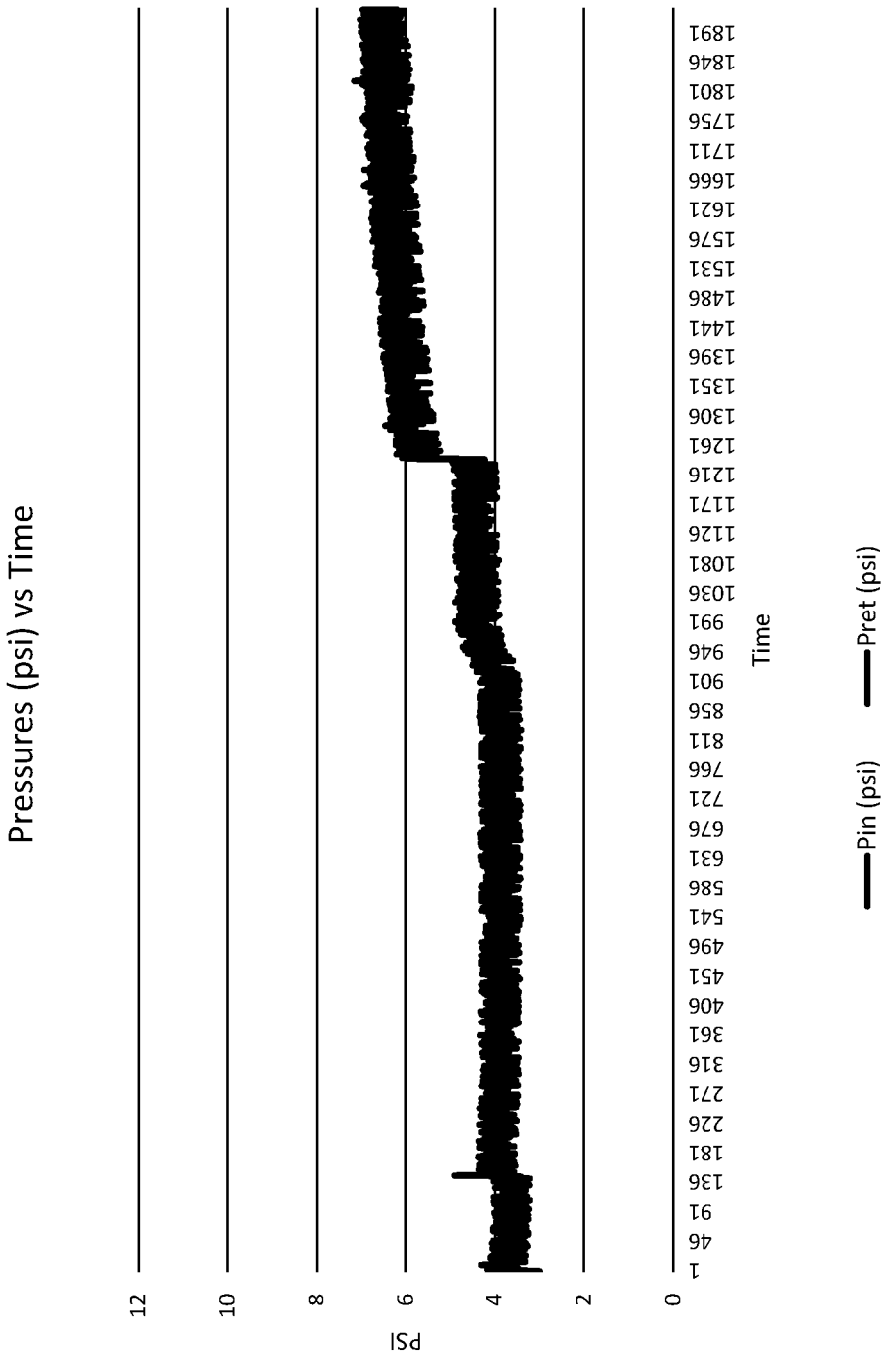

Pressures (psi) vs Time
PSI
12
10
8
6
4
2
0
Time
1
46
91
136
181
226
271
316
361
406
451
496
541
586
631
676
721
766
811
856
901
946
991
1036
1081
1126
1171
1216
1261
1306
1351
1396
1441
1486
1531
1576
1621
1666
1711
1756
1801
1846
1891
Pin (psi)
Pret (psi)

Fig. 25C

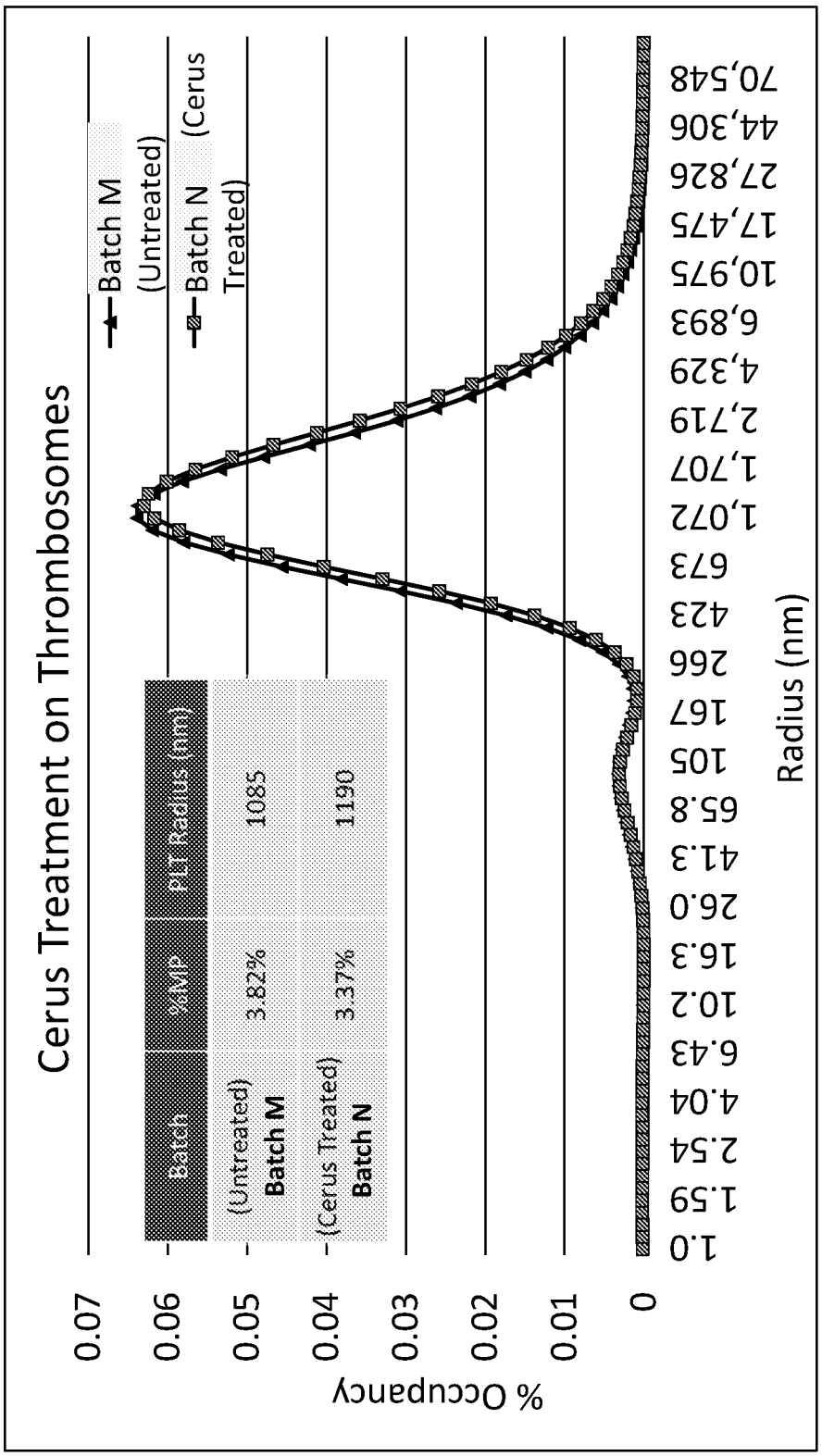

Cerus Treatment on Thrombosomes
Batch M (Untreated)
Batch N (Cerus Treated)
% Occupancy
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Radius (nm)
1.0
1.59
2.54
4.04
6.43
10.2
16.3
26.0
41.3
65.8
105
167
266
423
673
1,072
1,707
2,719
4,329
6,893
10,975
17,475
27,826
44,306
70,548
Batch
%MP
PLT Radius (nm)
(Untreated) Batch M
3.82%
1085
(Cerus Treated) Batch N
3.37%
1190

Fig. 26A

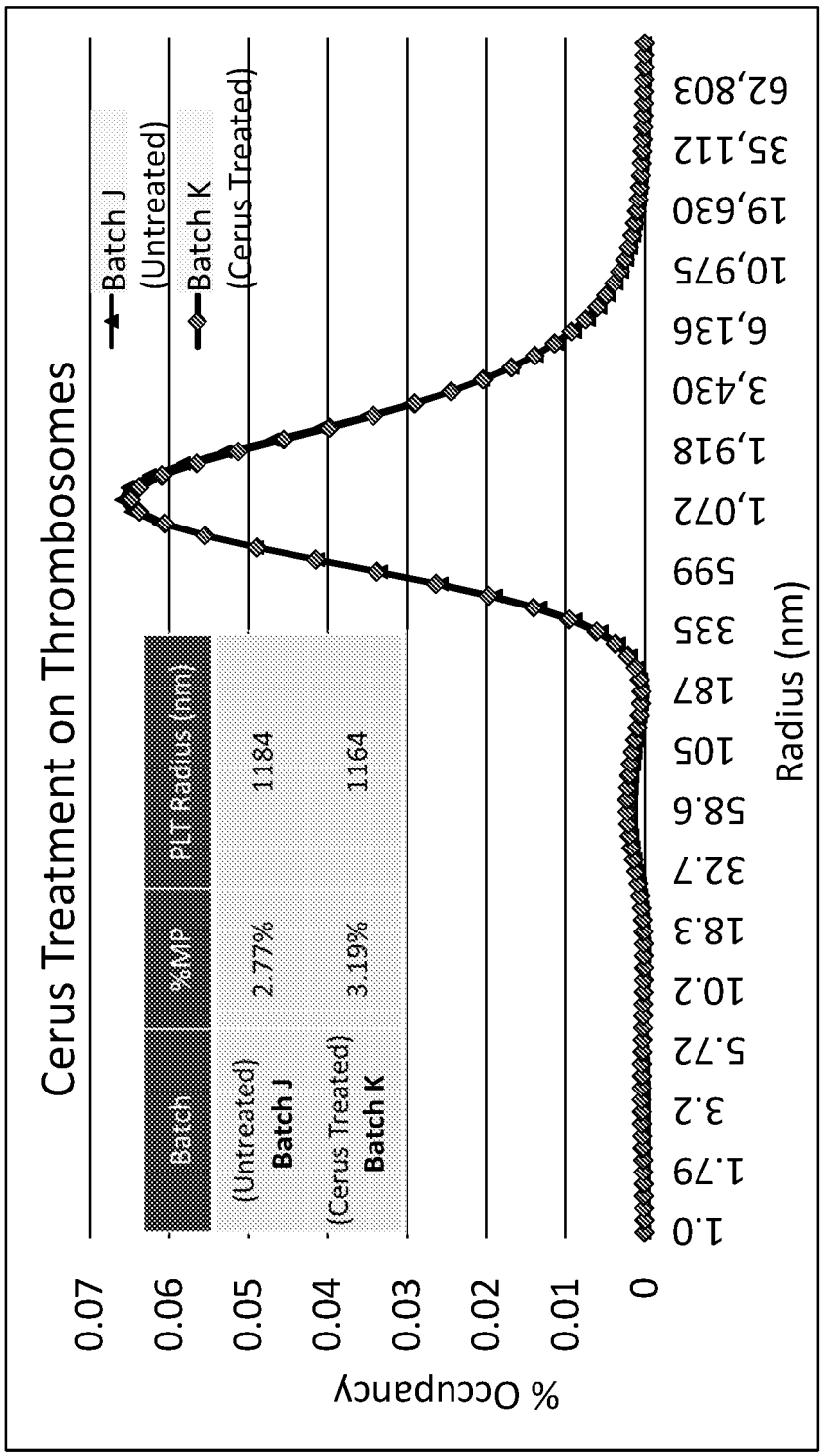

Cerus Treatment on Thrombosomes
Batch J (Untreated)
Batch K (Cerus Treated)
% Occupancy
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Batch
%MP
PLT Radius (nm)
(Untreated) Batch J
2.77%
1184
(Cerus Treated) Batch K
3.19%
1164
Radius (nm)
1.0
1.79
3.2
5.72
10.2
18.3
32.7
58.6
105
187
335
599
1,072
1,918
3,430
6,136
10,975
19,630
35,112
62,803

Fig. 26B

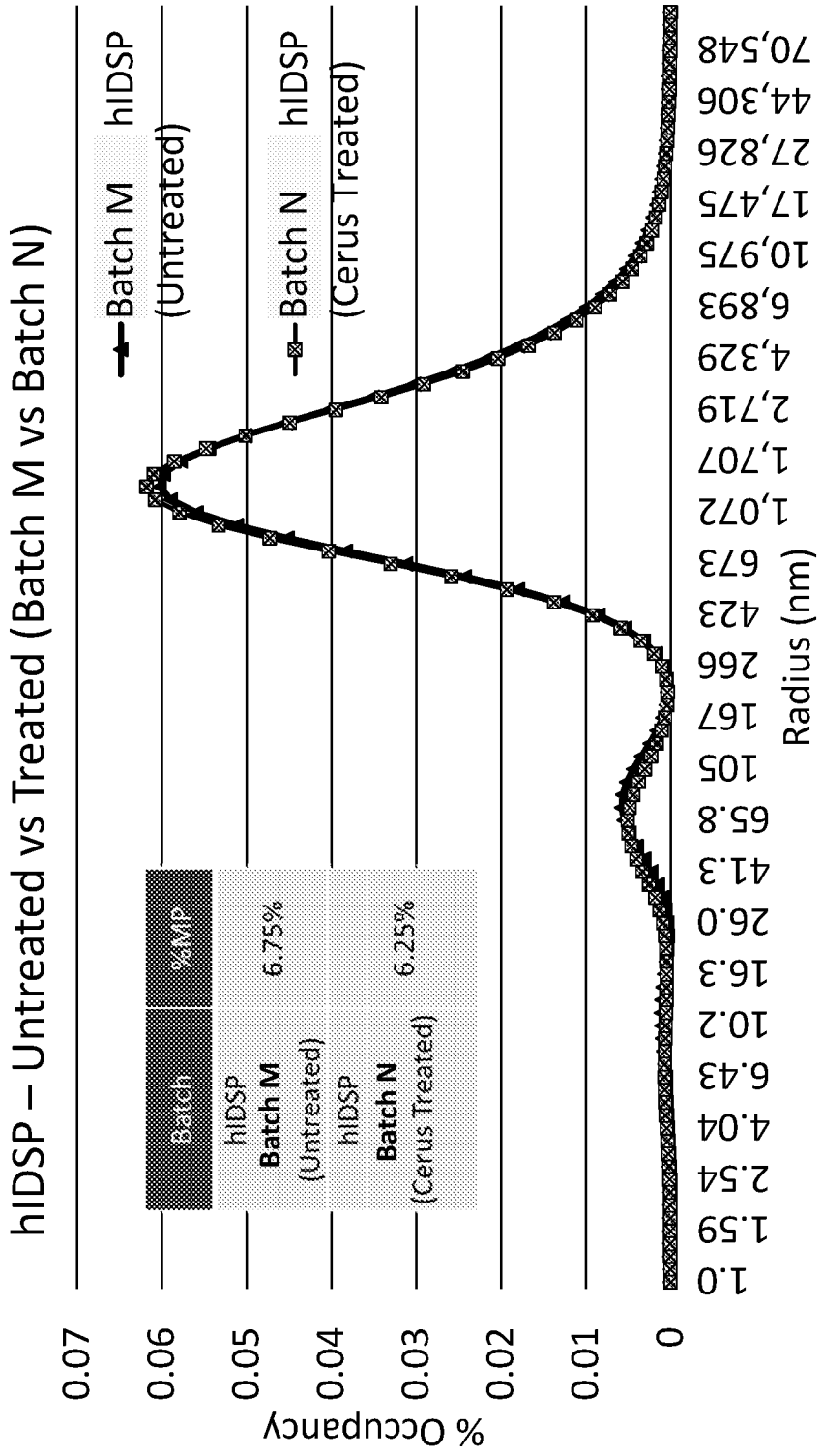

hIDSP – Untreated vs Treated (Batch M vs Batch N)
Batch M hIDSP (Untreated)
Batch N hIDSP (Cerus Treated)
% Occupancy
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Radius (nm)
1.0
1.59
2.54
4.04
6.43
10.2
16.3
26.0
41.3
65.8
105
167
266
423
673
1,072
1,707
2,719
4,329
6,893
10,975
17,475
27,826
44,306
70,548
Batch
PAMP
hIDSP Batch M (Untreated) 6.75%
hIDSP Batch N (Cerus Treated) 6.25%

**Fig. 27A**

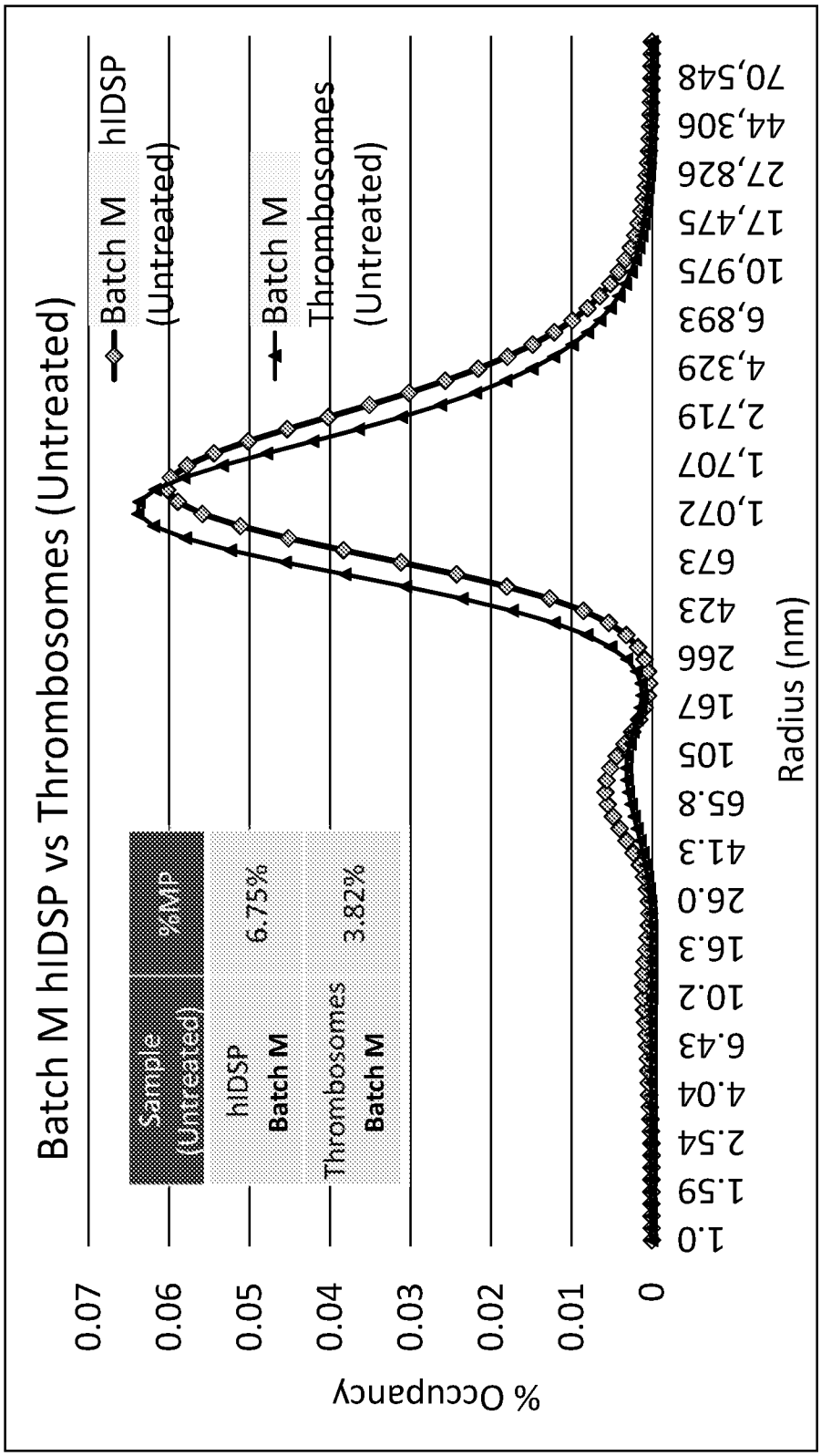

Batch M hIDSP vs Thrombosomes (Untreated)
Batch M hIDSP (Untreated)
Batch M Thrombosomes (Untreated)
Sample (Untreated)
%MP
hIDSP Batch M
6.75%
Thrombosomes Batch M
3.82%
% Occupancy
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Radius (nm)
1.0
1.59
2.54
4.04
6.43
10.2
16.3
26.0
41.3
65.8
105
167
266
423
673
1,072
1,707
2,719
4,329
6,893
10,975
17,475
27,826
44,306
70,548

Fig. 27B

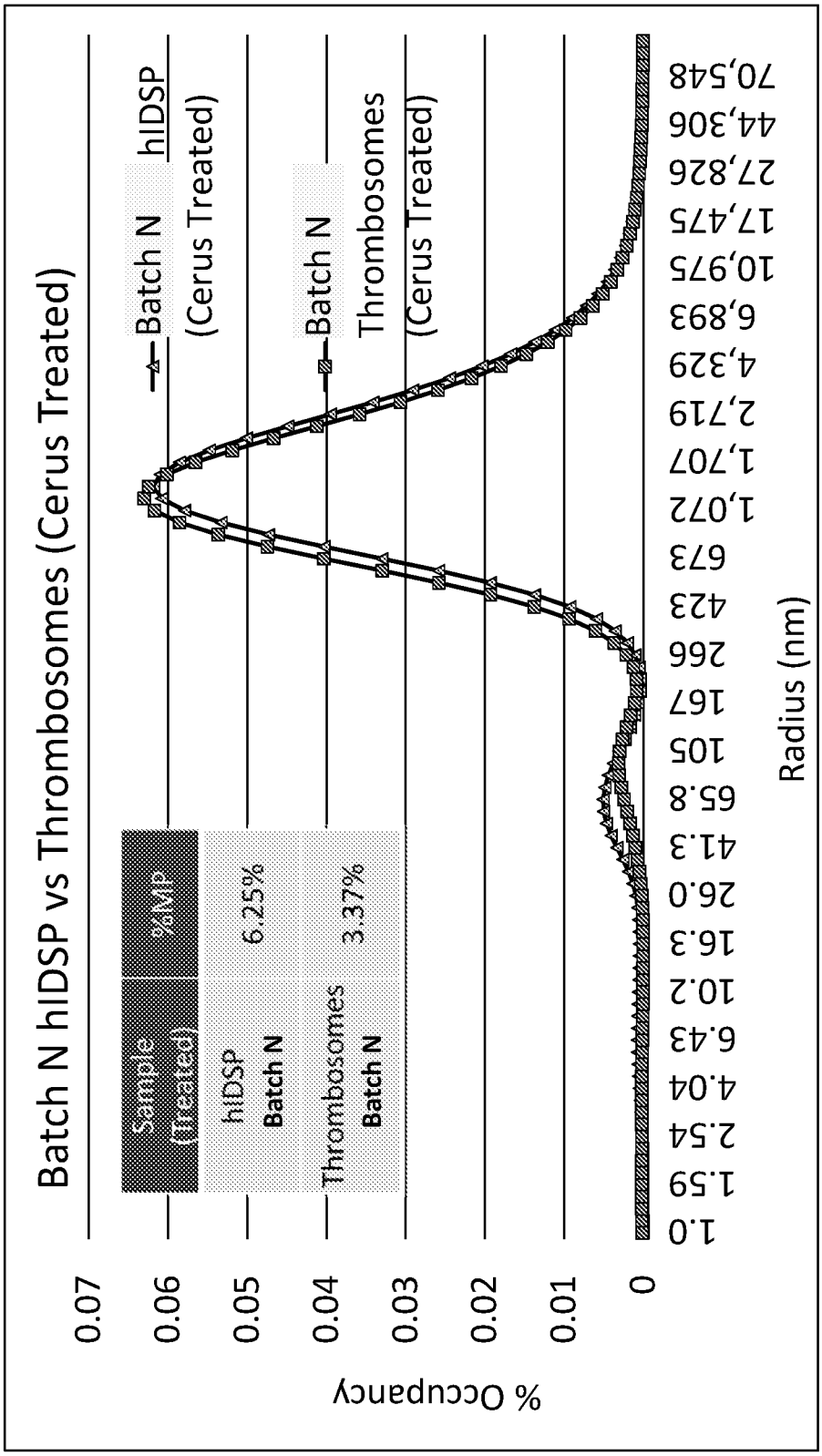

Batch N hIDSP vs Thrombosomes (Cerus Treated)
Sample (Treated)
%MP
hIDSP
Batch N
6.25%
Thrombosomes
Batch N
3.37%
Batch N hIDSP (Cerus Treated)
Batch N Thrombosomes (Cerus Treated)
% Occupancy
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Radius (nm)
1.0
1.59
2.54
4.04
6.43
10.2
16.3
26.0
41.3
65.8
105
167
266
423
673
1,072
1,707
2,719
4,329
6,893
10,975
17,475
27,826
44,306
70,548

Fig. 27C

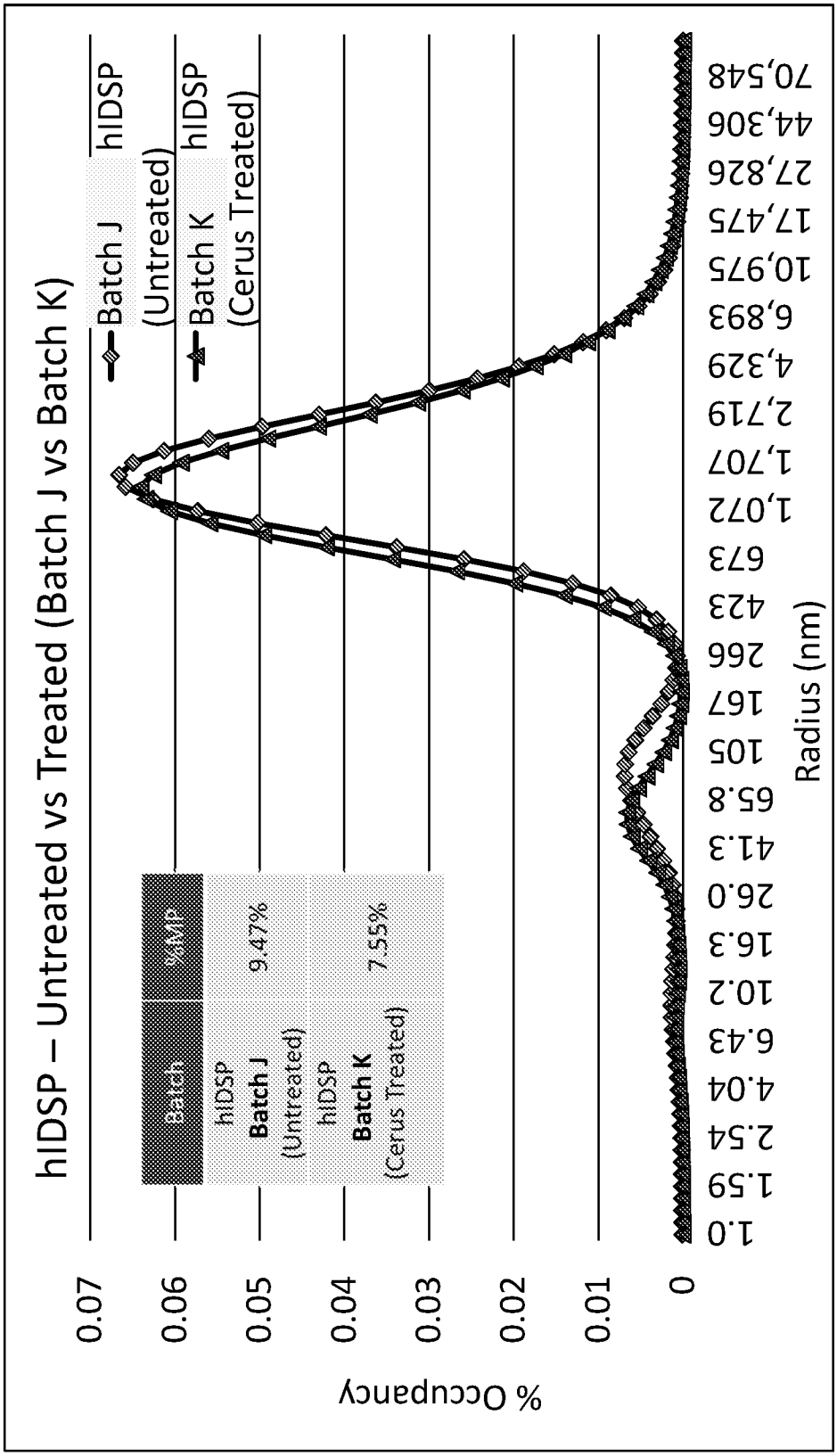

hIDSP – Untreated vs Treated (Batch J vs Batch K)
Batch J hIDSP (Untreated)
Batch K hIDSP (Cerus Treated)
% Occupancy
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Radius (nm)
1.0
1.59
2.54
4.04
6.43
10.2
16.3
26.0
41.3
65.8
105
167
266
423
673
1,072
1,707
2,719
4,329
6,893
10,975
17,475
27,826
44,306
70,548
Batch
AMP
hIDSP Batch J (Untreated) 9.47%
hIDSP Batch K (Cerus Treated) 7.55%

Fig. 28A

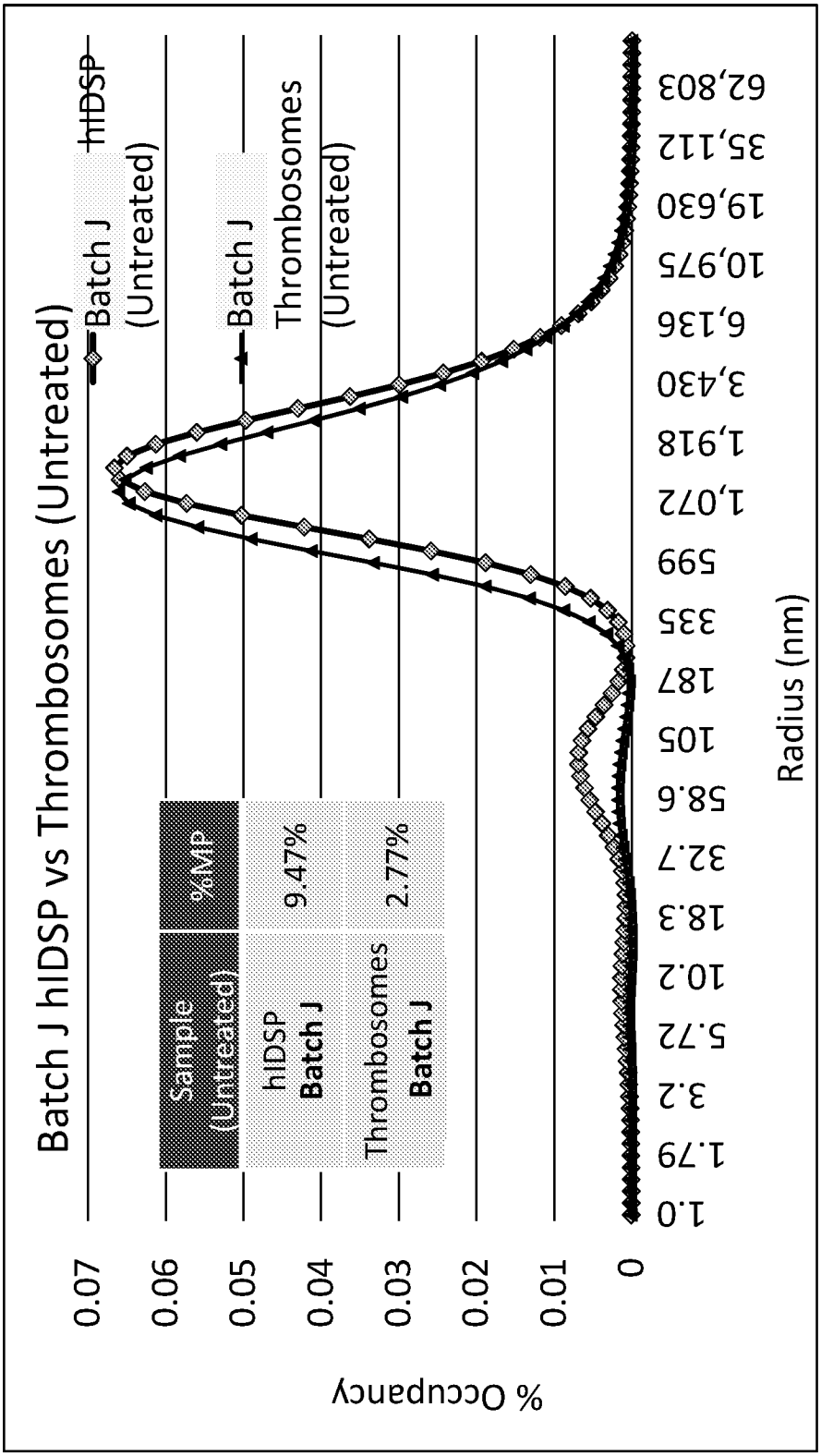

Batch J hIDSP vs Thrombosomes (Untreated)
hIDSP Batch J (Untreated)
Thrombosomes Batch J (Untreated)
% Occupancy
Radius (nm)
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
1.0
1.79
3.2
5.72
10.2
18.3
32.7
58.6
105
187
335
599
1,072
1,918
3,430
6,136
10,975
19,630
35,112
62,803
Sample (Untreated)
%MP
hIDSP Batch J
9.47%
Thrombosomes Batch J
2.77%

**Fig. 28B**

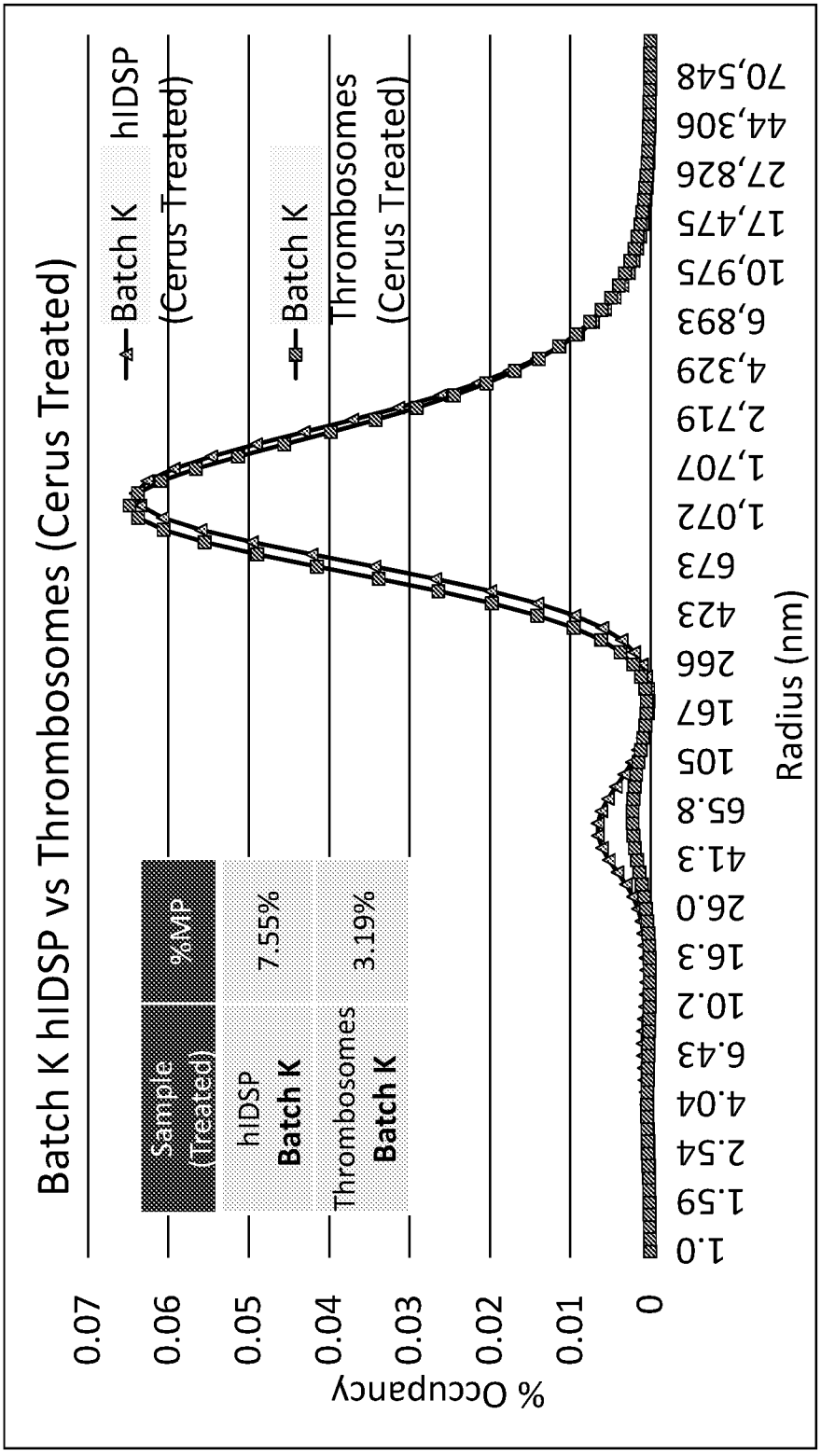

Batch K hIDSP vs Thrombosomes (Cerus Treated)
Batch K hIDSP (Cerus Treated)
Batch K Thrombosomes (Cerus Treated)
Sample (Treated)
%MP
hIDSP Batch K
7.55%
Thrombosomes Batch K
3.19%
% Occupancy
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Radius (nm)
1.0
1.59
2.54
4.04
6.43
10.2
16.3
26.0
41.3
65.8
105
167
266
423
673
1,072
1,707
2,719
4,329
6,893
10,975
17,475
27,826
44,306
70,548

Fig. 28C

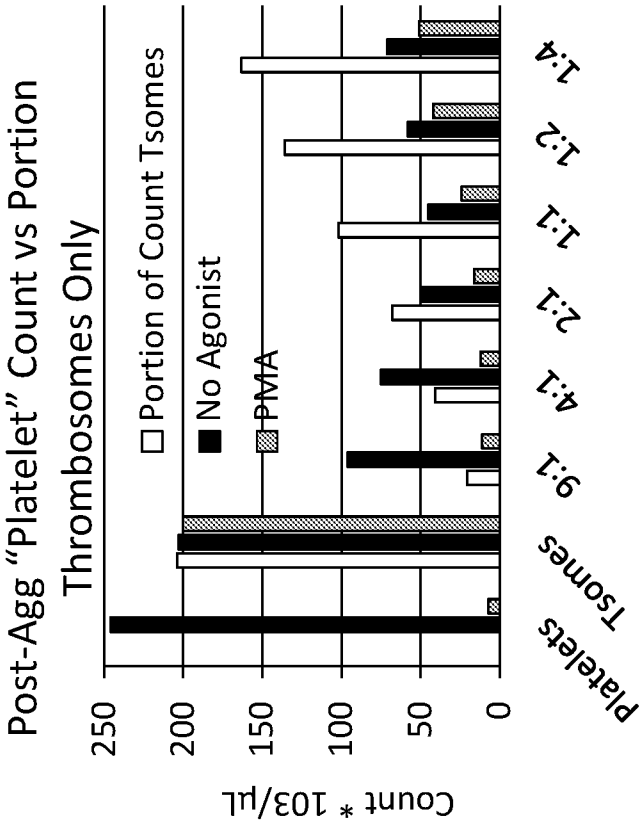



Fig. 29B

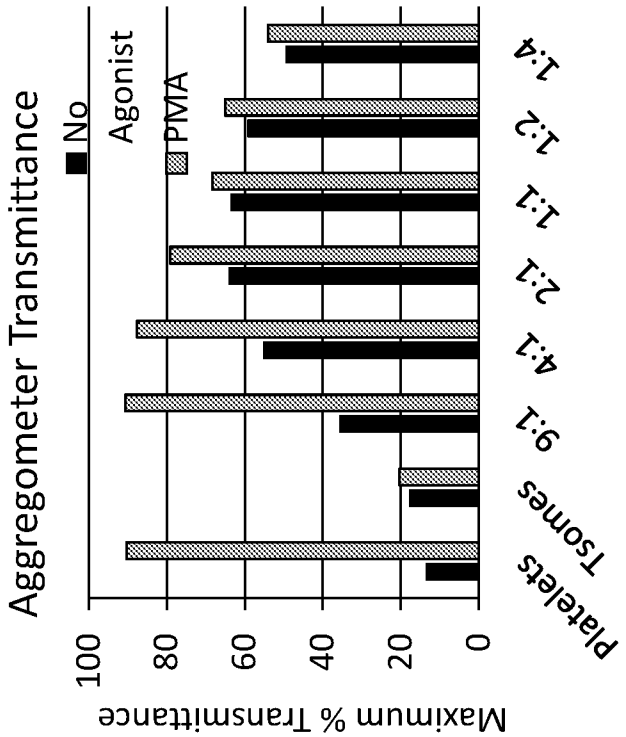



Fig. 29A

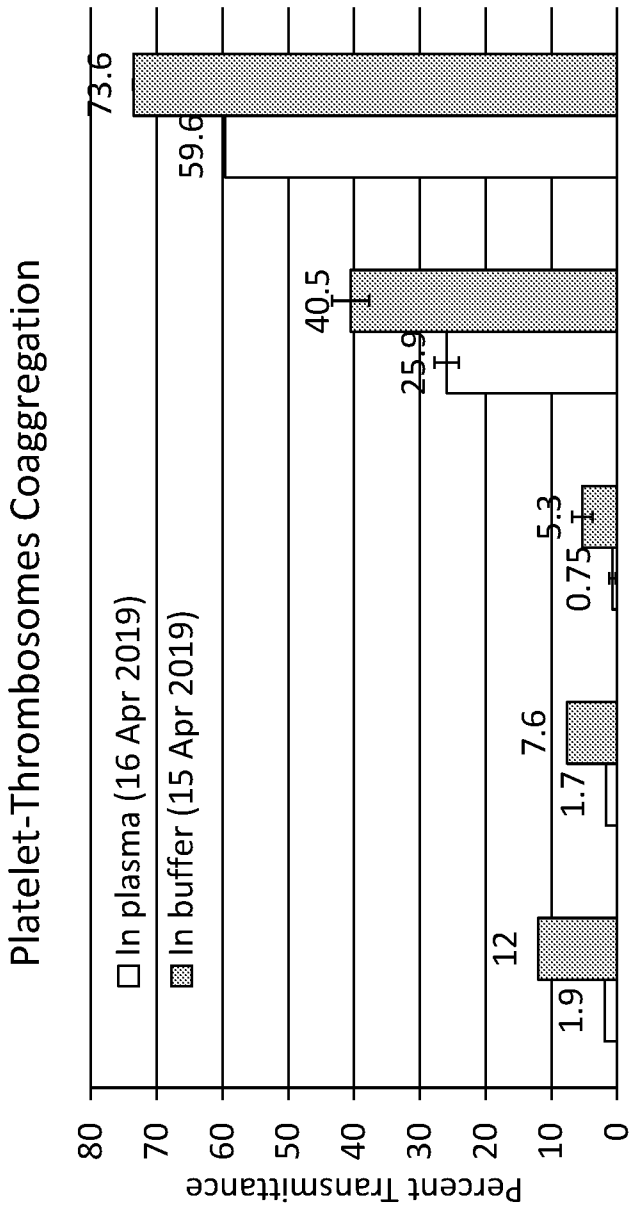

Platelet-Thrombosomes Coaggregation
In plasma (16 Apr 2019)
In buffer (15 Apr 2019)
Percent Transmittance
0
10
20
30
40
50
60
70
80
Platelets
Thrombosomes
Mixed NO Shear
Mixed WITH Shear
Mixed PMA
1.9
12
1.7
7.6
0.75
5.3
40.5
25.9
59.6
73.6

Fig. 29C

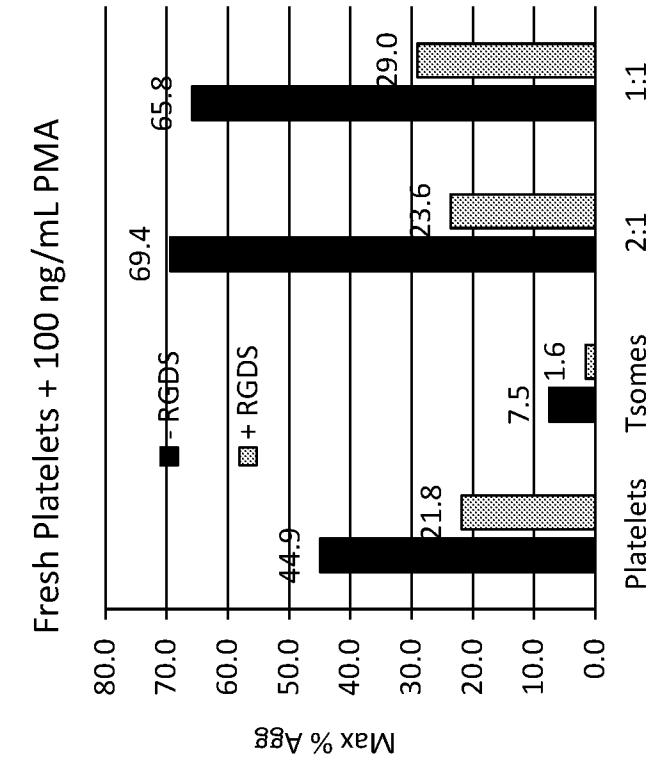

Fresh Platelets + 100 ng/mL PMA
Max % Agg
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
- RGDS
+ RGDS
69.4
65.8
44.9
29.0
23.6
21.8
7.5
1.6
Platelets
Tsomes
2:1
1:1

Fig. 31

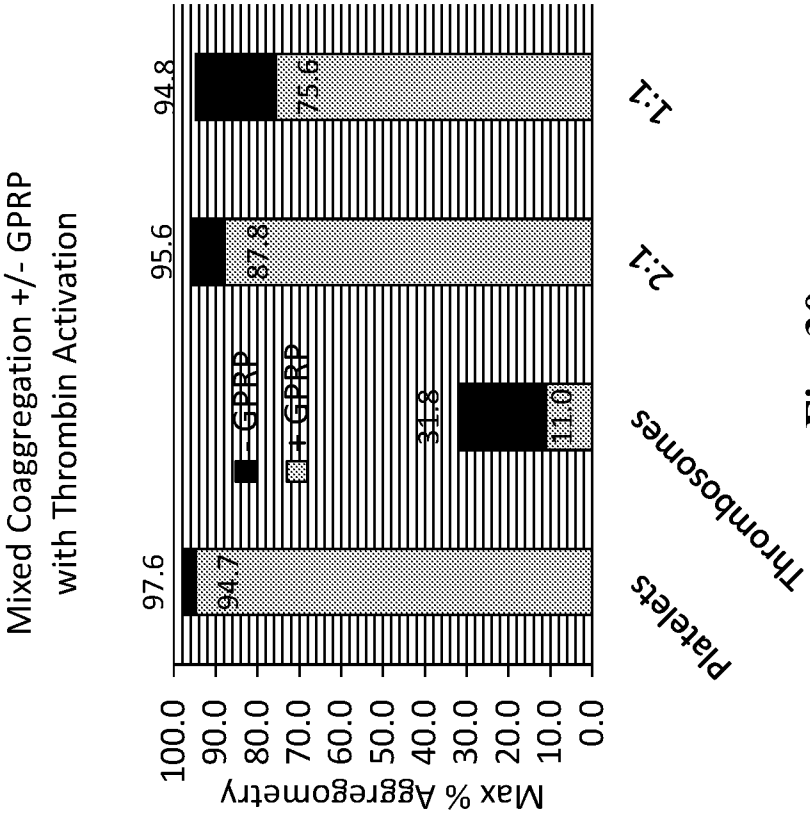

Mixed Coaggregation +/- GPRP
with Thrombin Activation
Max % Aggregometry
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
- GPRP
+ GPRP
97.6
94.7
95.6
87.8
94.8
75.6
31.8
11.0
Platelets
Thromboosomes
2:1
1:1

Fig. 30

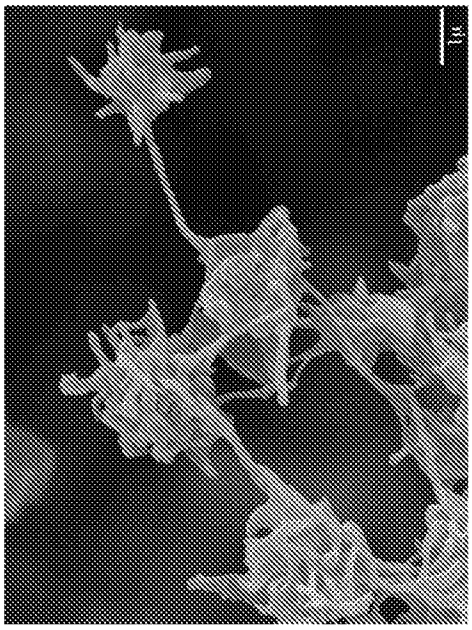


Fig. 32B

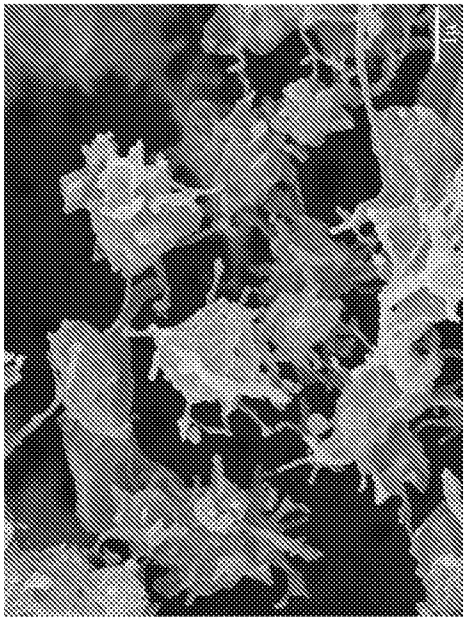


Fig. 32D

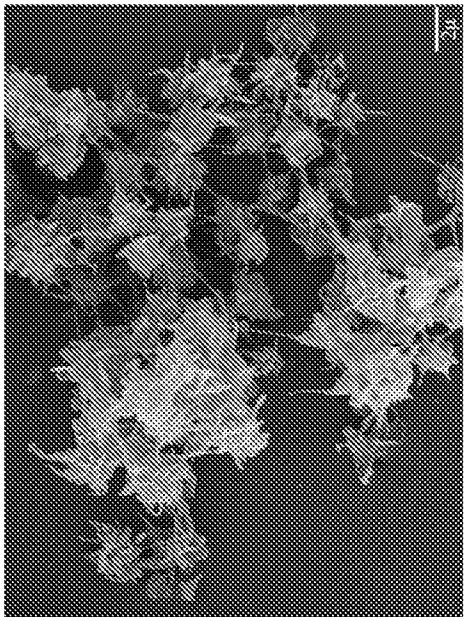


Fig. 32A

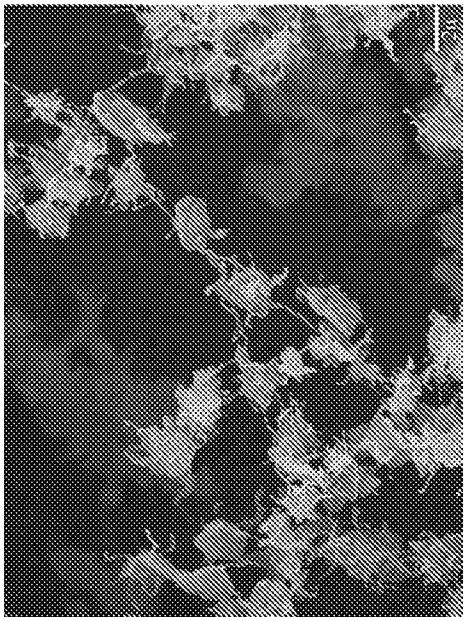


Fig. 32C

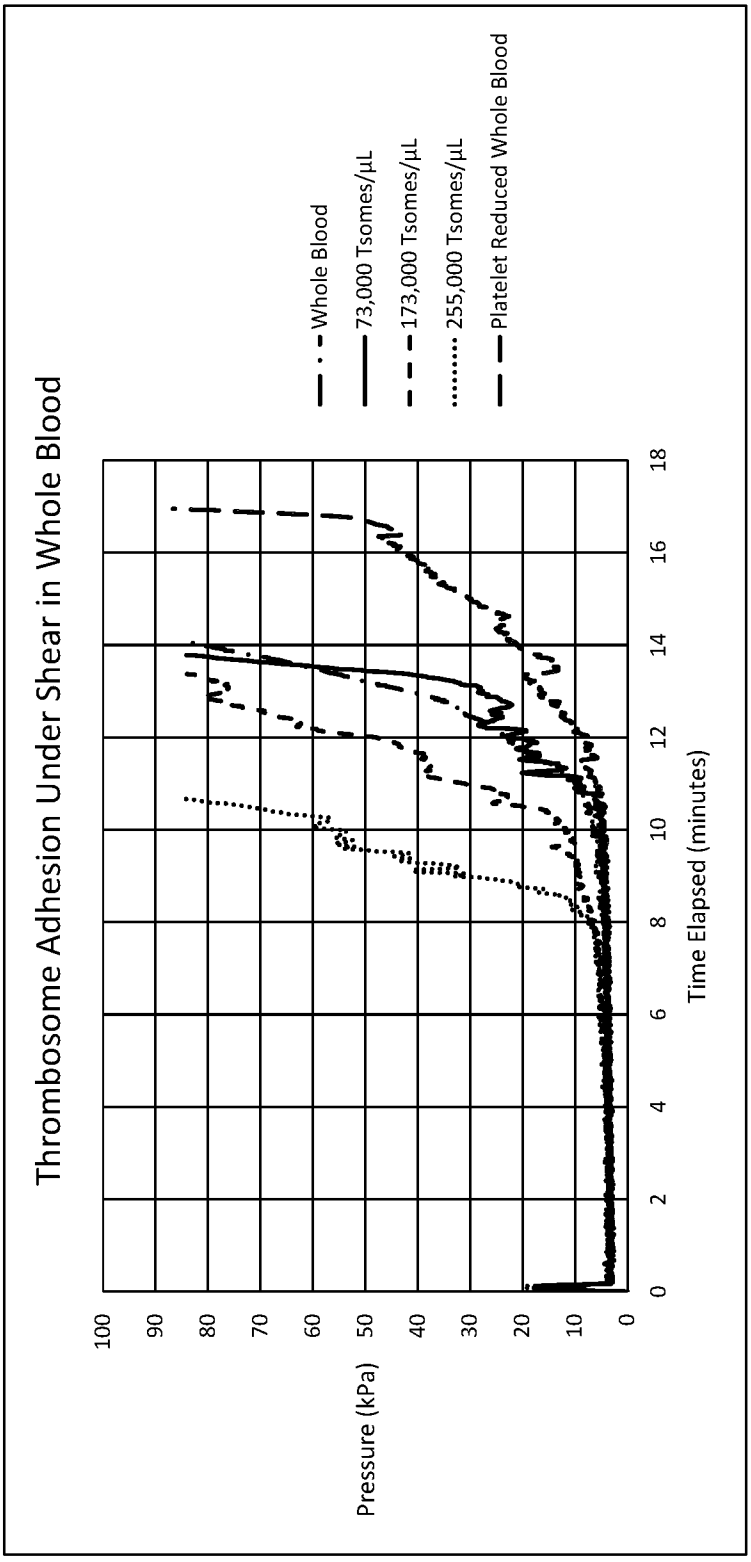

Thrombosome Adhesion Under Shear in Whole Blood
Whole Blood
73,000 Tsomes/µL
173,000 Tsomes/µL
255,000 Tsomes/µL
Platelet Reduced Whole Blood
Pressure (kPa)
100
90
80
70
60
50
40
30
20
10
0
Time Elapsed (minutes)
0
2
4
6
8
10
12
14
16
18

Fig. 33A

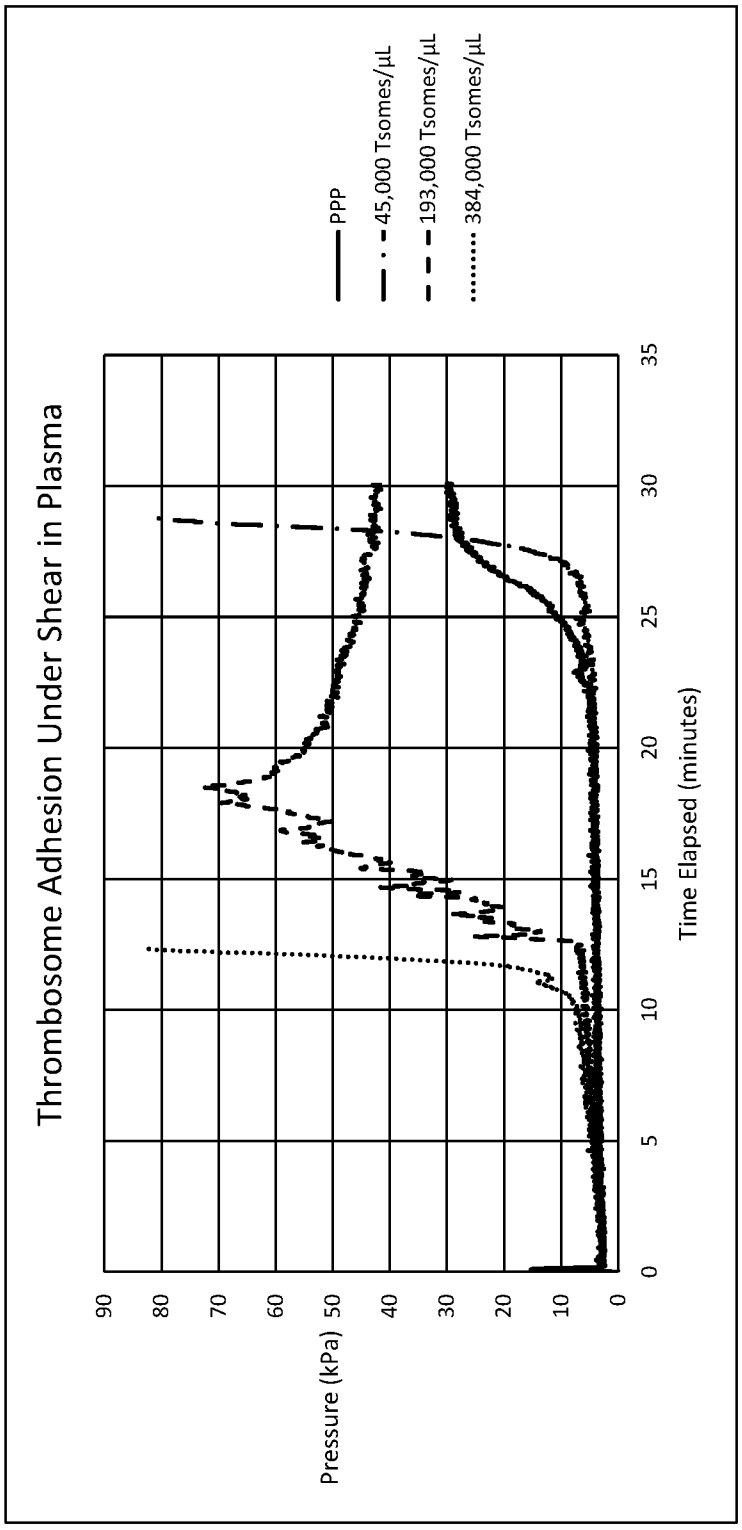

Thrombosome Adhesion Under Shear in Plasma
PPP
45,000 Tsomes/µL
193,000 Tsomes/µL
384,000 Tsomes/µL
Pressure (kPa)
0
10
20
30
40
50
60
70
80
90
Time Elapsed (minutes)
0
5
10
15
20
25
30
35

Fig. 33B

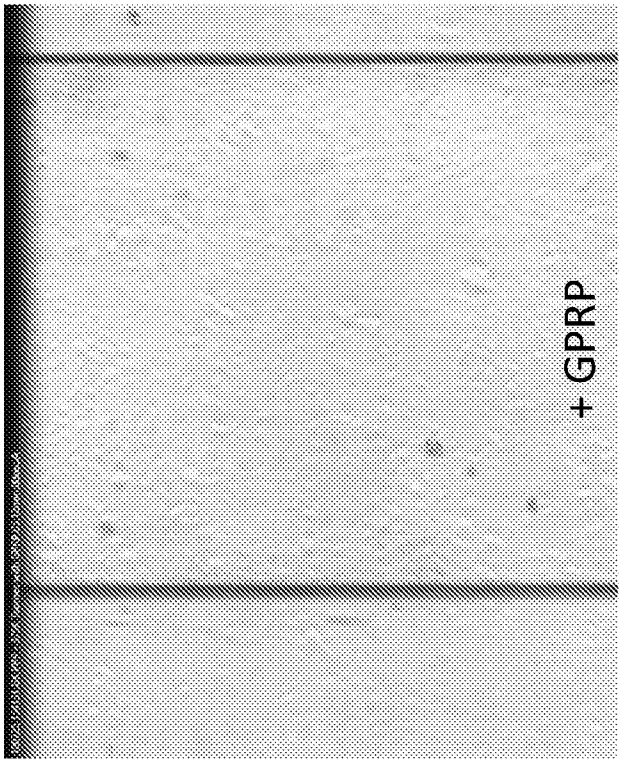



Fig. 33D

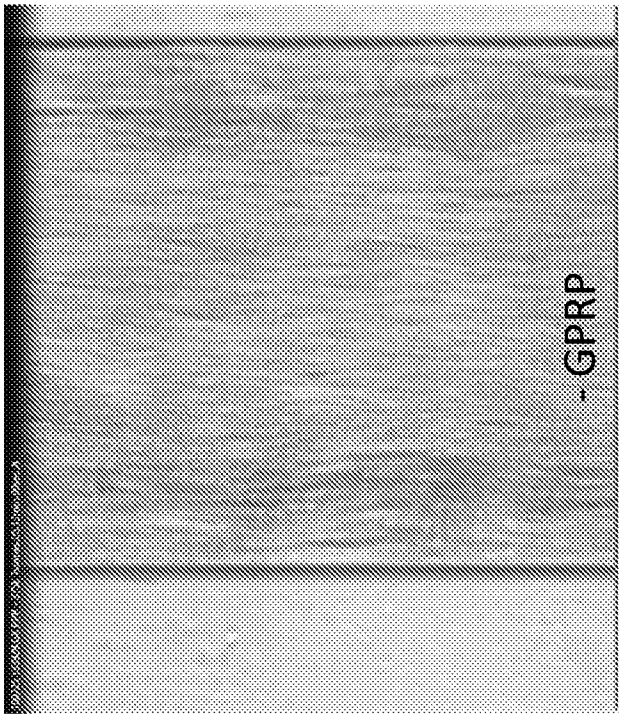



Fig. 33C

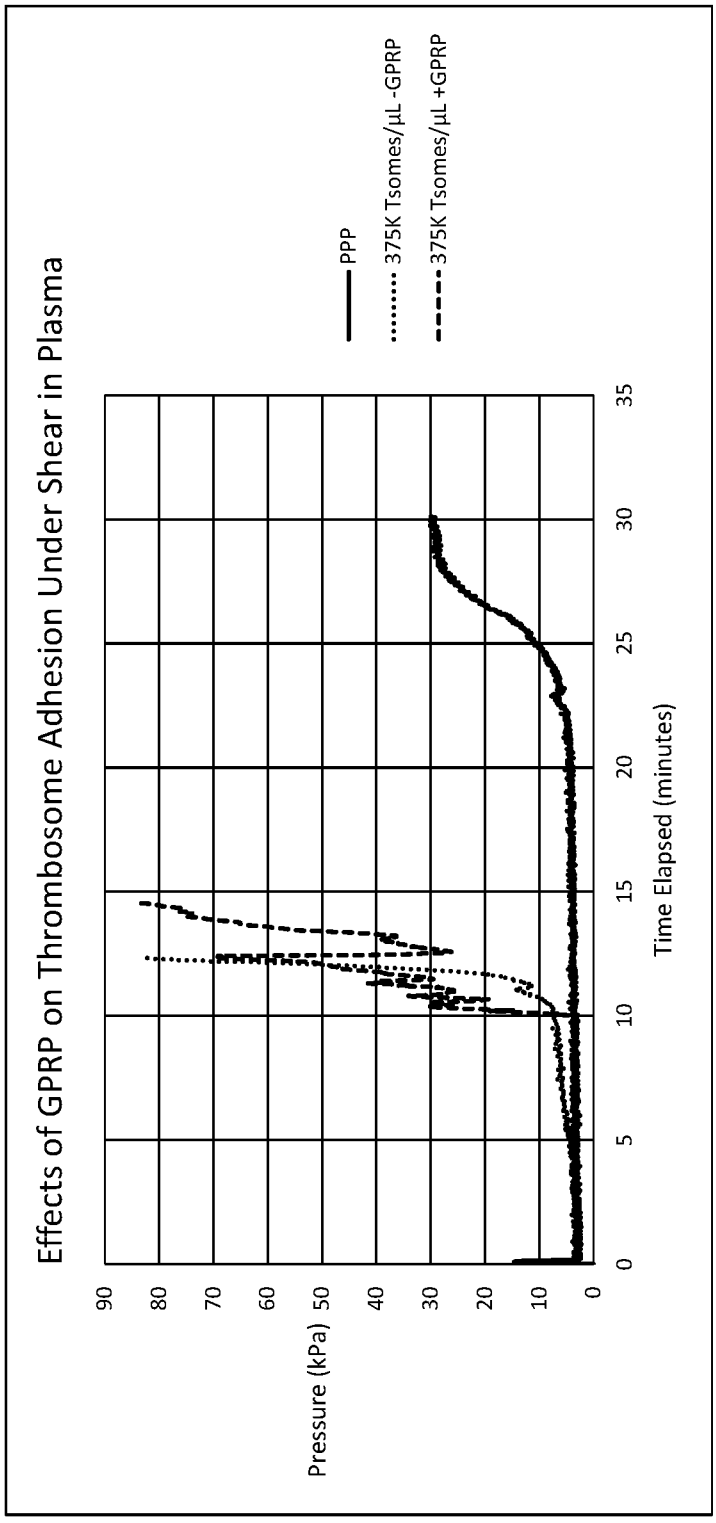

Effects of GPRP on Thrombosome Adhesion Under Shear in Plasma
PPP
375K Tsomes/µL -GPRP
375K Tsomes/µL +GPRP
Pressure (kPa)
0
10
20
30
40
50
60
70
80
90
Time Elapsed (minutes)
0
5
10
15
20
25
30
35

Fig. 33E