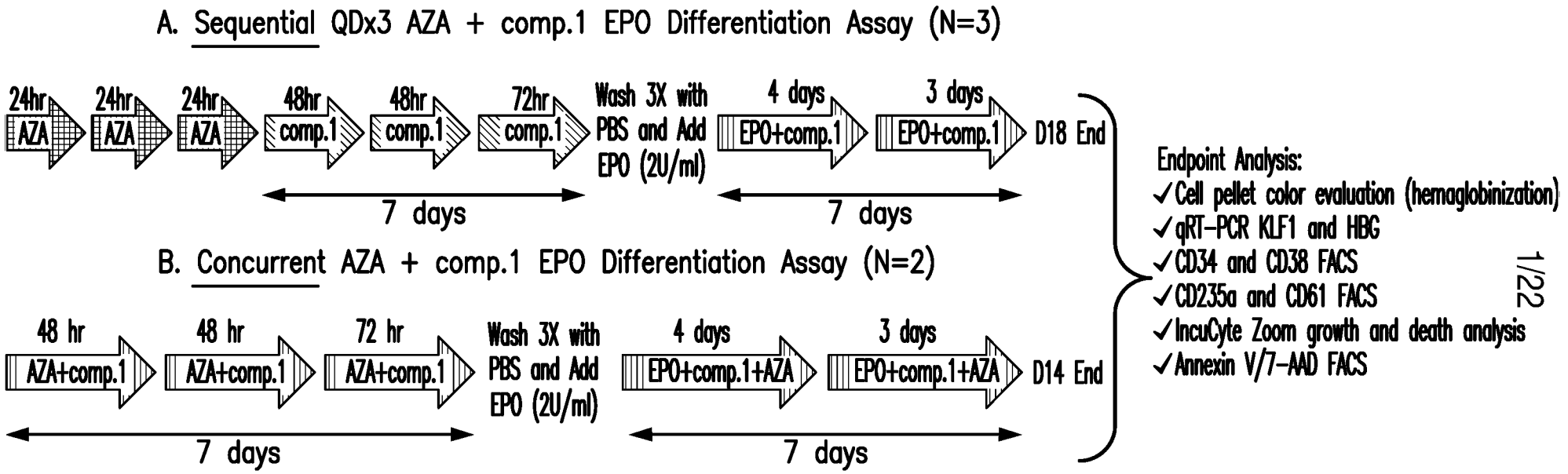
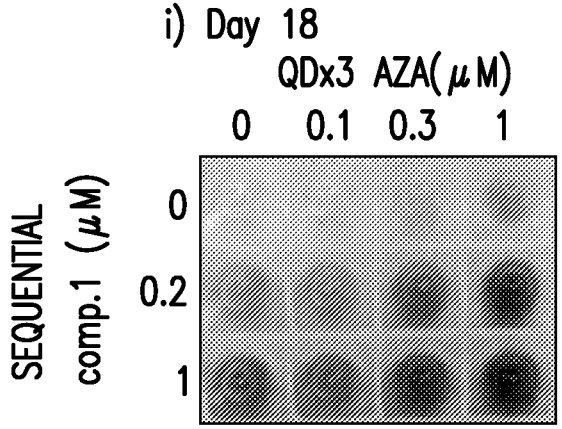
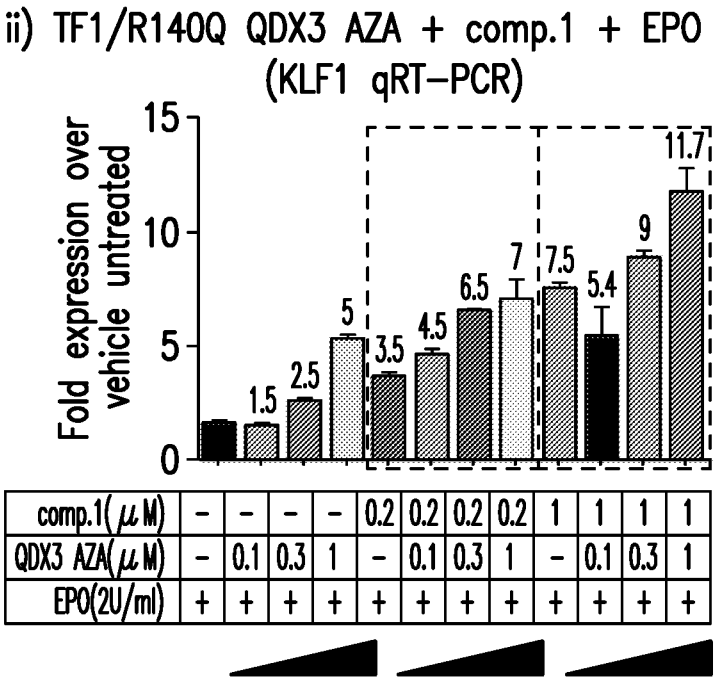

A. Sequential QDx3 AZA + comp.1 EPO Differentiation Assay (N=3)
24hr AZA
24hr AZA
24hr AZA
48hr comp.1
48hr comp.1
72hr comp.1
7 days
Wash 3X with PBS and Add EPO (2U/ml)
4 days EPO+comp.1
3 days EPO+comp.1
7 days
D18 End
B. Concurrent AZA + comp.1 EPO Differentiation Assay (N=2)
48 hr AZA+comp.1
48 hr AZA+comp.1
72 hr AZA+comp.1
7 days
Wash 3X with PBS and Add EPO (2U/ml)
4 days EPO+comp.1+AZA
3 days EPO+comp.1+AZA
7 days
D14 End
Endpoint Analysis:
✓Cell pellet color evaluation (hemaglobinization)
✓qRT-PCR KLF1 and HBG
✓CD34 and CD38 FACS
✓CD235a and CD61 FACS
✓IncuCyte Zoom growth and death analysis
✓Annexin V/7-AAD FACS

FIG. 1


i) Day 18
QDx3 AZA(μM)
0 0.1 0.3 1
SEQUENTIAL comp.1 (μM)
0
0.2
1


ii) TF1/R140Q QDX3 AZA + comp.1 + EPO Day 18
(KLF1 qRT-PCR)
Fold expression over vehicle untreated
15
10
5
0
1.5
2.5
5
3.5
4.5
6.5
7
7.5
5.4
9
11.7
comp.1(μM) - - - - 0.2 0.2 0.2 0.2 1 1 1 1
QDX3 AZA(μM) - 0.1 0.3 1 - 0.1 0.3 1 - 0.1 0.3 1
EPO(2U/ml) + + + + + + + + + + + +

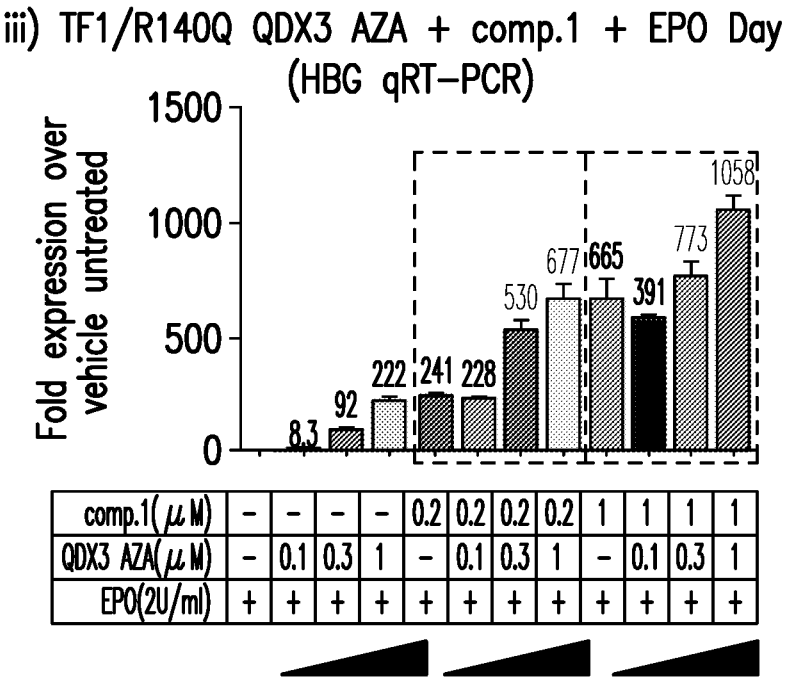

iii) TF1/R140Q QDX3 AZA + comp.1 + EPO Day 18
(HBG qRT-PCR)
Fold expression over vehicle untreated
1500
1000
500
0
8.3
92
222
241
228
530
677
665
391
773
1058
comp.1(μM) - - - - 0.2 0.2 0.2 0.2 1 1 1 1
QDX3 AZA(μM) - 0.1 0.3 1 - 0.1 0.3 1 - 0.1 0.3 1
EPO(2U/ml) + + + + + + + + + + + +

FIG. 2A

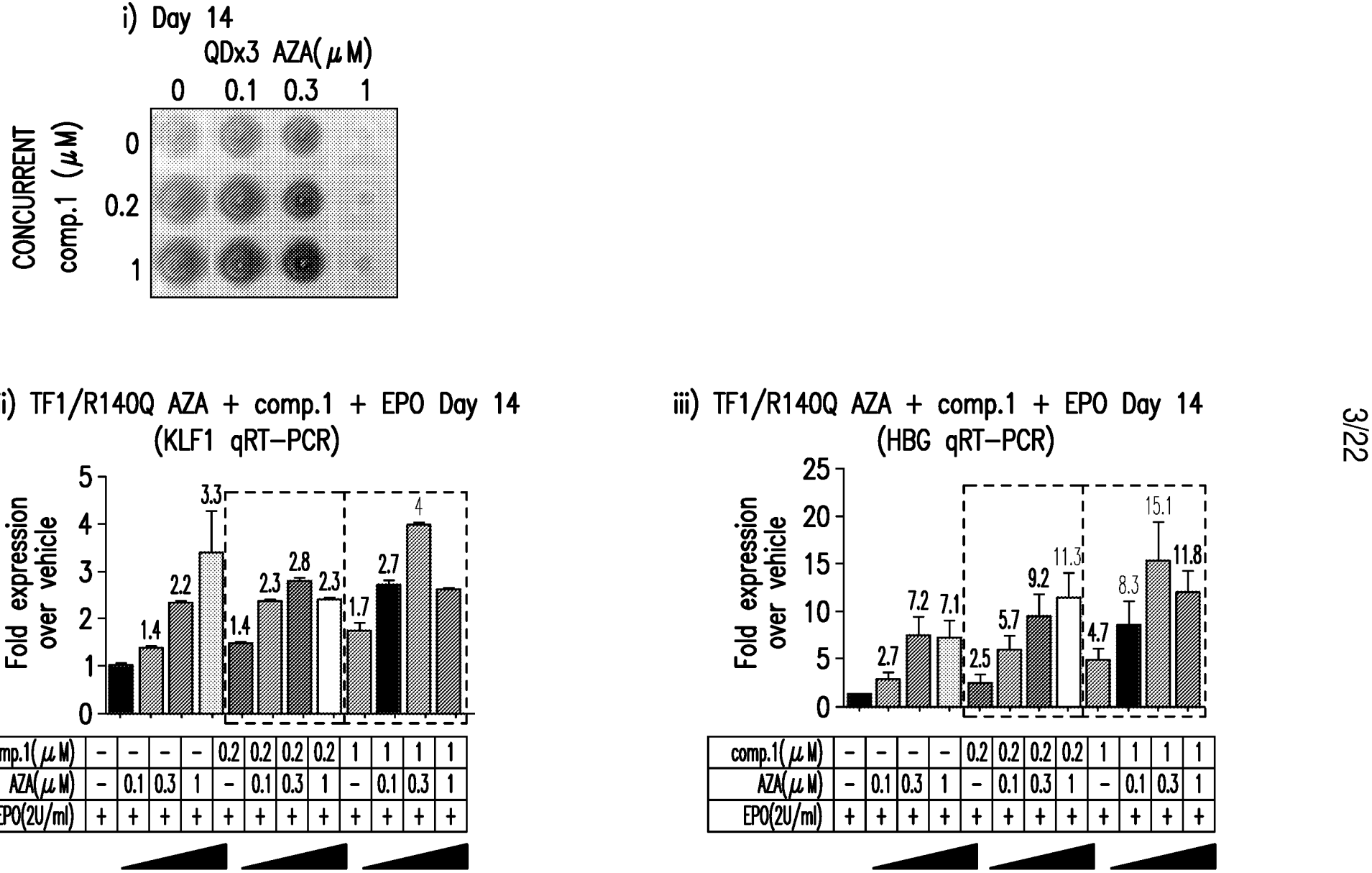

i) Day 14
QDx3 AZA(μM)
0 0.1 0.3 1
CONCURRENT comp.1 (μM)
0
0.2
1
ii) TF1/R140Q AZA + comp.1 + EPO Day 14
(KLF1 qRT-PCR)
Fold expression over vehicle
0 1 2 3 4 5
1.4 2.2 3.3 1.4 2.3 2.8 2.3 1.7 2.7 4
comp.1(μM) - - - - 0.2 0.2 0.2 0.2 1 1 1 1
AZA(μM) - 0.1 0.3 1 - 0.1 0.3 1 - 0.1 0.3 1
EPO(2U/ml) + + + + + + + + + + + +
iii) TF1/R140Q AZA + comp.1 + EPO Day 14
(HBG qRT-PCR)
Fold expression over vehicle
0 5 10 15 20 25
2.7 7.2 7.1 2.5 5.7 9.2 11.3 4.7 8.3 15.1 11.8
comp.1(μM) - - - - 0.2 0.2 0.2 0.2 1 1 1 1
AZA(μM) - 0.1 0.3 1 - 0.1 0.3 1 - 0.1 0.3 1
EPO(2U/ml) + + + + + + + + + + + +

FIG. 2B

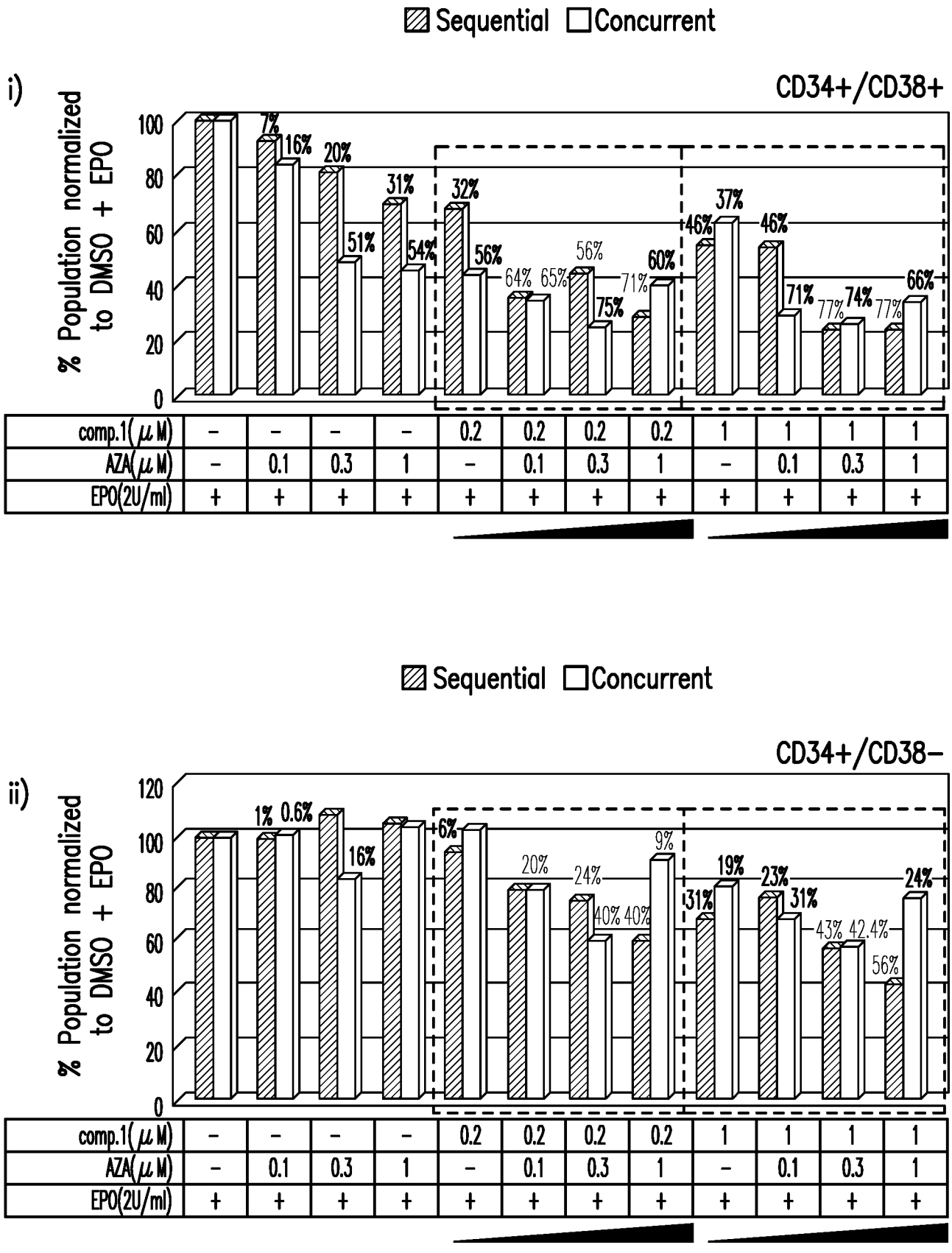

Sequential
Concurrent
i)
CD34+/CD38+
% Population normalized to DMSO + EPO
100
80
60
40
20
0
7%
16%
20%
51%
31%
54%
32%
56%
64%
65%
56%
75%
71%
60%
46%
37%
46%
71%
77%
74%
77%
66%
comp.1(μM) – – – – 0.2 0.2 0.2 0.2 1 1 1 1
AZA(μM) – 0.1 0.3 1 – 0.1 0.3 1 – 0.1 0.3 1
EPO(2U/ml) + + + + + + + + + + + +
Sequential
Concurrent
ii)
CD34+/CD38−
% Population normalized to DMSO + EPO
120
100
80
60
40
20
0
1%
0.6%
16%
6%
20%
24%
40%
40%
9%
31%
19%
23%
31%
43%
42.4%
56%
24%
comp.1(μM) – – – – 0.2 0.2 0.2 0.2 1 1 1 1
AZA(μM) – 0.1 0.3 1 – 0.1 0.3 1 – 0.1 0.3 1
EPO(2U/ml) + + + + + + + + + + + +

FIG. 3

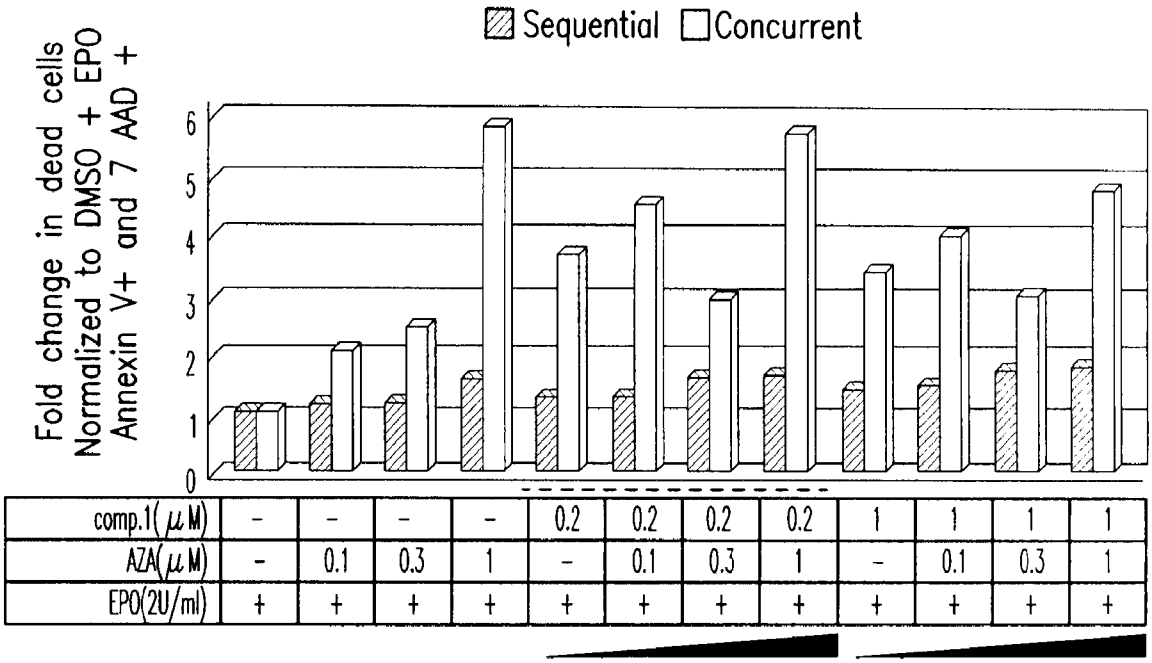

Sequential
Concurrent
Fold change in dead cells
Normalized to DMSO + EPO
Annexin V+ and 7 AAD +
0
1
2
3
4
5
6
comp.1(μM) - - - - 0.2 0.2 0.2 0.2 1 1 1 1
AZA(μM) - 0.1 0.3 1 - 0.1 0.3 1 - 0.1 0.3 1
EPO(2U/ml) + + + + + + + + + + + +

FIG. 4

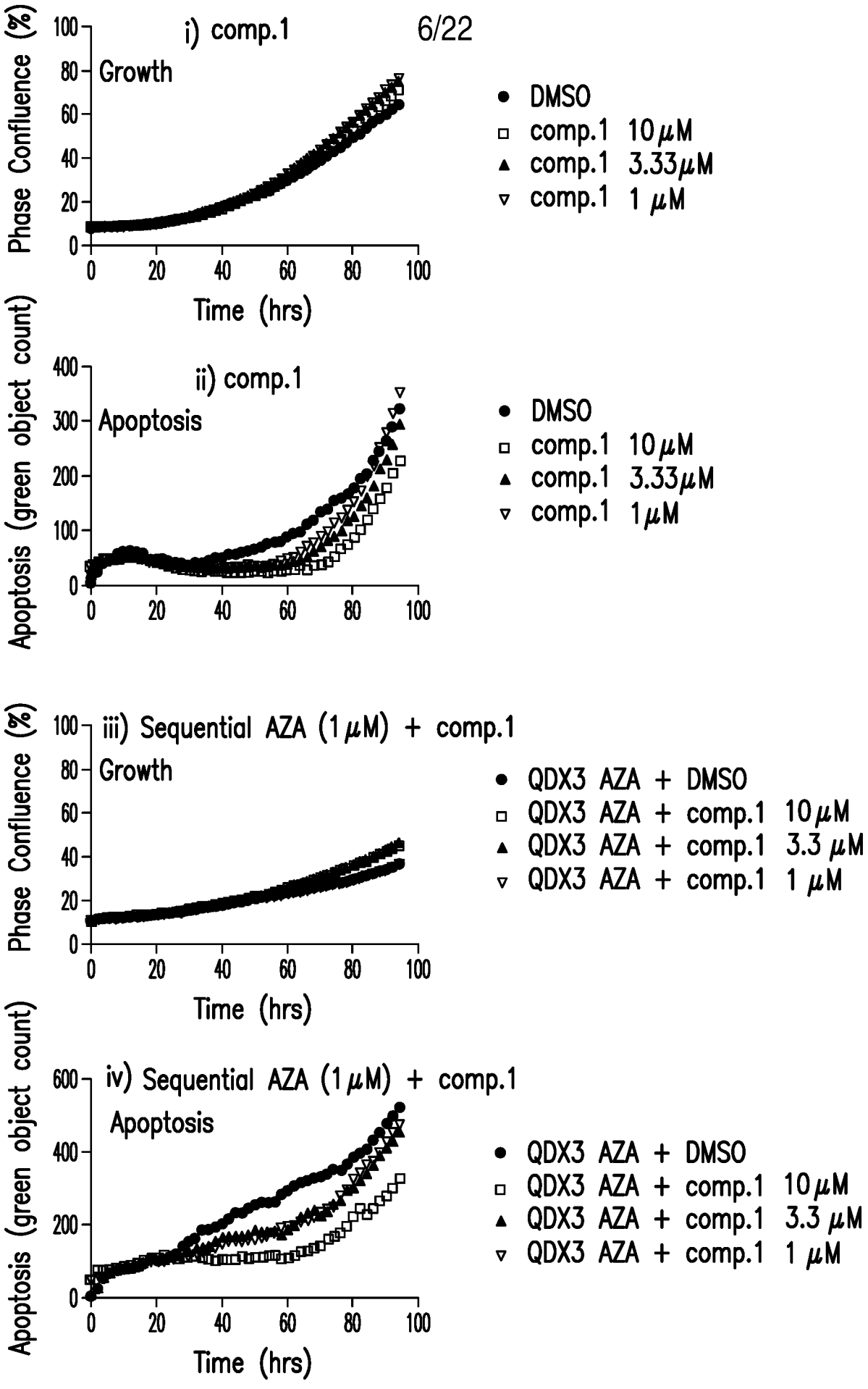

i) comp.1
Growth
Phase Confluence (%)
Time (hrs)
DMSO
comp.1 10μM
comp.1 3.33μM
comp.1 1 μM
ii) comp.1
Apoptosis
Apoptosis (green object count)
Time (hrs)
DMSO
comp.1 10μM
comp.1 3.33μM
comp.1 1μM
iii) Sequential AZA (1μM) + comp.1
Growth
Phase Confluence (%)
Time (hrs)
QDX3 AZA + DMSO
QDX3 AZA + comp.1 10μM
QDX3 AZA + comp.1 3.3 μM
QDX3 AZA + comp.1 1 μM
iv) Sequential AZA (1μM) + comp.1
Apoptosis
Apoptosis (green object count)
Time (hrs)
QDX3 AZA + DMSO
QDX3 AZA + comp.1 10μM
QDX3 AZA + comp.1 3.3 μM
QDX3 AZA + comp.1 1 μM

FIG. 5

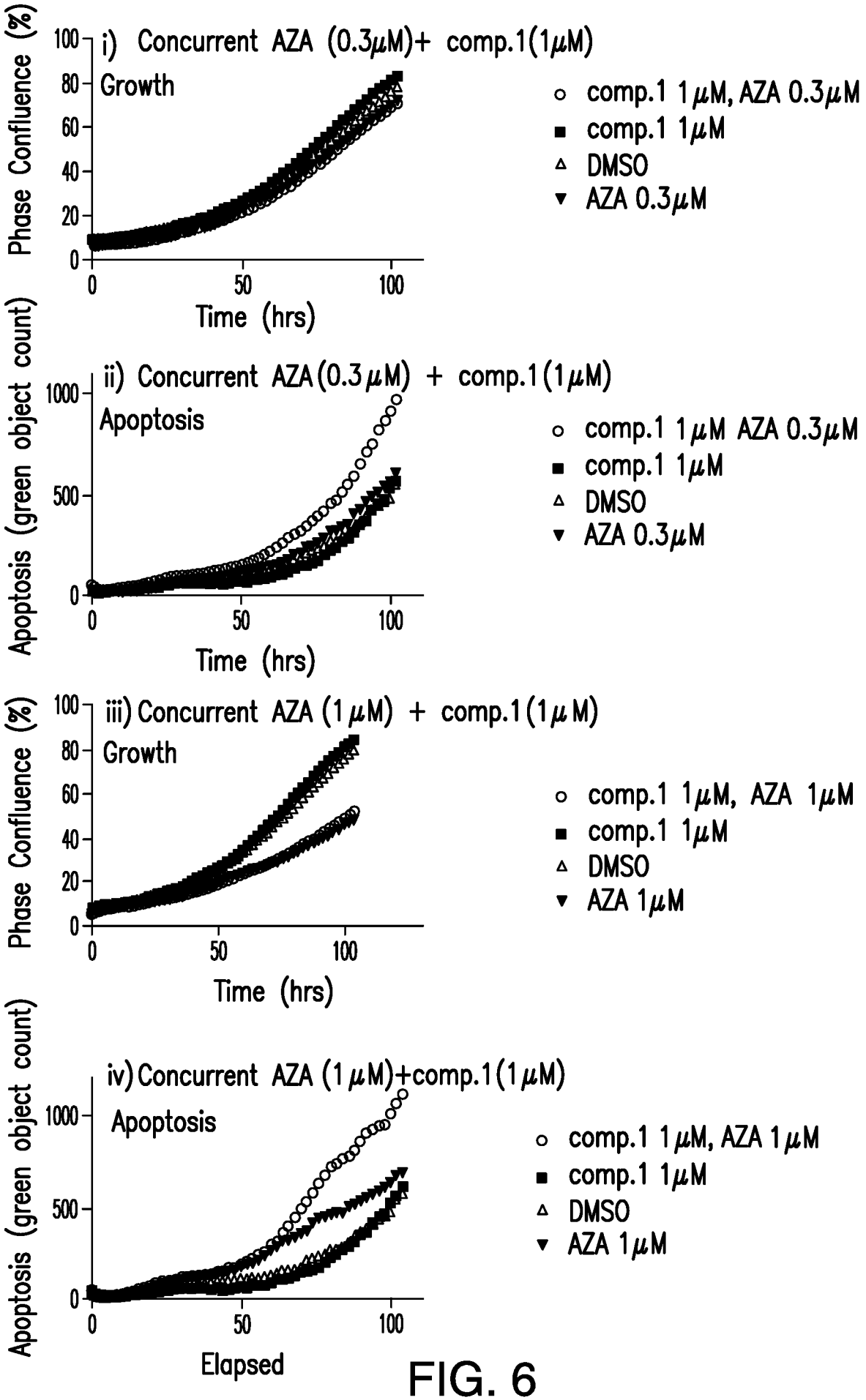

i) Concurrent AZA (0.3μM)+ comp.1(1μM)
Growth
Phase Confluence (%)
Time (hrs)
comp.1 1μM, AZA 0.3μM
comp.1 1μM
DMSO
AZA 0.3μM
ii) Concurrent AZA(0.3μM) + comp.1(1μM)
Apoptosis
Apoptosis (green object count)
Time (hrs)
comp.1 1μM AZA 0.3μM
comp.1 1μM
DMSO
AZA 0.3μM
iii) Concurrent AZA (1μM) + comp.1(1μM)
Growth
Phase Confluence (%)
Time (hrs)
comp.1 1μM, AZA 1μM
comp.1 1μM
DMSO
AZA 1μM
iv) Concurrent AZA (1μM)+comp.1(1μM)
Apoptosis
Apoptosis (green object count)
Elapsed
comp.1 1μM, AZA 1μM
comp.1 1μM
DMSO
AZA 1μM

FIG. 6

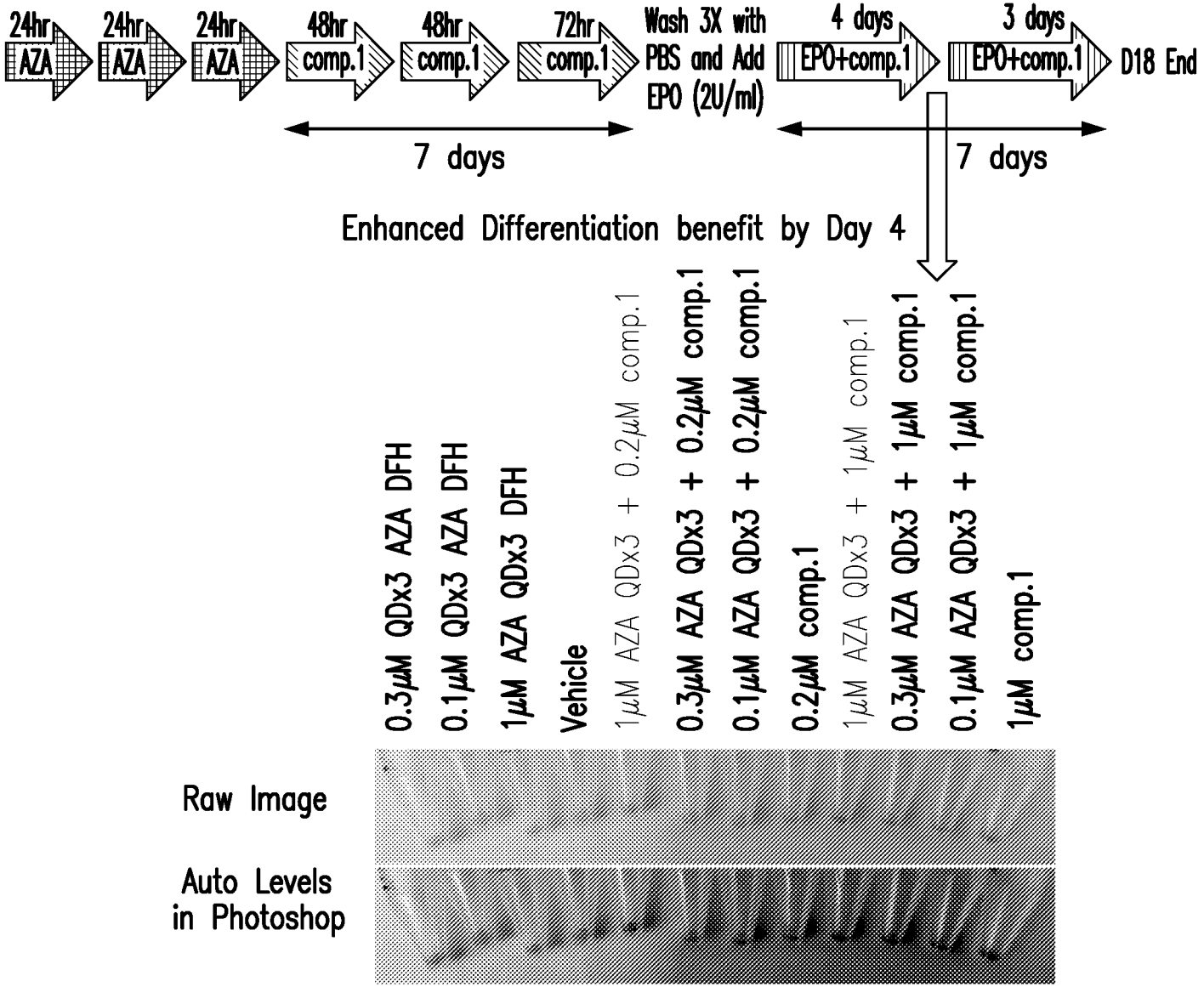

24hr AZA
24hr AZA
24hr AZA
48hr comp.1
48hr comp.1
72hr comp.1
Wash 3X with PBS and Add EPO (2U/ml)
4 days EPO+comp.1
3 days EPO+comp.1
D18 End
7 days
7 days
Enhanced Differentiation benefit by Day 4
0.3μM QDx3 AZA DFH
0.1μM QDx3 AZA DFH
1μM AZA QDx3 DFH
Vehicle
1μM AZA QDx3 + 0.2μM comp.1
0.3μM AZA QDx3 + 0.2μM comp.1
0.1μM AZA QDx3 + 0.2μM comp.1
0.2μM comp.1
1μM AZA QDx3 + 1μM comp.1
0.3μM AZA QDx3 + 1μM comp.1
0.1μM AZA QDx3 + 1μM comp.1
1μM comp.1
Raw Image
Auto Levels in Photoshop

FIG. 7

$CD34^+/CD38^-$ =Hematopoietic stem cell
$CD34^+/CD38^+$=Hematopoietic progenitor cell

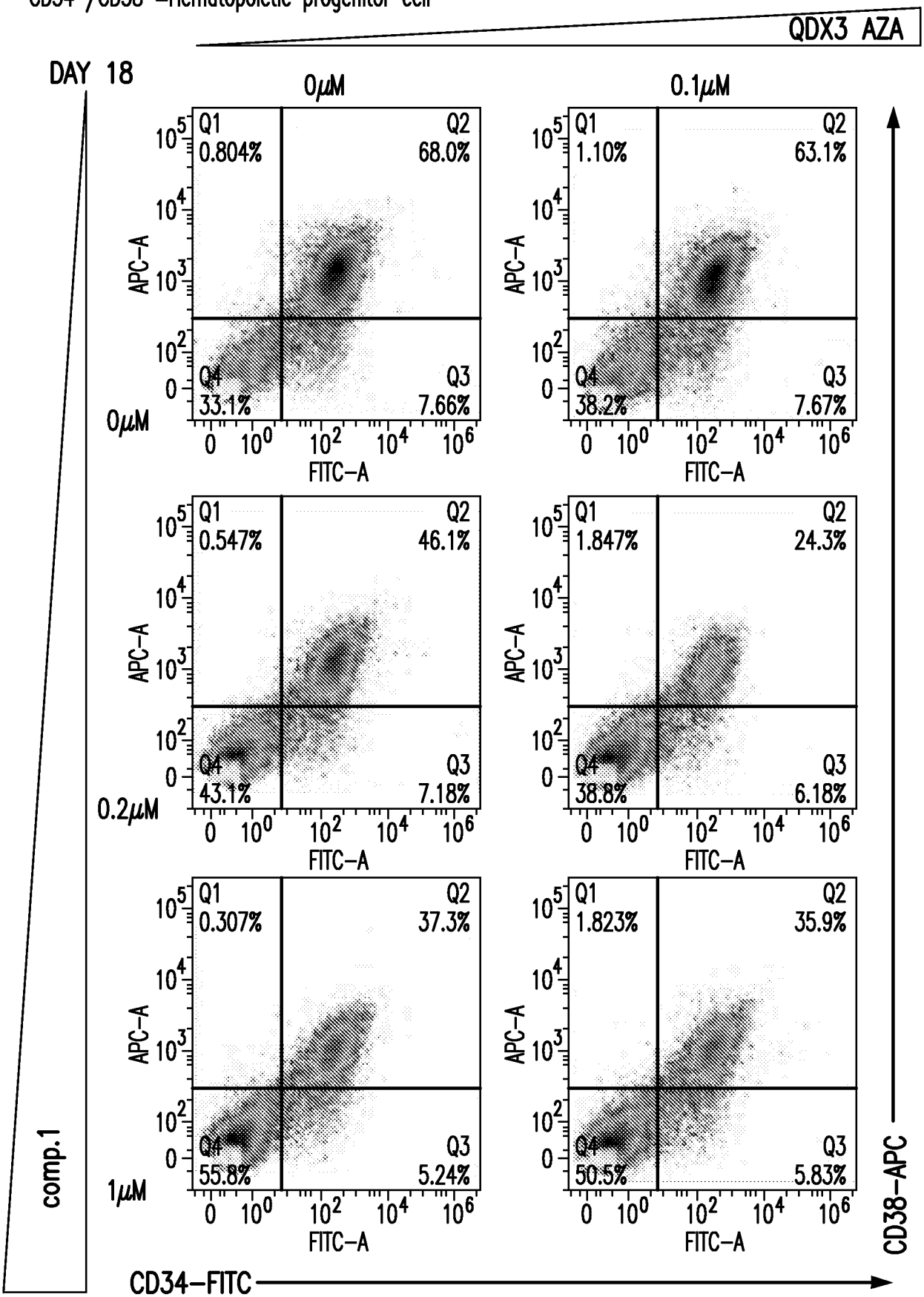



FIG. 8A

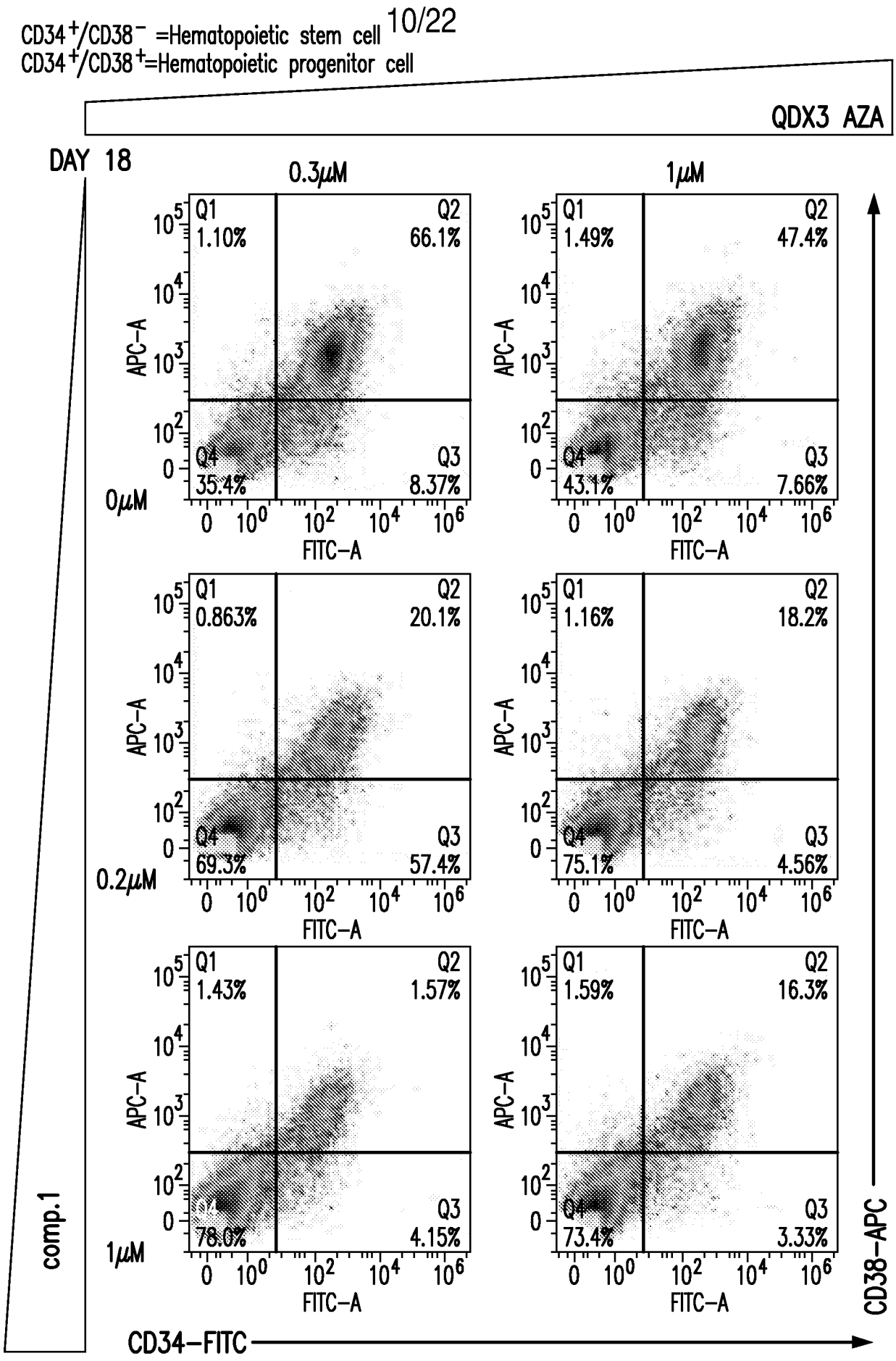



FIG. 8A (continued)

$CD34^+/CD38^-$ =Hematopoietic stem cell
$CD34^+/CD38^+$=Hematopoietic progenitor cell

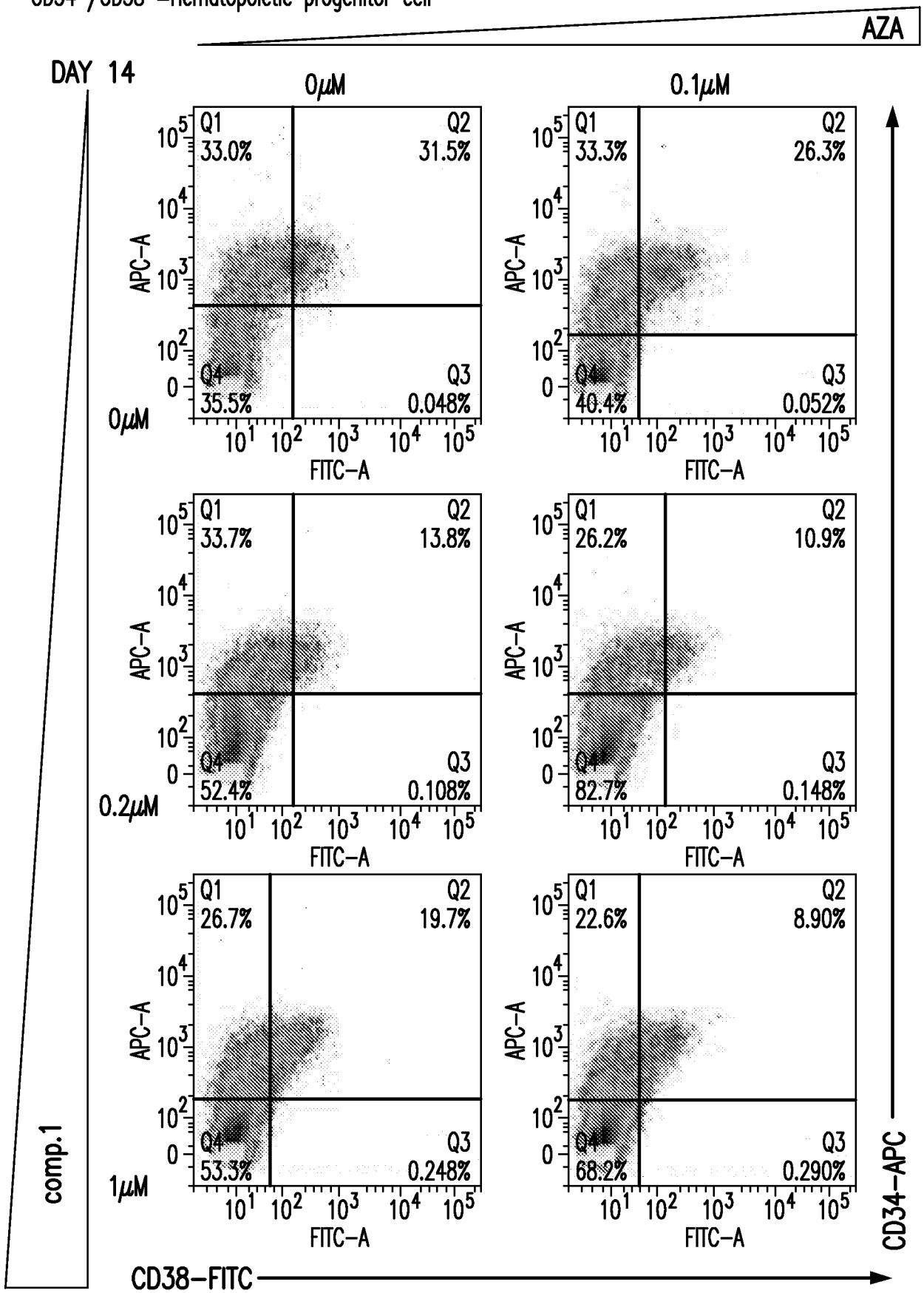



FIG. 8B

$CD34^+/CD38^-$ =Hematopoietic stem cell
$CD34^+/CD38^+$=Hematopoietic progenitor cell

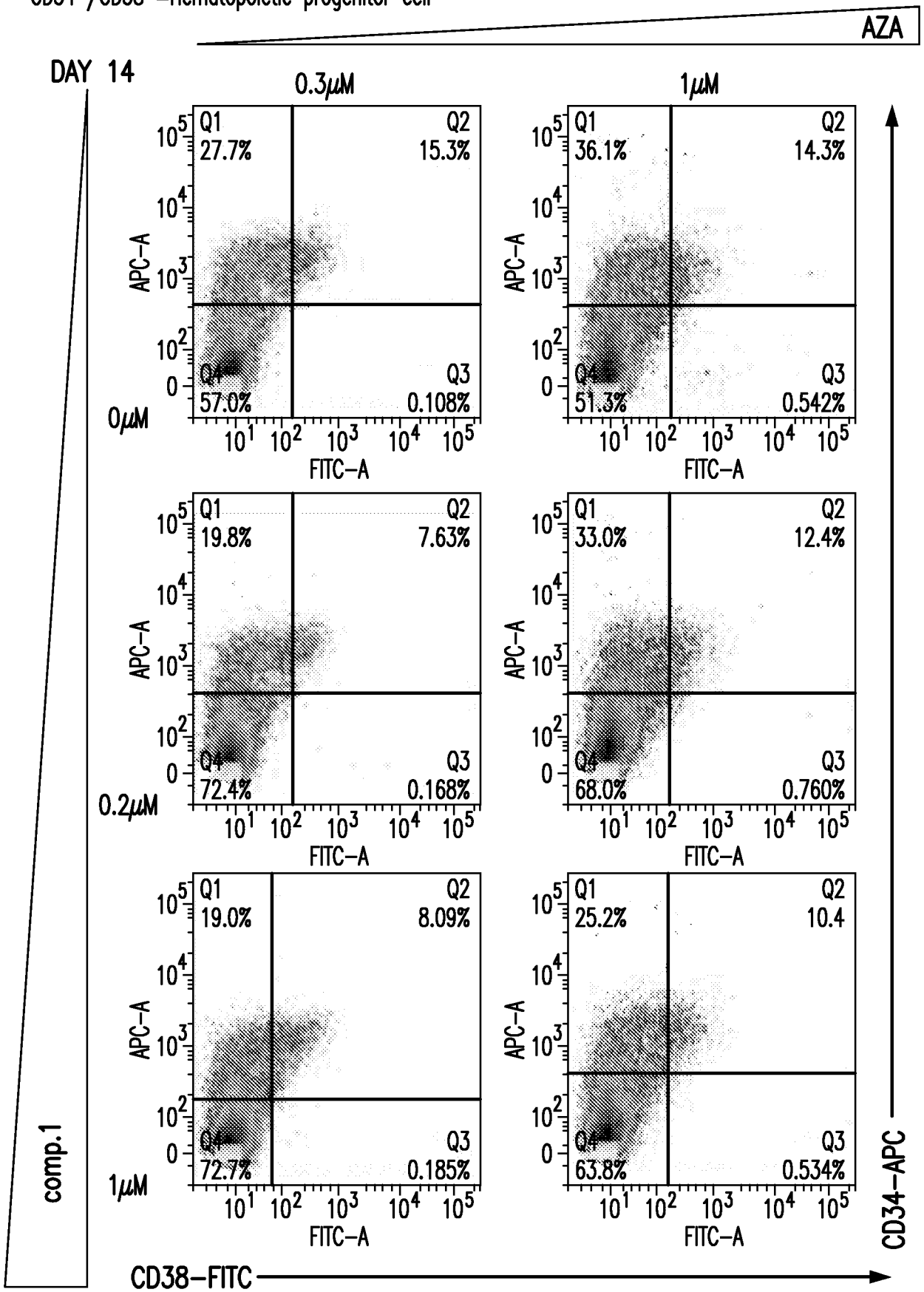



FIG. 8B (continued)

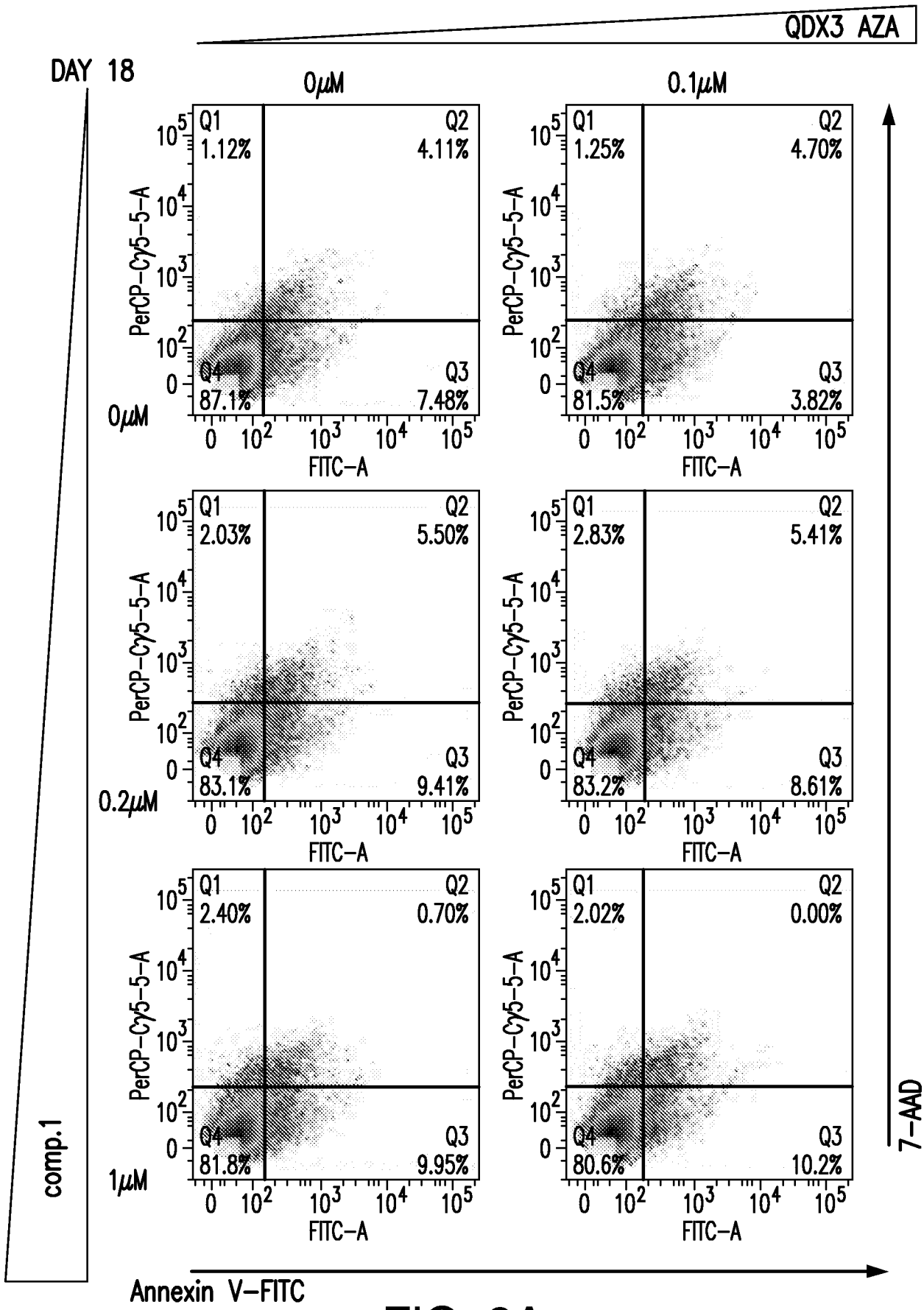

QDX3 AZA
DAY 18
0μM
0.1μM
0μM
0.2μM
1μM
Q1 1.12%
Q2 4.11%
Q4 87.1%
Q3 7.48%
Q1 1.25%
Q2 4.70%
Q4 81.5%
Q3 3.82%
Q1 2.03%
Q2 5.50%
Q4 83.1%
Q3 9.41%
Q1 2.83%
Q2 5.41%
Q4 83.2%
Q3 8.61%
Q1 2.40%
Q2 0.70%
Q4 81.8%
Q3 9.95%
Q1 2.02%
Q2 0.00%
Q4 80.6%
Q3 10.2%
PerCP-Cy5-5-A
FITC-A
comp.1
7-AAD
Annexin V-FITC

FIG. 9A

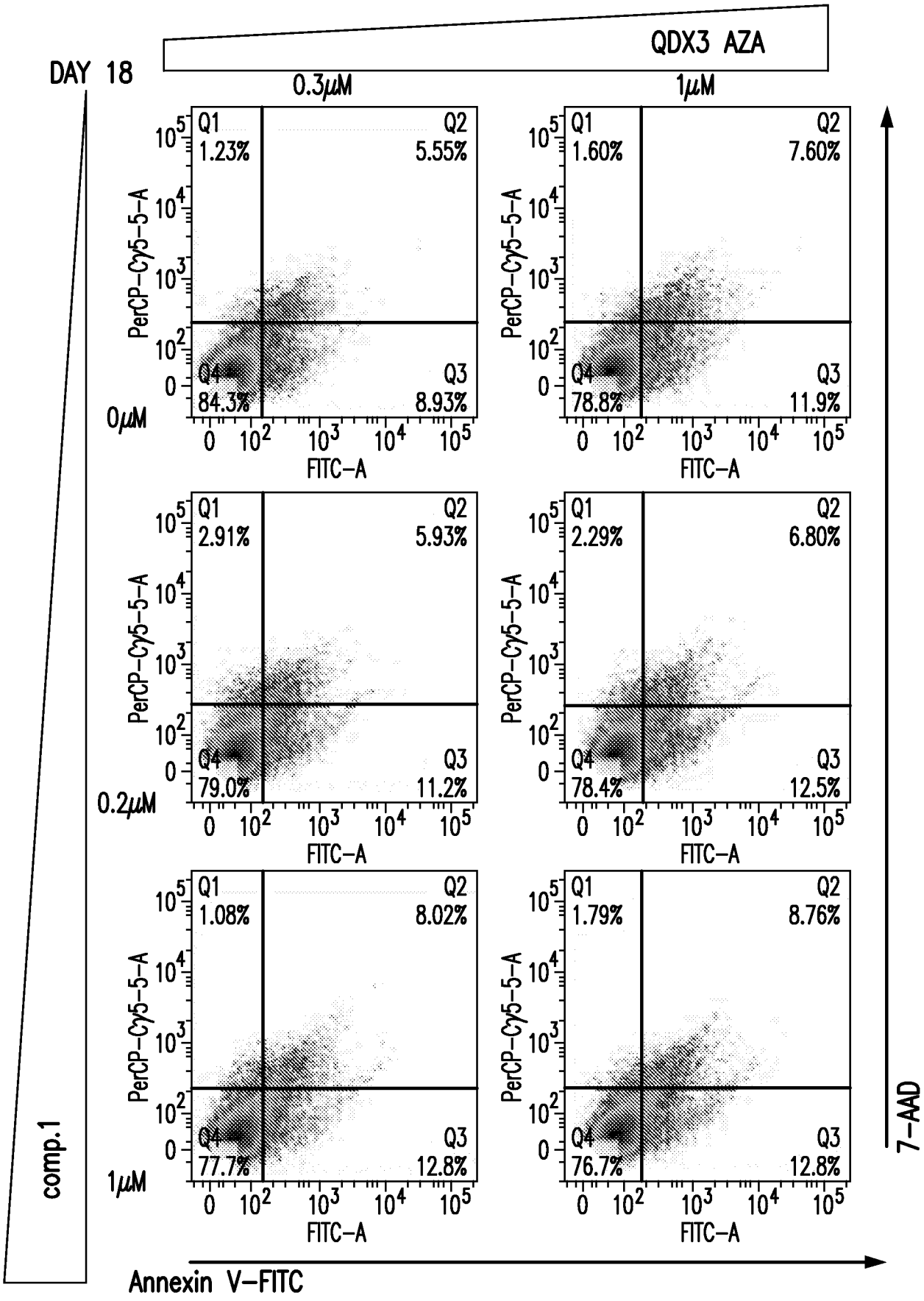



FIG. 9A (continued)

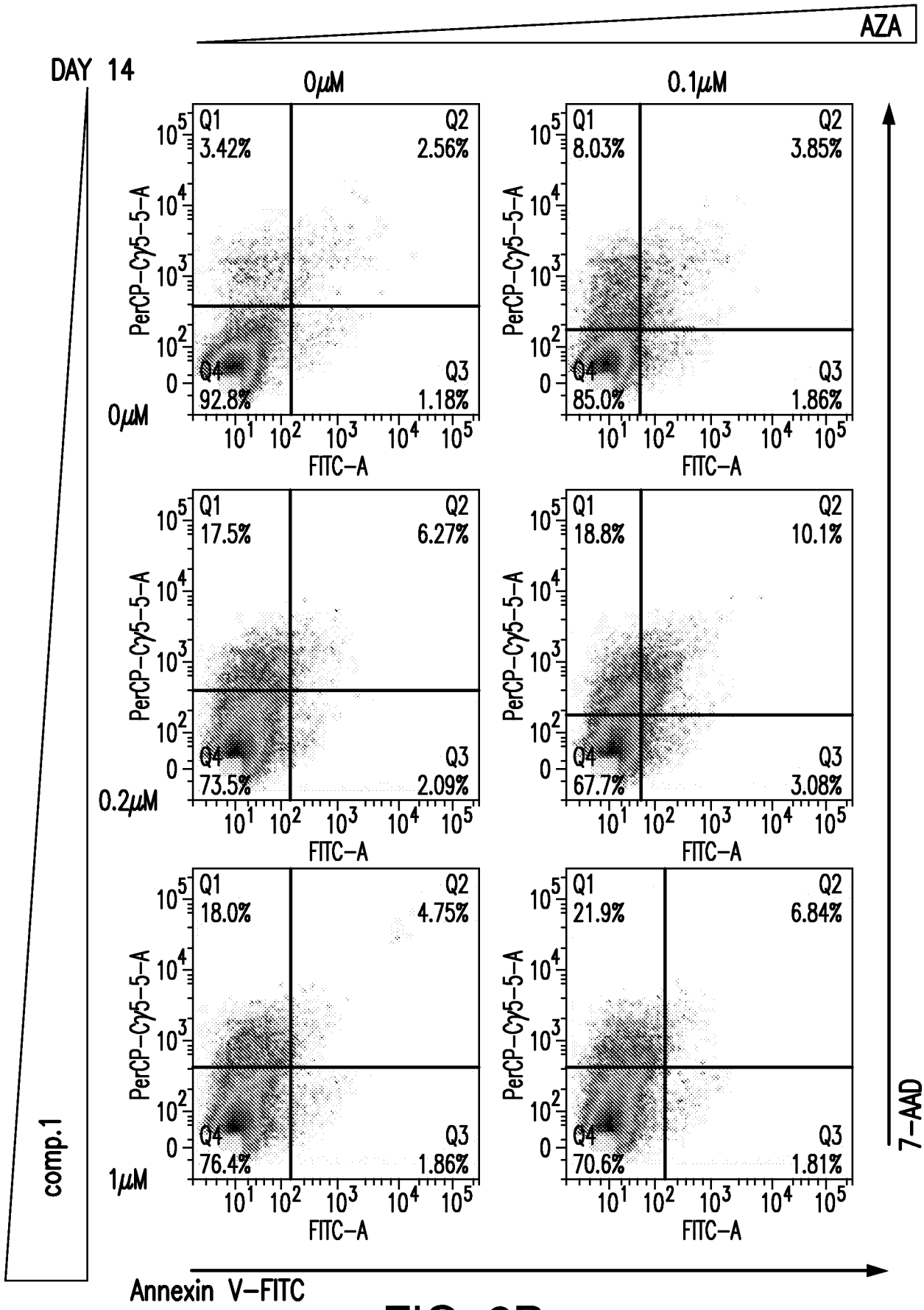

AZA
DAY 14
0μM
0.1μM
0.2μM
1μM
Q1
3.42%
Q2
2.56%
Q4
92.8%
Q3
1.18%
Q1
8.03%
Q2
3.85%
Q4
85.0%
Q3
1.86%
Q1
17.5%
Q2
6.27%
Q4
73.5%
Q3
2.09%
Q1
18.8%
Q2
10.1%
Q4
67.7%
Q3
3.08%
Q1
18.0%
Q2
4.75%
Q4
76.4%
Q3
1.86%
Q1
21.9%
Q2
6.84%
Q4
70.6%
Q3
1.81%
PerCP-Cy5-5-A
FITC-A
comp.1
7-AAD
Annexin V-FITC

FIG. 9B

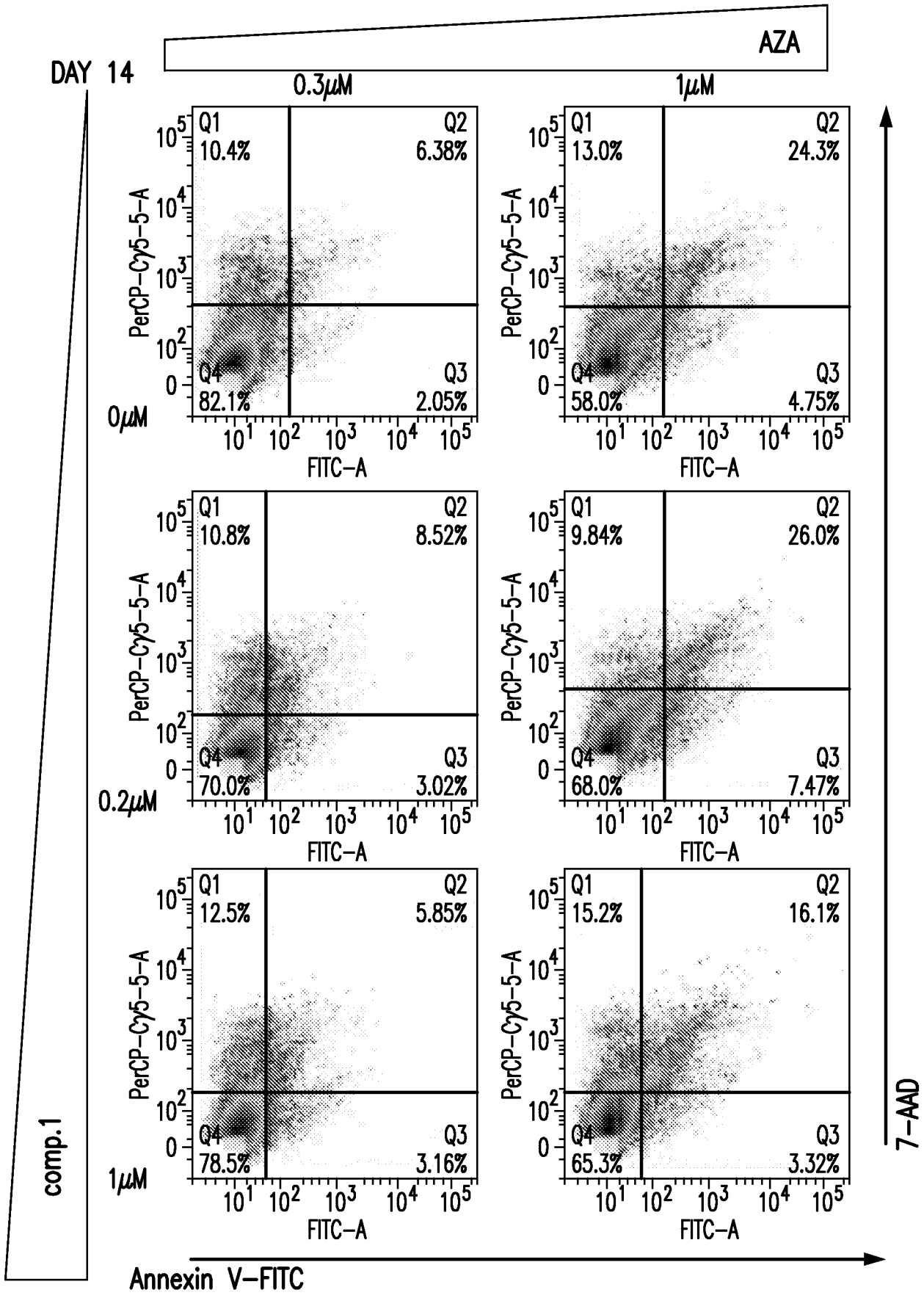



FIG. 9B (continued)

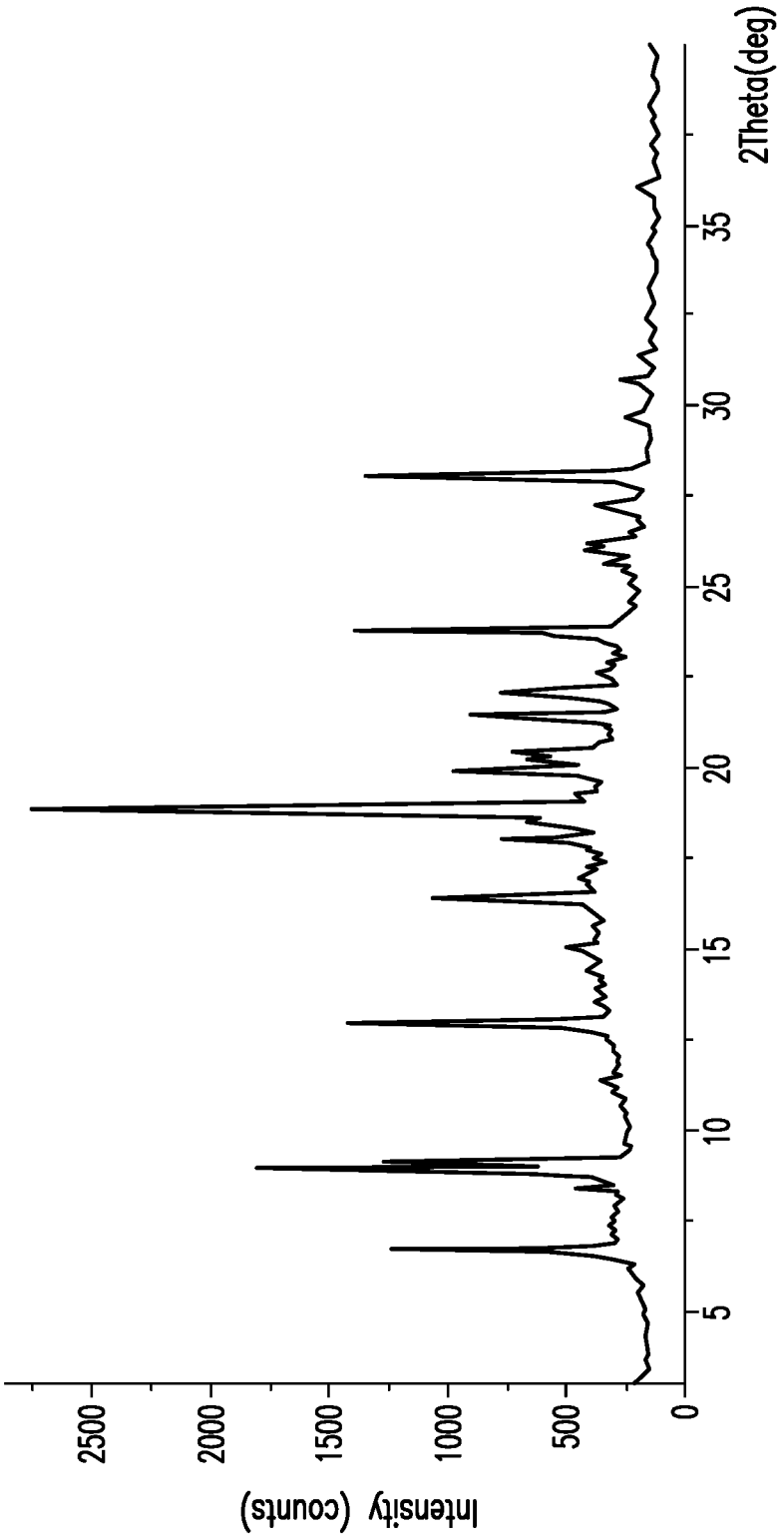

Intensity (counts)
0
500
1000
1500
2000
2500
5
10
15
20
25
30
35
2Theta(deg)

FIG. 10

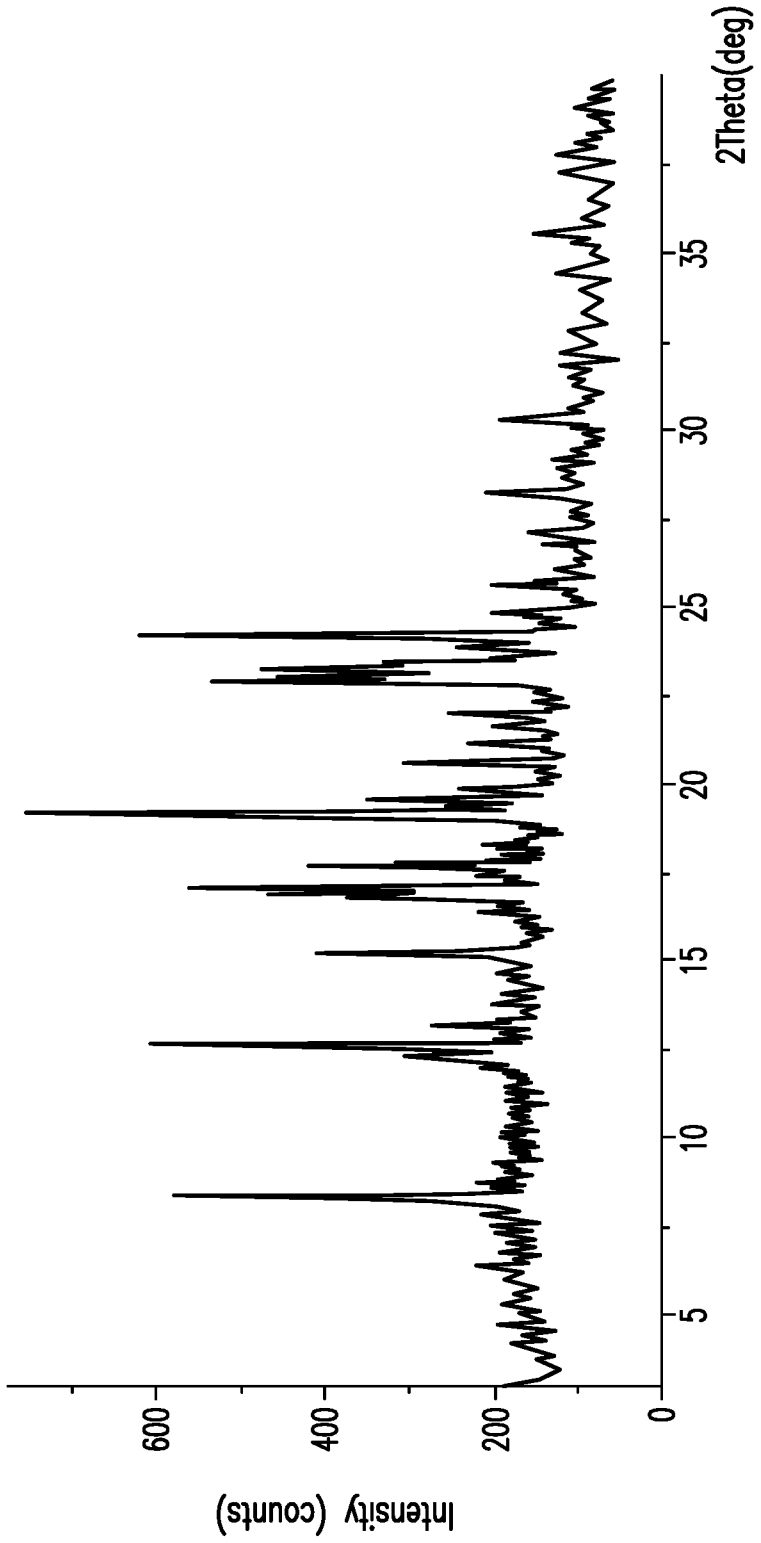



FIG. 11

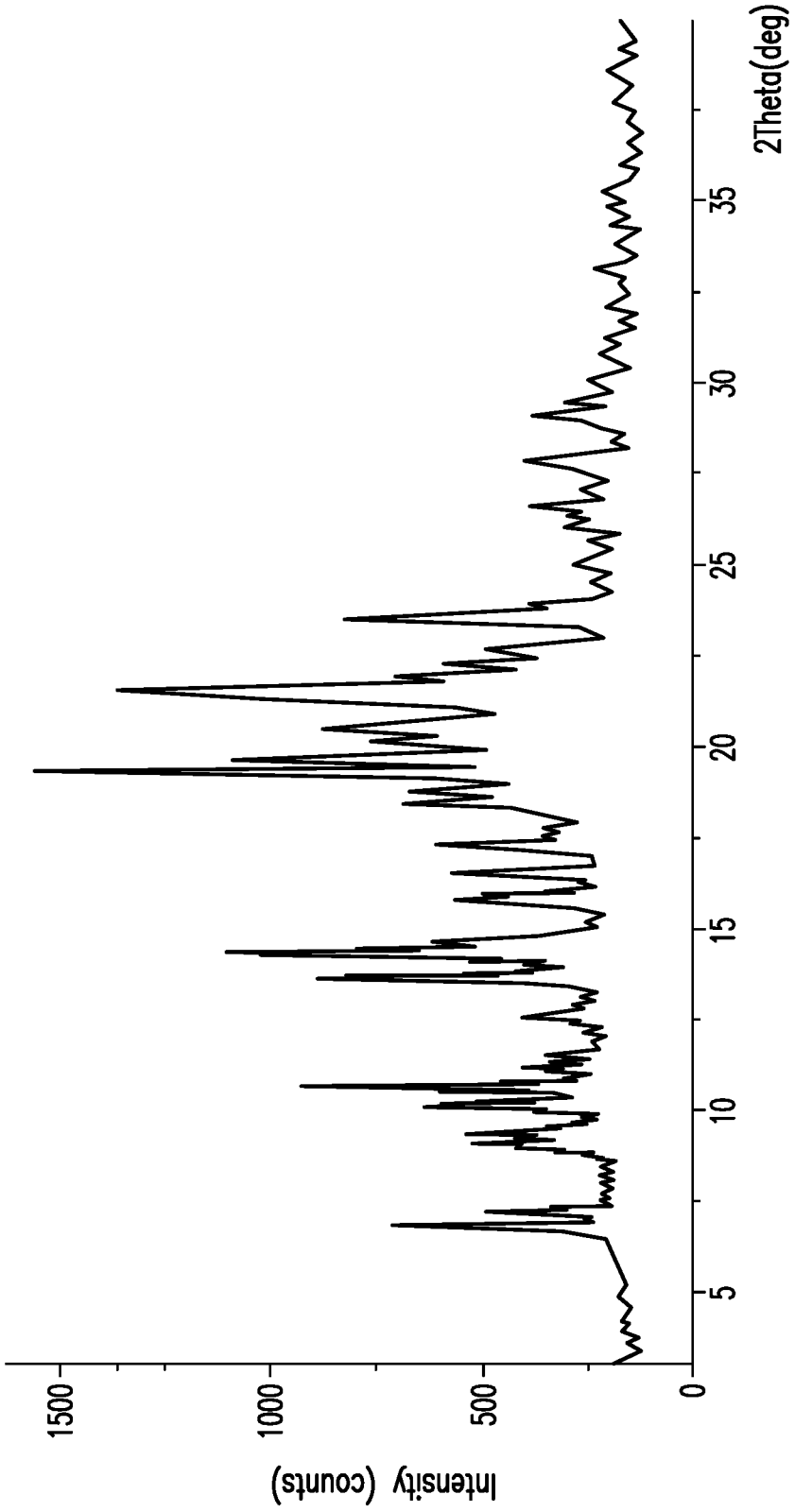



FIG. 12

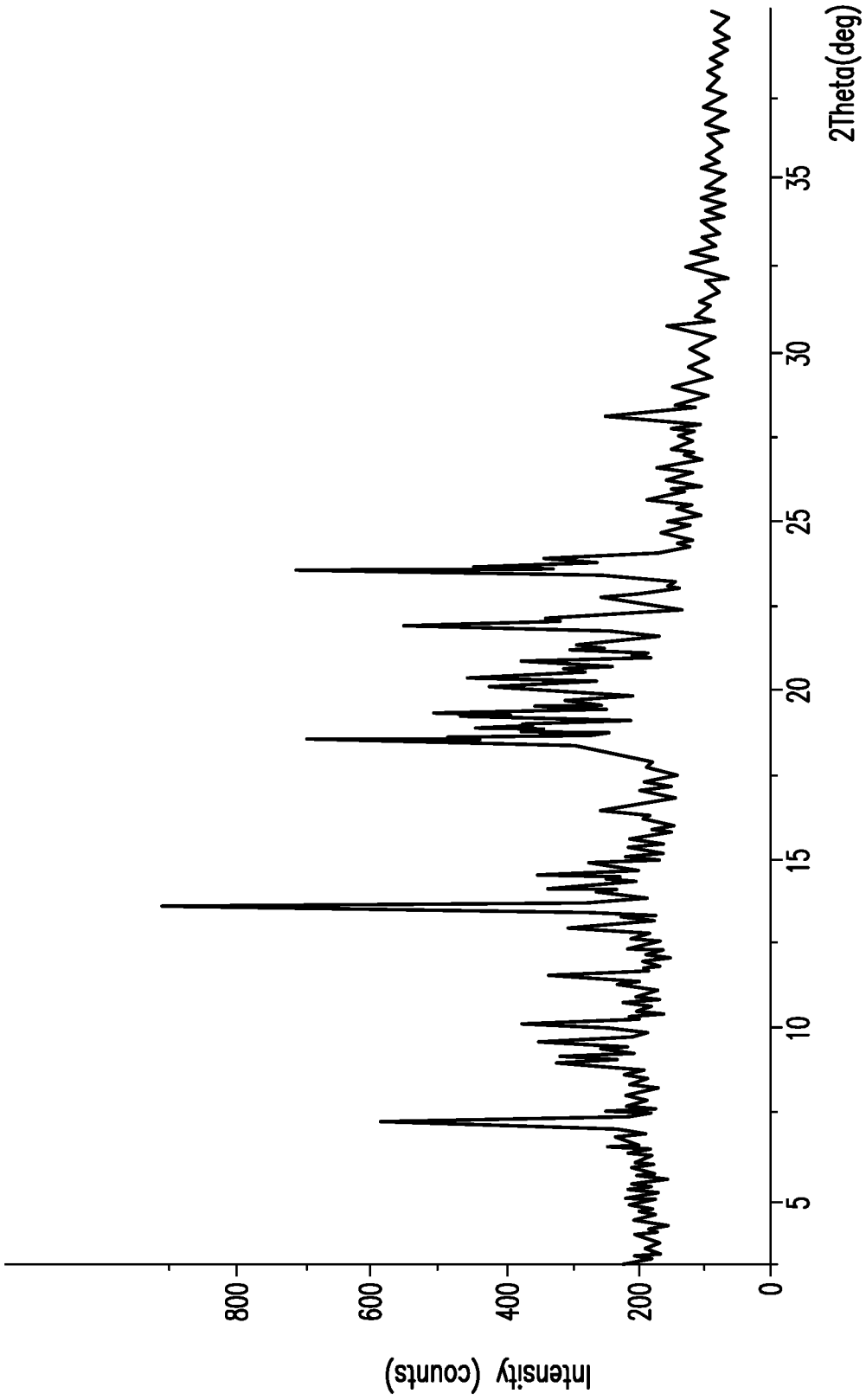

Intensity (counts)
0
200
400
600
800
5
10
15
20
25
30
35
2Theta(deg)

FIG. 13

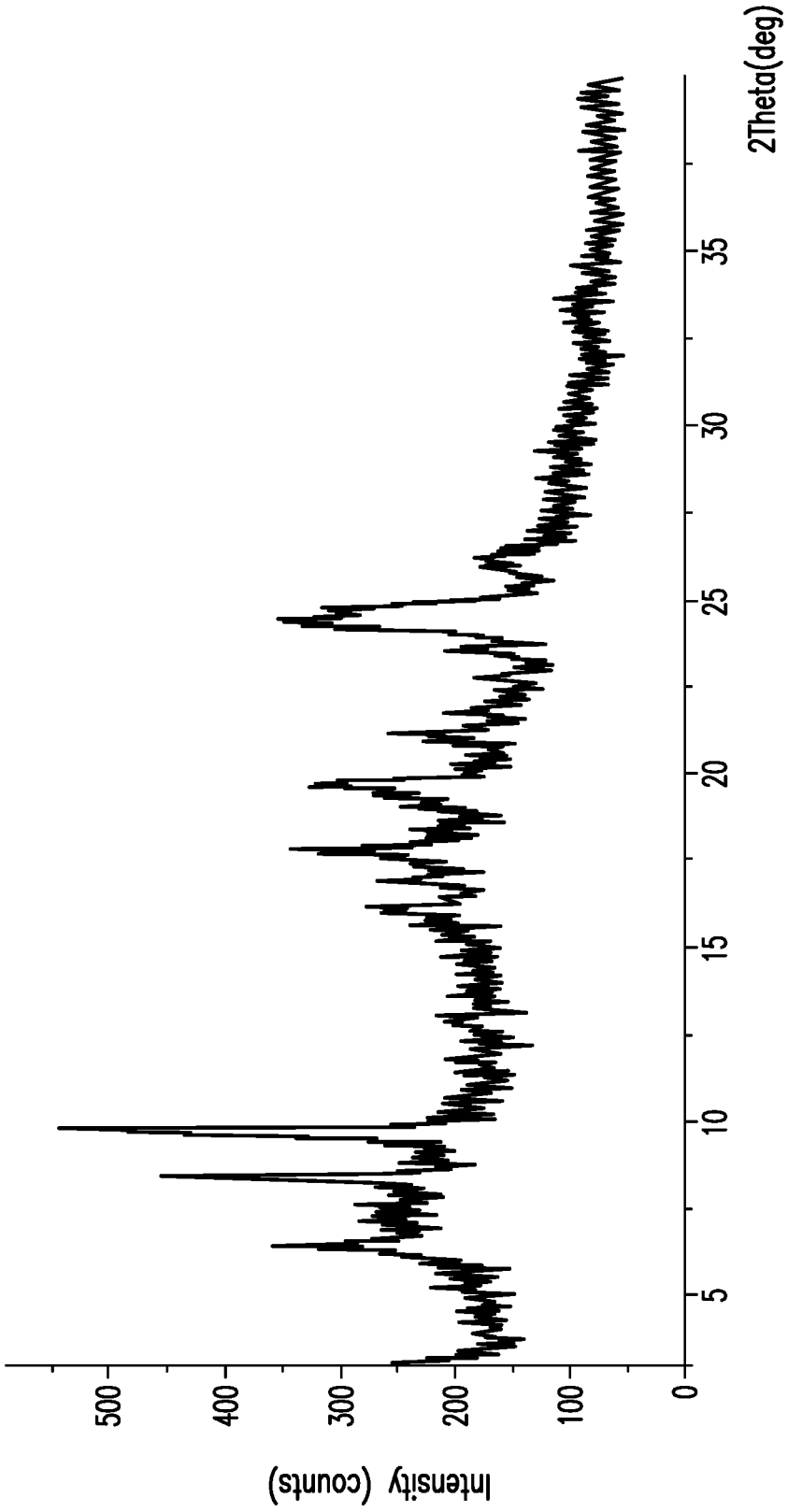

Intensity (counts)
0
100
200
300
400
500
5
10
15
20
25
30
35
2Theta(deg)

FIG. 14

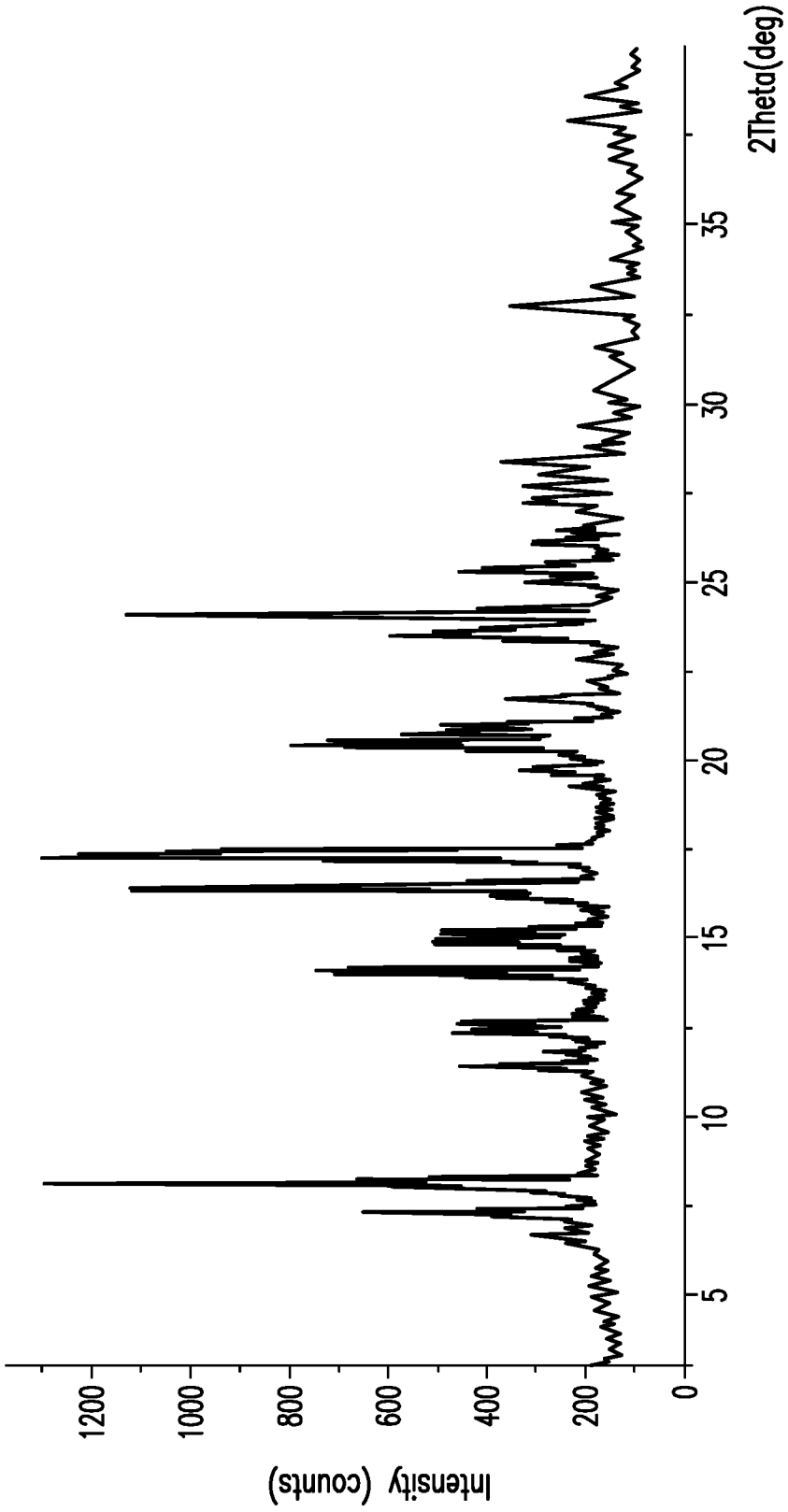

Intensity (counts)
0
200
400
600
800
1000
1200
5
10
15
20
25
30
35
2Theta(deg)

FIG. 15