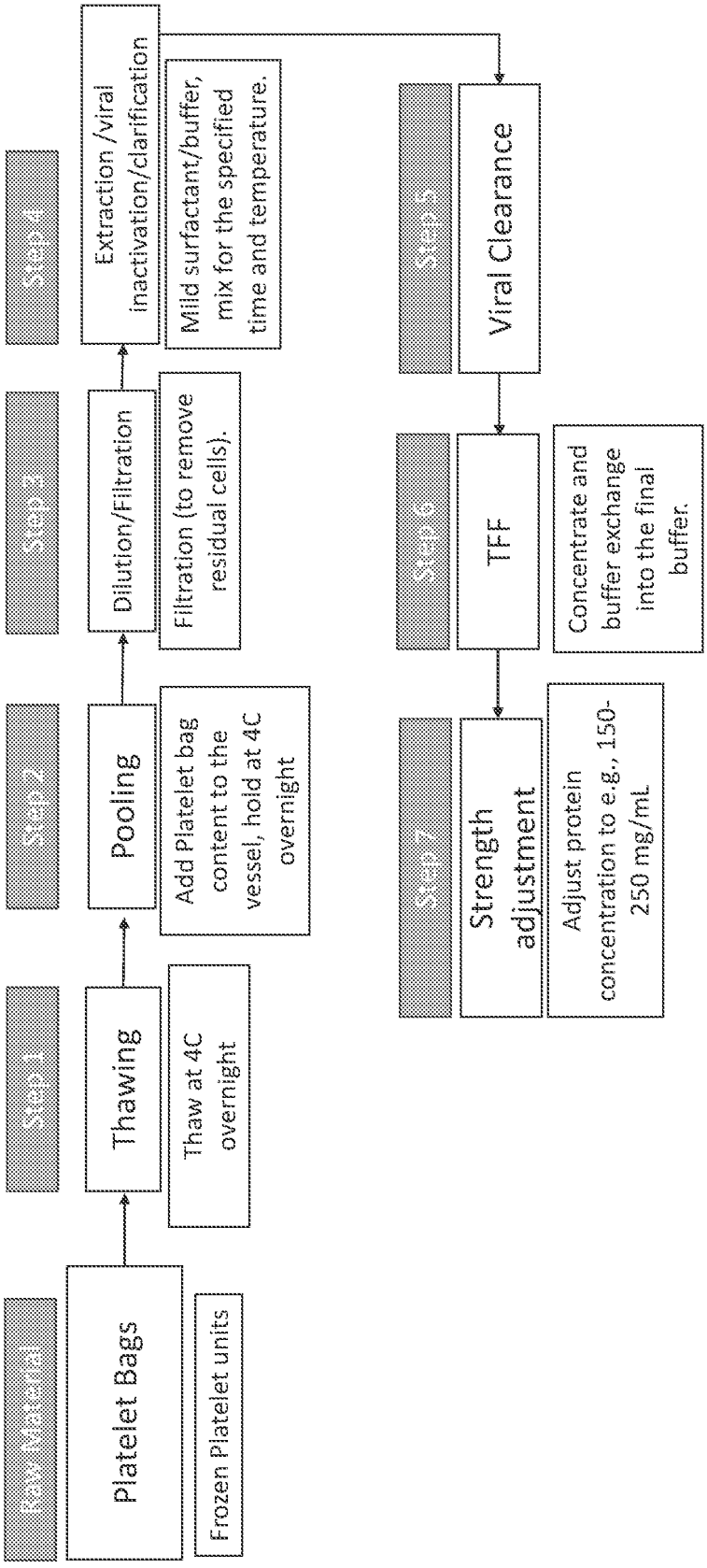
Raw Material
Platelet Bags
Frozen Platelet units
Step 1
Thawing
Thaw at 4C overnight
Step 2
Pooling
Add Platelet bag content to the vessel, hold at 4C overnight
Step 3
Dilution/Filtration
Filtration (to remove residual cells).
Step 4
Extraction /viral inactivation/clarification
Mild surfactant/buffer, mix for the specified time and temperature.
Step 5
Viral Clearance
Step 6
TFF
Concentrate and buffer exchange into the final buffer.
Step 7
Strength adjustment
Adjust protein concentration to e.g., 150-250 mg/mL

FIG. 1

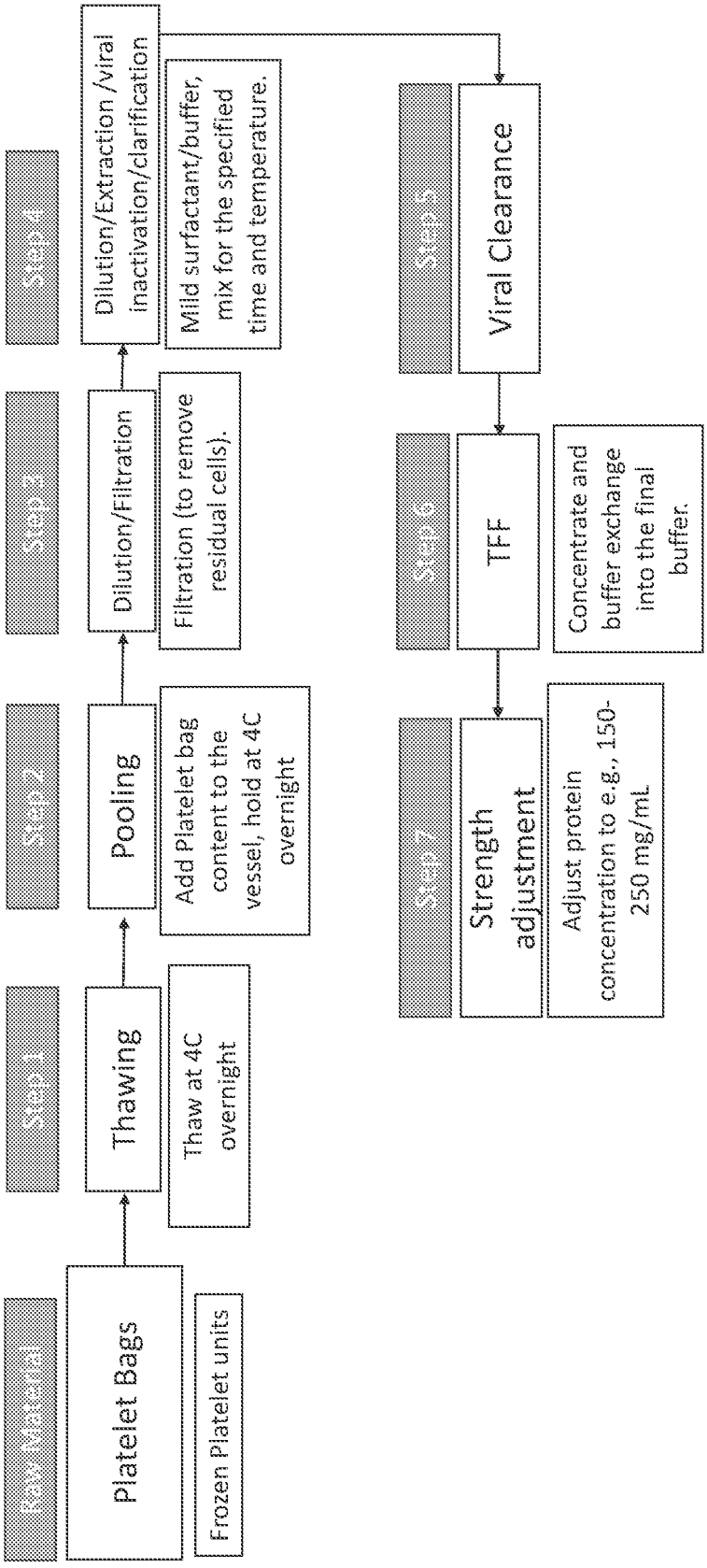
Raw Material
Platelet Bags
Frozen Platelet units
Step 1
Thawing
Thaw at 4C overnight
Step 2
Pooling
Add Platelet bag content to the vessel, hold at 4C overnight
Step 3
Dilution/Filtration
Filtration (to remove residual cells).
Step 4
Dilution/Extraction /viral inactivation/clarification
Mild surfactant/buffer, mix for the specified time and temperature.
Step 5
Viral Clearance
Step 6
TFF
Concentrate and buffer exchange into the final buffer.
Step 7
Strength adjustment
Adjust protein concentration to e.g., 150-250 mg/mL

FIG. 2

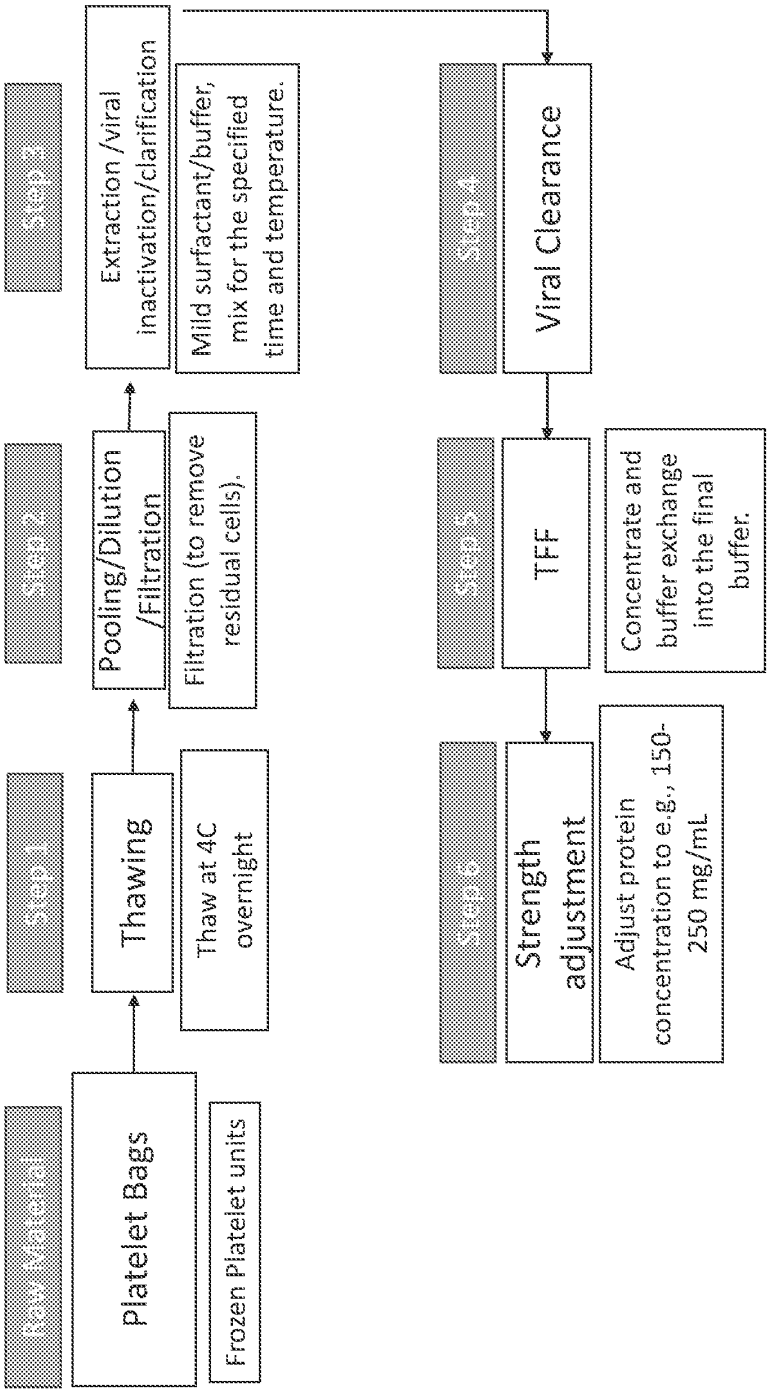
Raw Material
Platelet Bags
Frozen Platelet units
Step 1
Thawing
Thaw at 4C overnight
Step 2
Pooling/Dilution /Filtration
Filtration (to remove residual cells).
Step 3
Extraction /viral inactivation/clarification
Mild surfactant/buffer, mix for the specified time and temperature.
Step 4
Viral Clearance
Step 5
TFF
Concentrate and buffer exchange into the final buffer.
Step 6
Strength adjustment
Adjust protein concentration to e.g., 150-250 mg/mL

FIG. 3

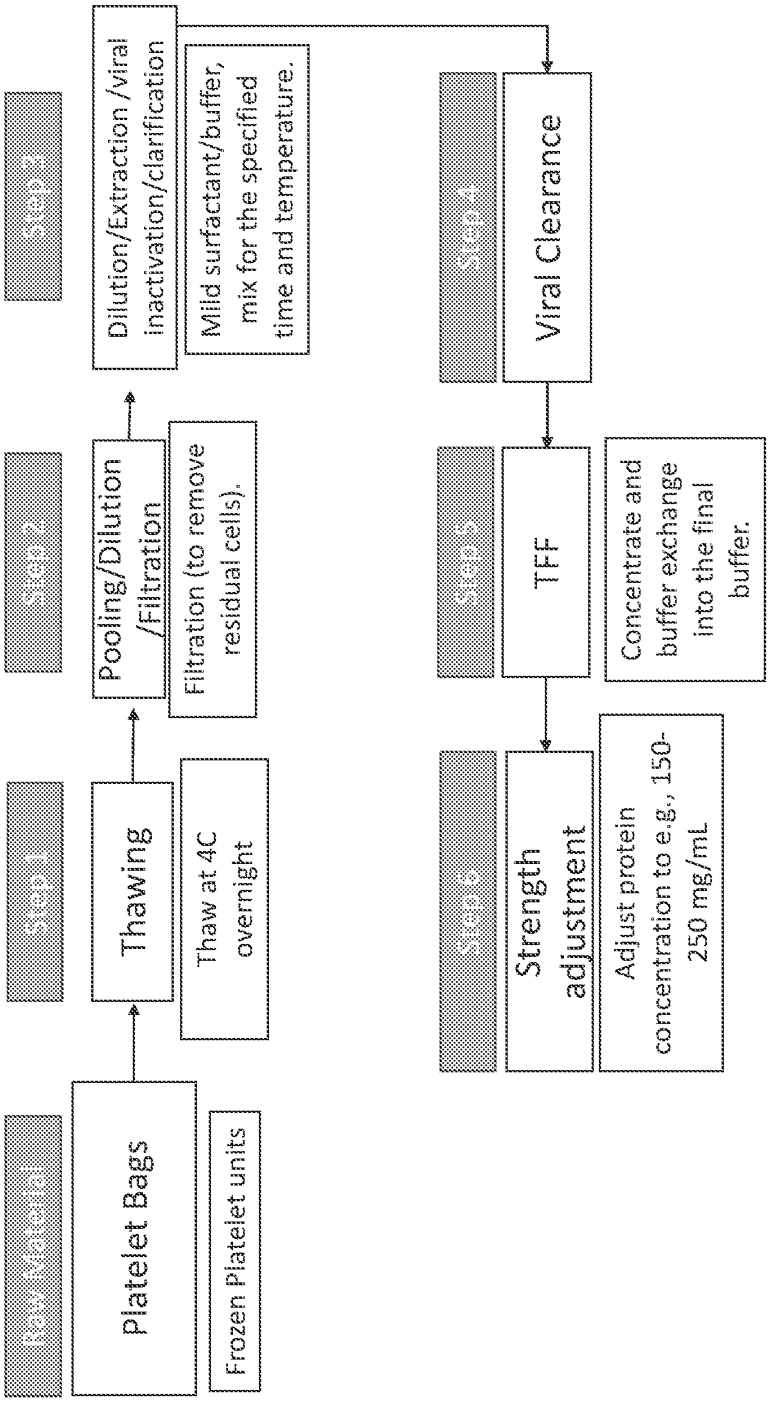
Raw Material
Platelet Bags
Frozen Platelet units
Step 1
Thawing
Thaw at 4C overnight
Step 2
Pooling/Dilution /Filtration
Filtration (to remove residual cells).
Step 3
Dilution/Extraction /viral inactivation/clarification
Mild surfactant/buffer, mix for the specified time and temperature.
Step 4
Viral Clearance
Step 5
TFF
Concentrate and buffer exchange into the final buffer.
Step 6
Strength adjustment
Adjust protein concentration to e.g., 150-250 mg/mL

FIG. 4

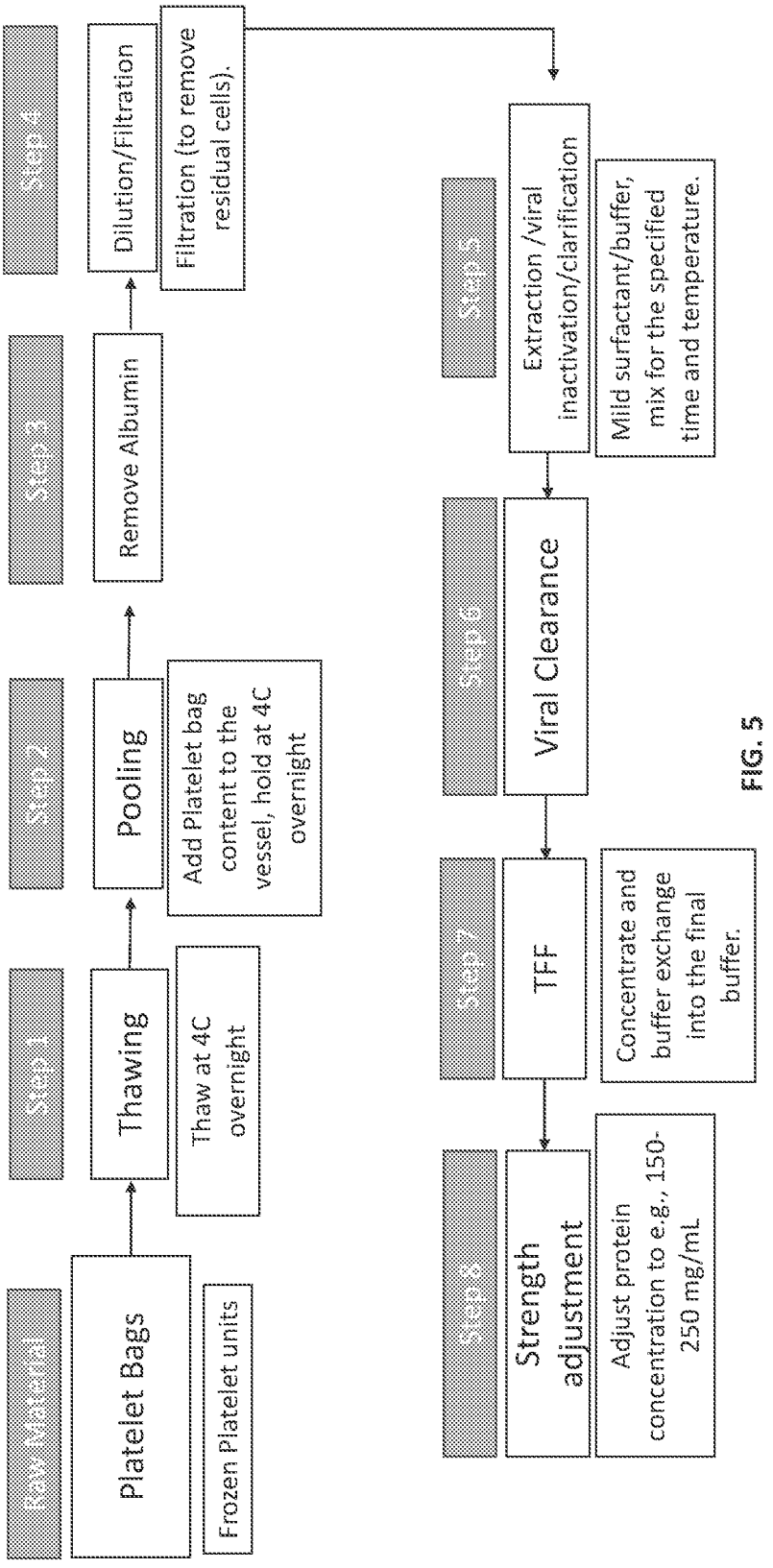
Raw Material
Platelet Bags
Frozen Platelet units
Step 1
Thawing
Thaw at 4C overnight
Step 2
Pooling
Add Platelet bag content to the vessel, hold at 4C overnight
Step 3
Remove Albumin
Step 4
Dilution/Filtration
Filtration (to remove residual cells).
Step 5
Extraction /Viral inactivation/clarification
Mild surfactant/buffer, mix for the specified time and temperature.
Step 6
Viral Clearance
Step 7
TFF
Concentrate and buffer exchange into the final buffer.
Step 8
Strength adjustment
Adjust protein concentration to e.g., 150-250 mg/mL

FIG. 5

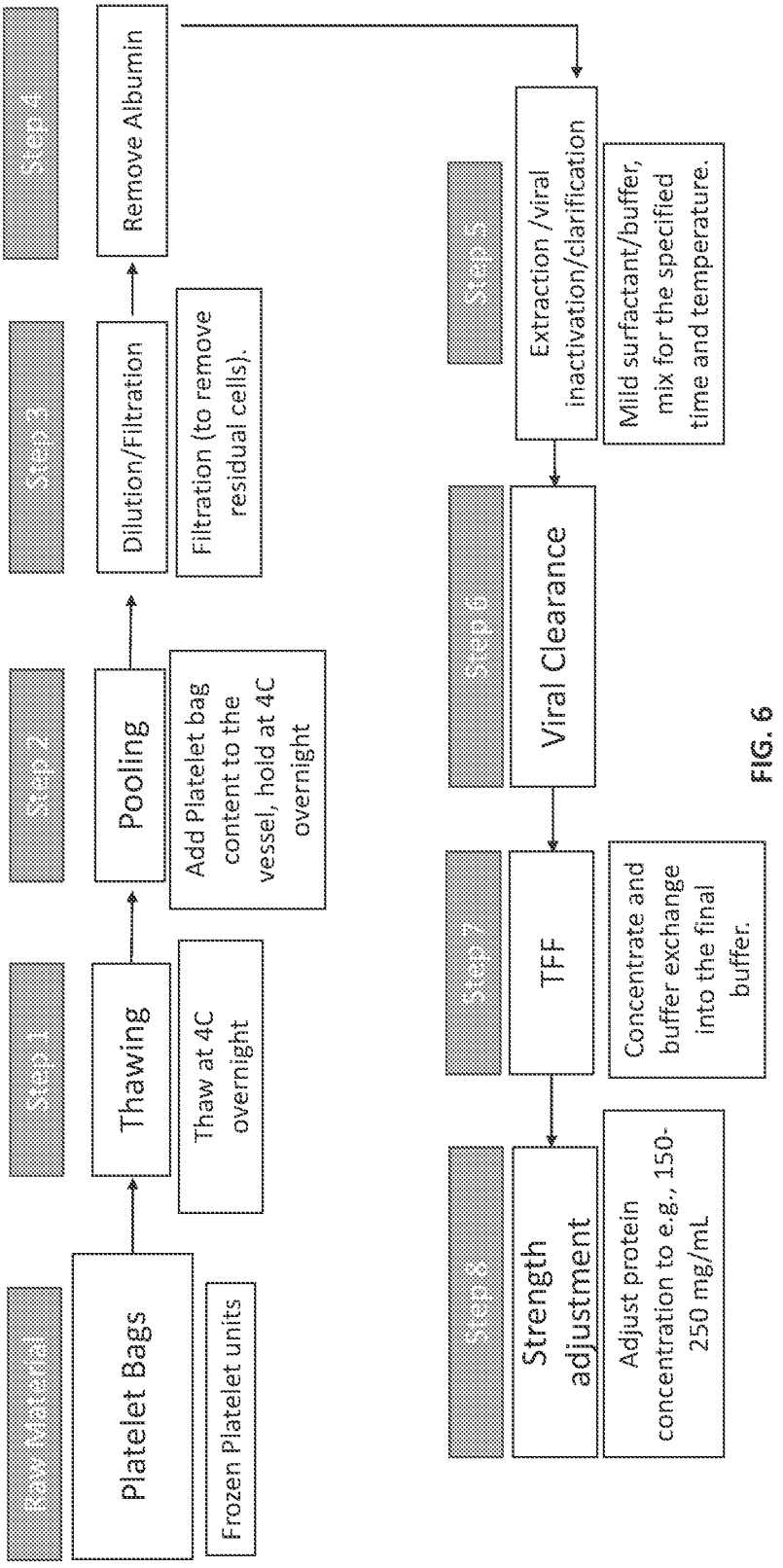
Raw Material
Platelet Bags
Frozen Platelet units
Step 1
Thawing
Thaw at 4C overnight
Step 2
Pooling
Add Platelet bag content to the vessel, hold at 4C overnight
Step 3
Dilution/Filtration
Filtration (to remove residual cells).
Step 4
Remove Albumin
Step 5
Extraction /Viral inactivation/clarification
Mild surfactant/buffer, mix for the specified time and temperature.
Step 6
Viral Clearance
Step 7
TFF
Concentrate and buffer exchange into the final buffer.
Step 8
Strength adjustment
Adjust protein concentration to e.g., 150-250 mg/mL

FIG. 6

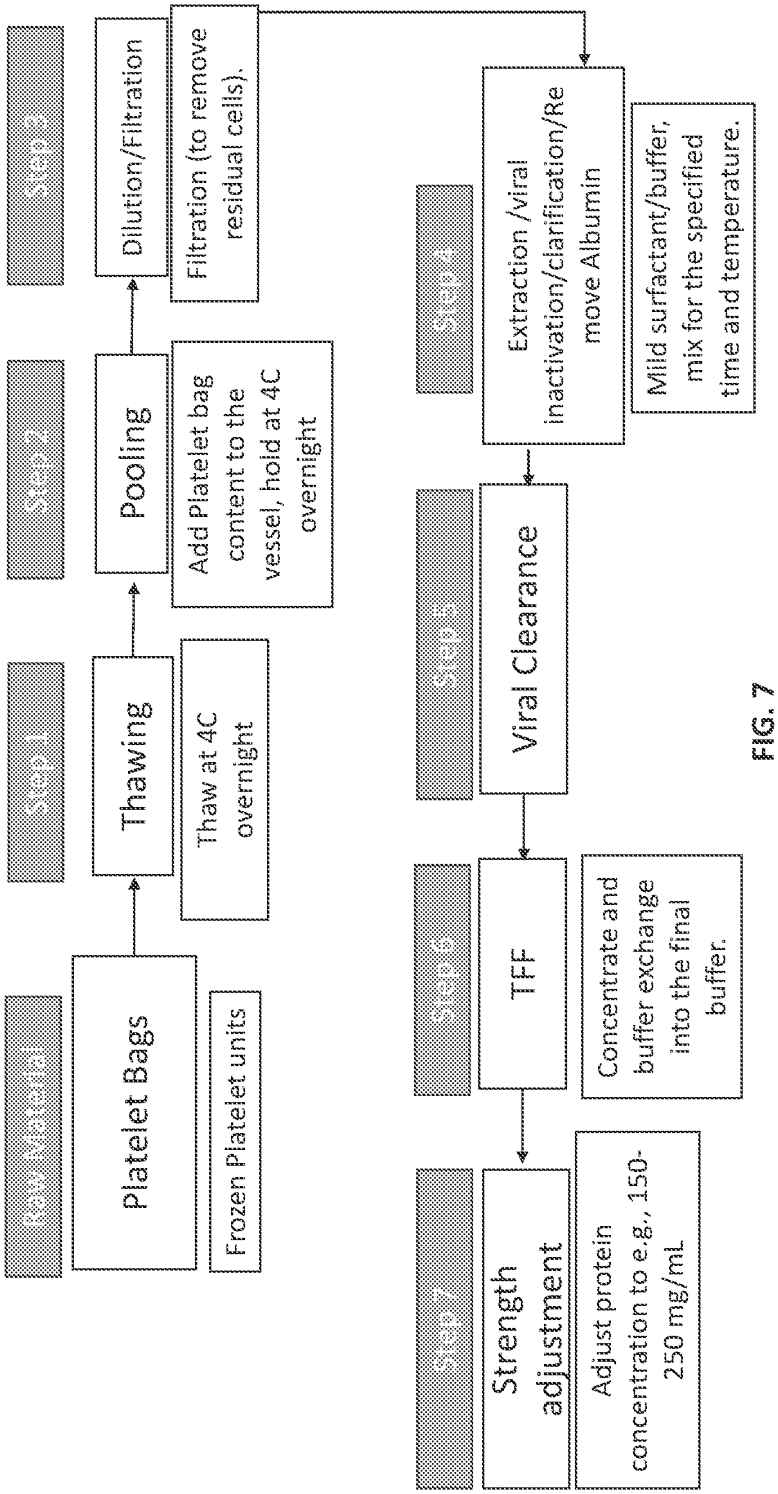
Raw Material
Platelet Bags
Frozen Platelet units
Step 1
Thawing
Thaw at 4C overnight
Step 2
Pooling
Add Platelet bag content to the vessel, hold at 4C overnight
Step 3
Dilution/Filtration
Filtration (to remove residual cells).
Step 4
Extraction /viral inactivation/clarification/Remove Albumin
Mild surfactant/buffer, mix for the specified time and temperature.
Step 5
Viral Clearance
Step 6
TFF
Concentrate and buffer exchange into the final buffer.
Step 7
Strength adjustment
Adjust protein concentration to e.g., 150-250 mg/mL

FIG. 7

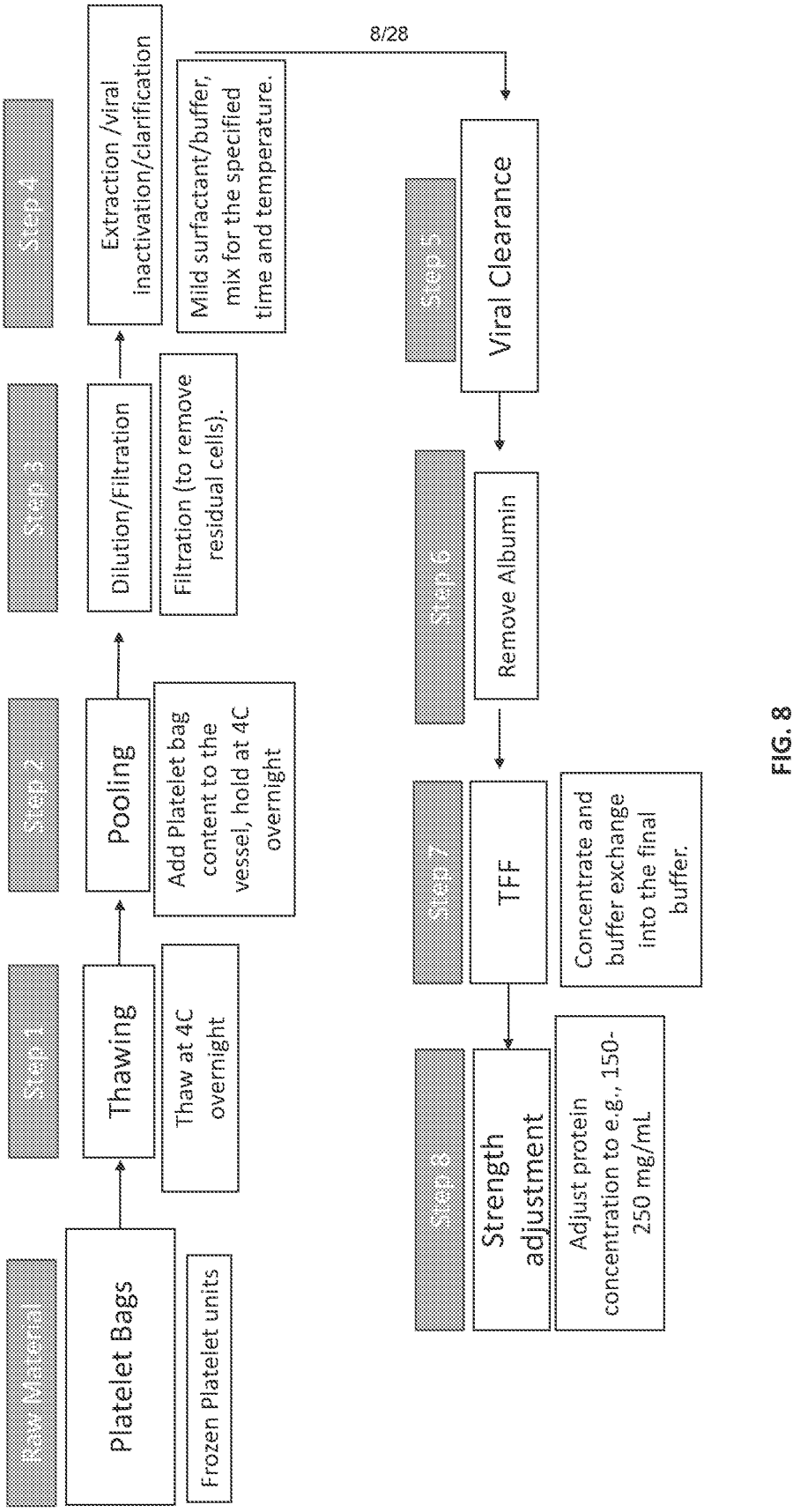
Raw Material
Platelet Bags
Frozen Platelet units
Step 1
Thawing
Thaw at 4C overnight
Step 2
Pooling
Add Platelet bag content to the vessel, hold at 4C overnight
Step 3
Dilution/Filtration
Filtration (to remove residual cells).
Step 4
Extraction /viral inactivation/clarification
Mild surfactant/buffer, mix for the specified time and temperature.
Step 5
Viral Clearance
Step 6
Remove Albumin
Step 7
TFF
Concentrate and buffer exchange into the final buffer.
Step 8
Strength adjustment
Adjust protein concentration to e.g., 150-250 mg/mL

FIG. 8

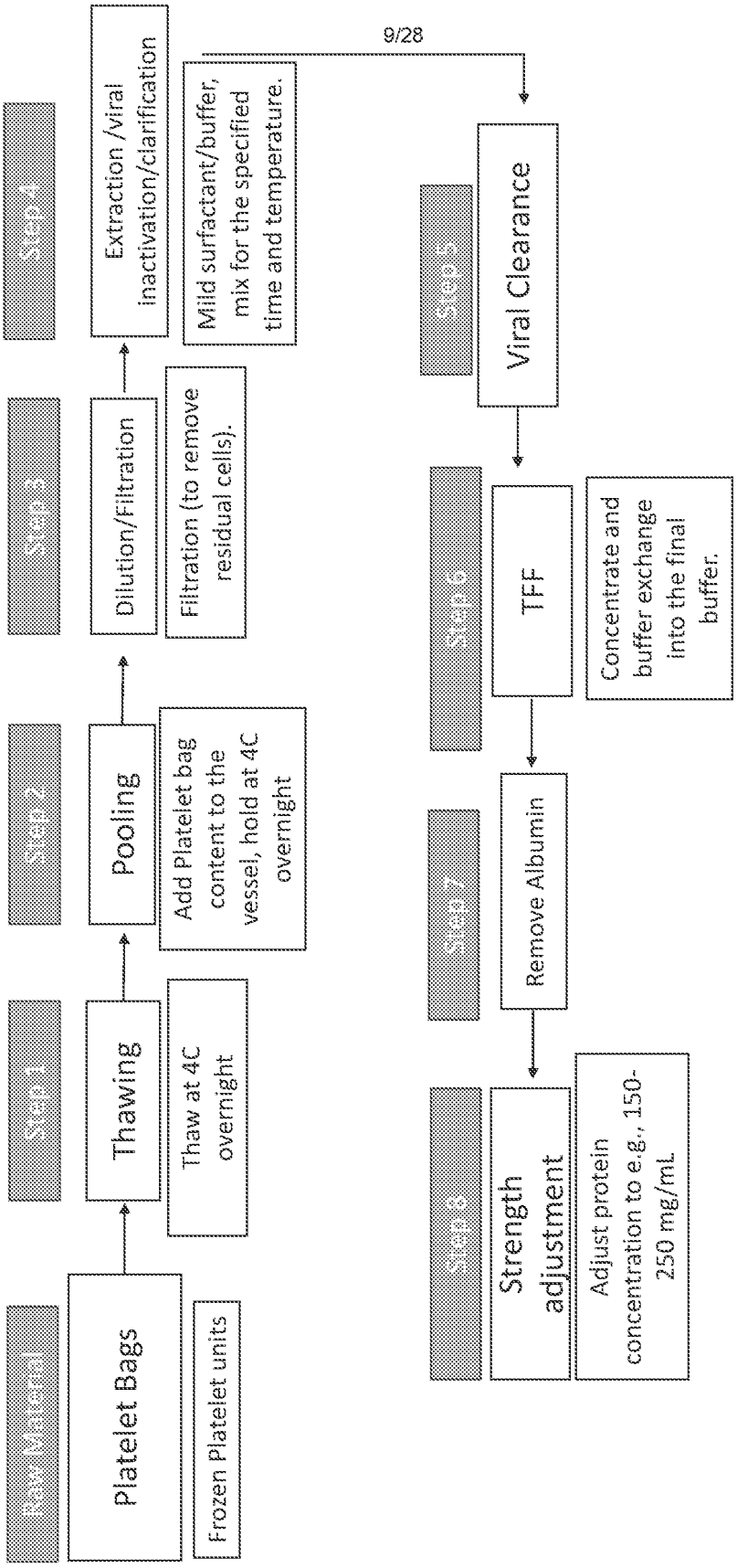
Raw Material
Platelet Bags
Frozen Platelet units
Step 1
Thawing
Thaw at 4C overnight
Step 2
Pooling
Add Platelet bag content to the vessel, hold at 4C overnight
Step 3
Dilution/Filtration
Filtration (to remove residual cells).
Step 4
Extraction /viral inactivation/clarification
Mild surfactant/buffer, mix for the specified time and temperature.
Step 5
Viral Clearance
Step 6
TFF
Concentrate and buffer exchange into the final buffer.
Step 7
Remove Albumin
Step 8
Strength adjustment
Adjust protein concentration to e.g., 150-250 mg/mL

FIG. 9

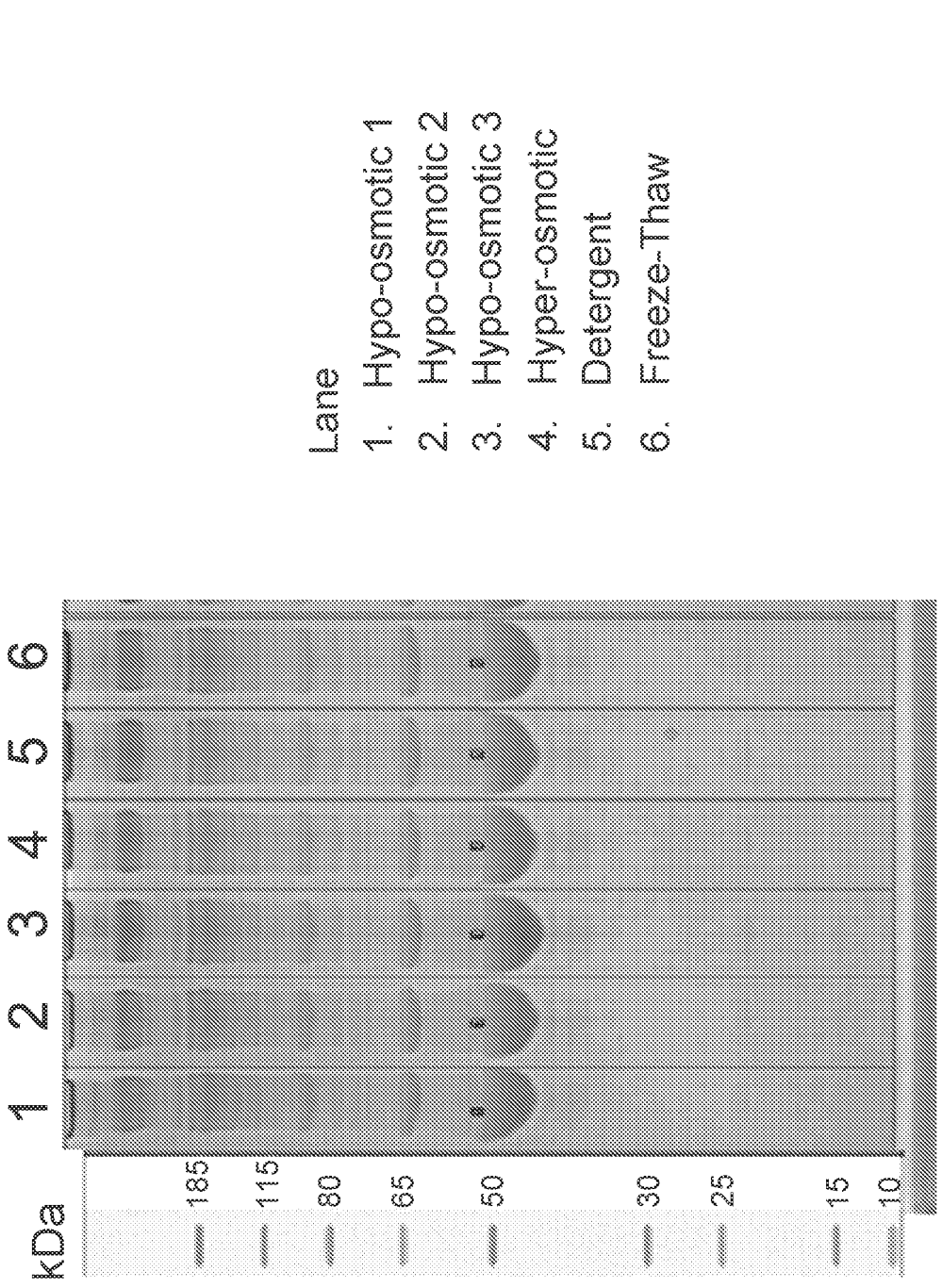
Lane
1. Hypo-osmotic 1
2. Hypo-osmotic 2
3. Hypo-osmotic 3
4. Hyper-osmotic
5. Detergent
6. Freeze-Thaw
kDa
185
115
80
65
50
30
25
15
10
1
2
3
4
5
6

FIG. 10

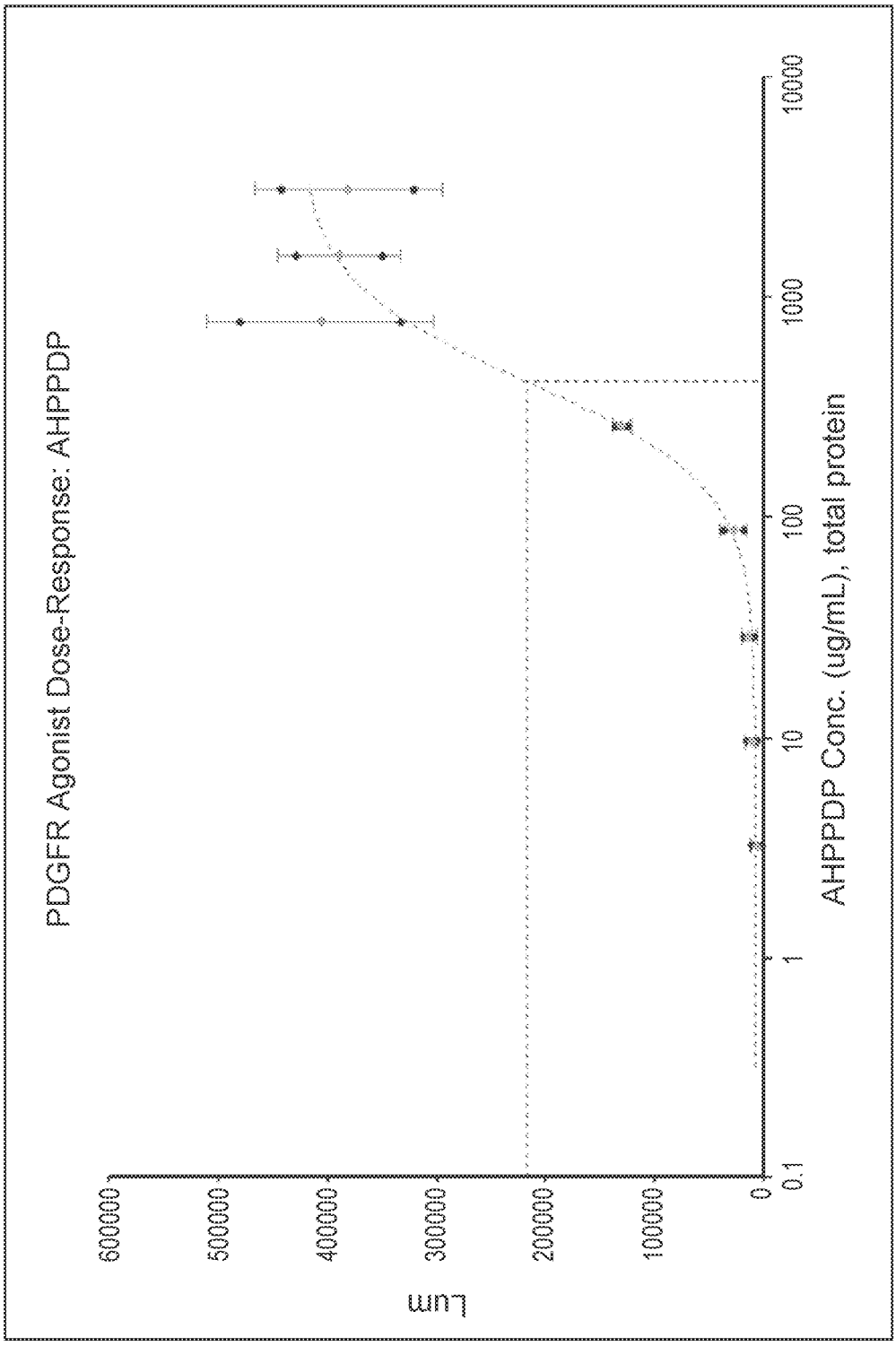
PDGFR Agonist Dose-Response: AHPPDP
Lum
600000
500000
400000
300000
200000
100000
0
0.1
1
10
100
1000
10000
AHPPDP Conc. (ug/mL), total protein

FIG. 11

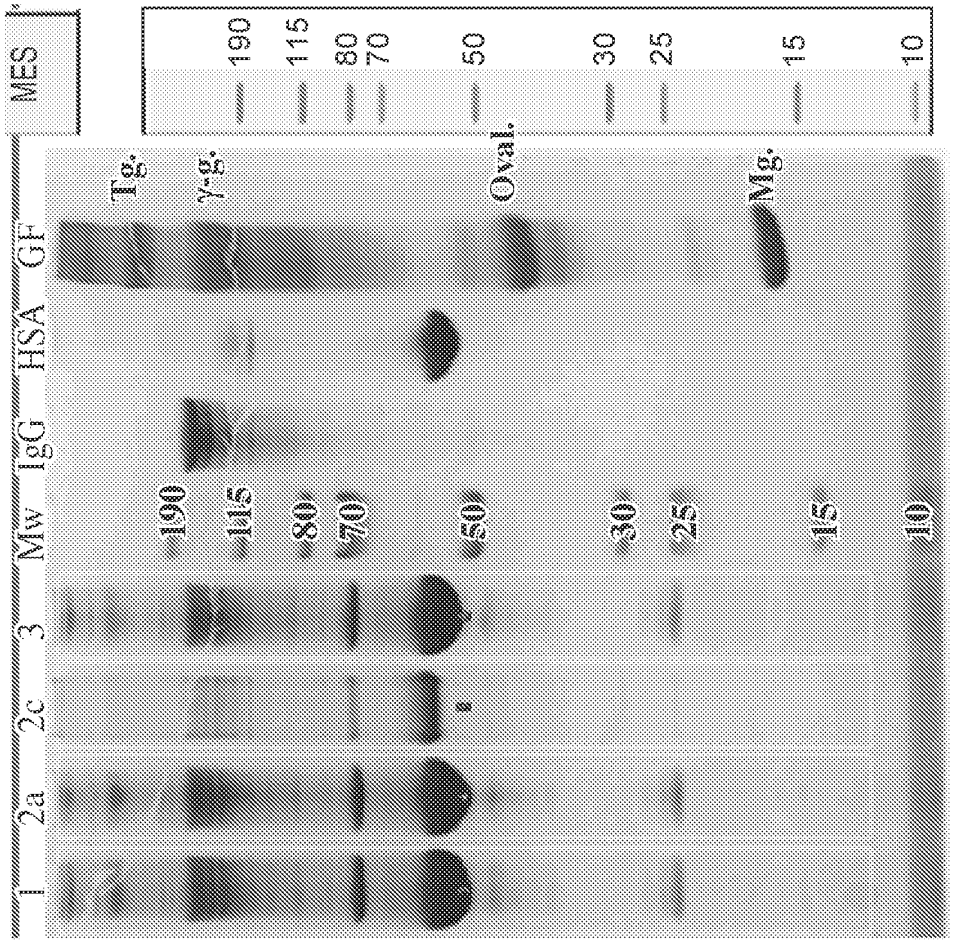

FIG. 12

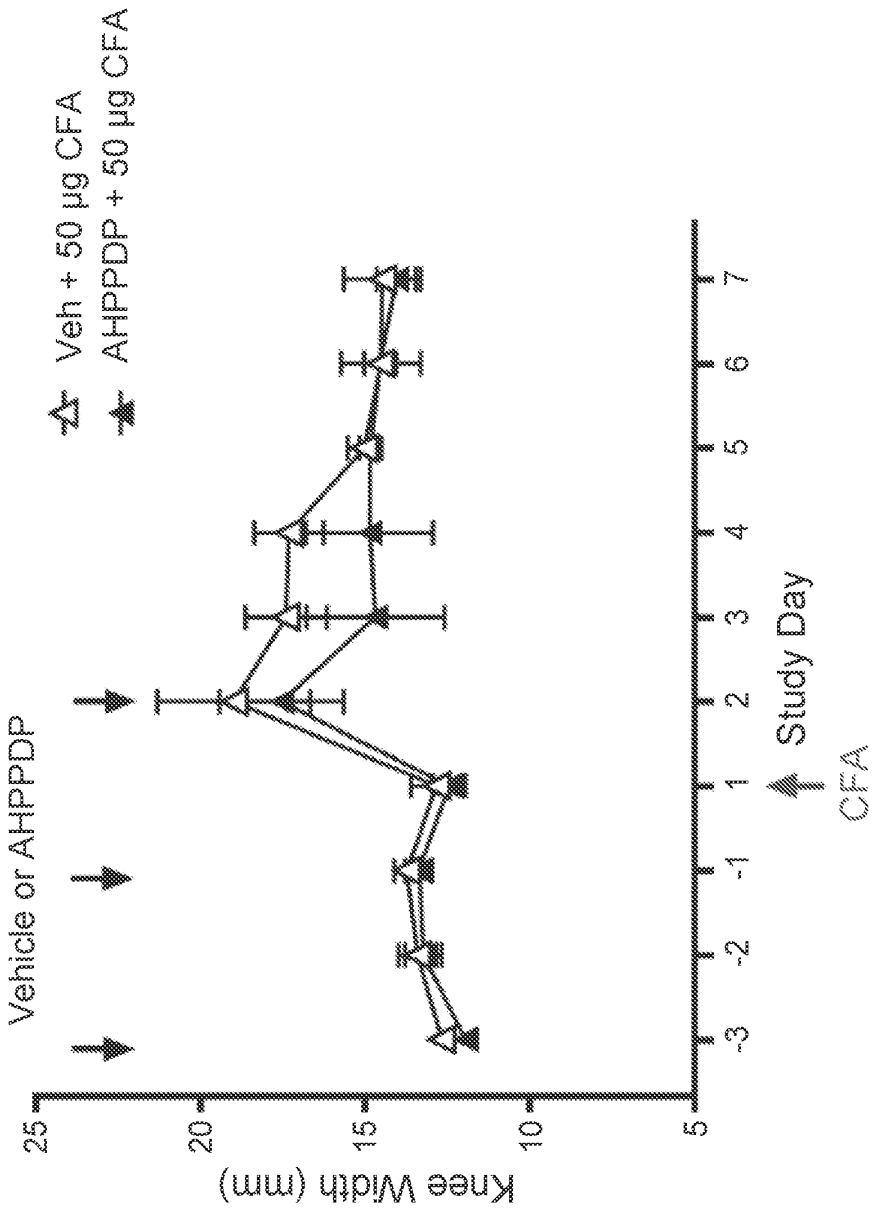
Veh + 50 µg CFA
AHPPDP + 50 µg CFA
Vehicle or AHPPDP
Knee Width (mm)
25
20
15
10
5
-3
-2
-1
1
2
3
4
5
6
7
Study Day
CFA

FIG. 13A

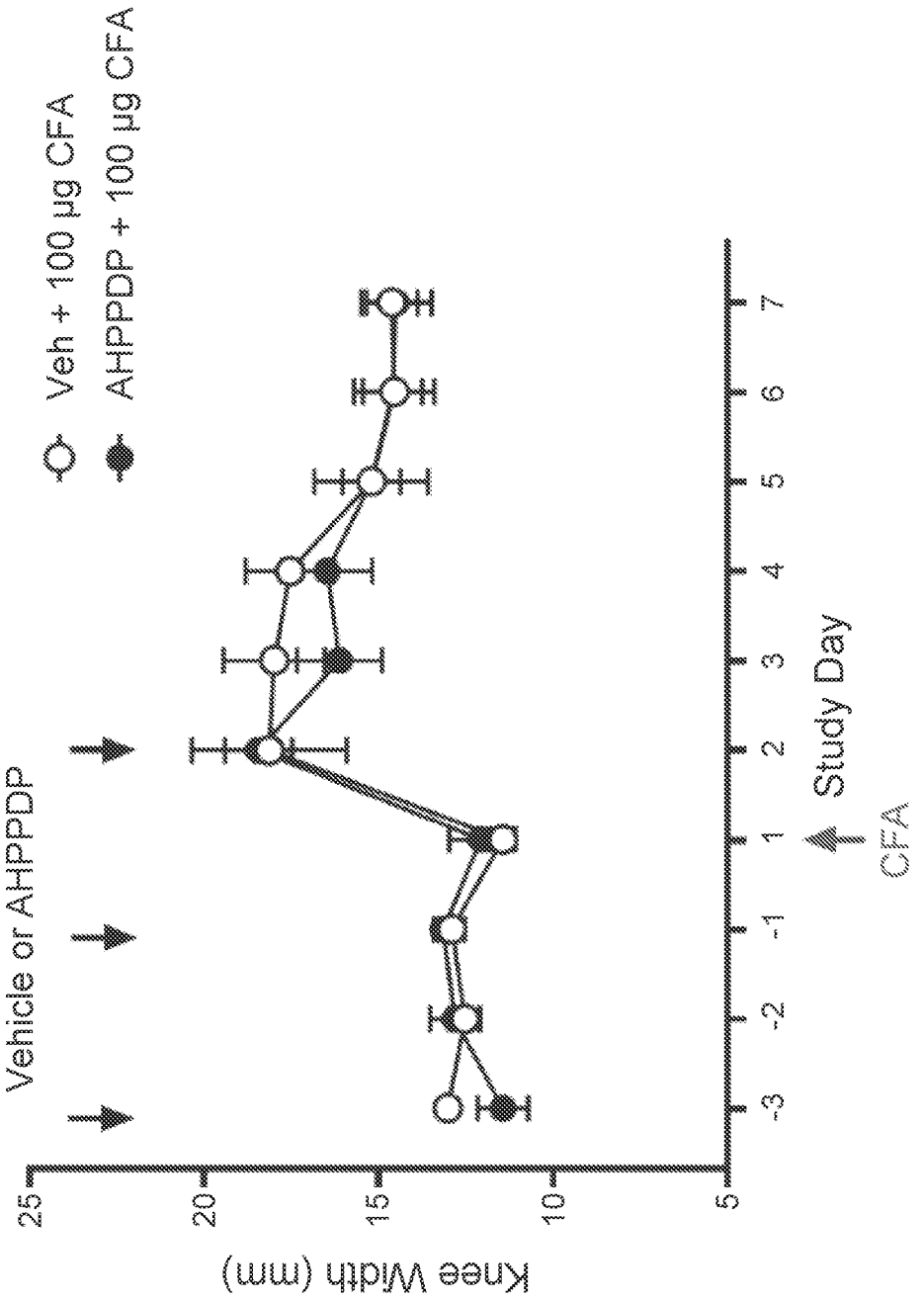
Veh + 100 µg CFA
AHPPDP + 100 µg CFA
Vehicle or AHPPDP
CFA
Study Day
Knee Width (mm)
25
20
15
10
5
-3
-2
-1
1
2
3
4
5
6
7

FIG. 13B

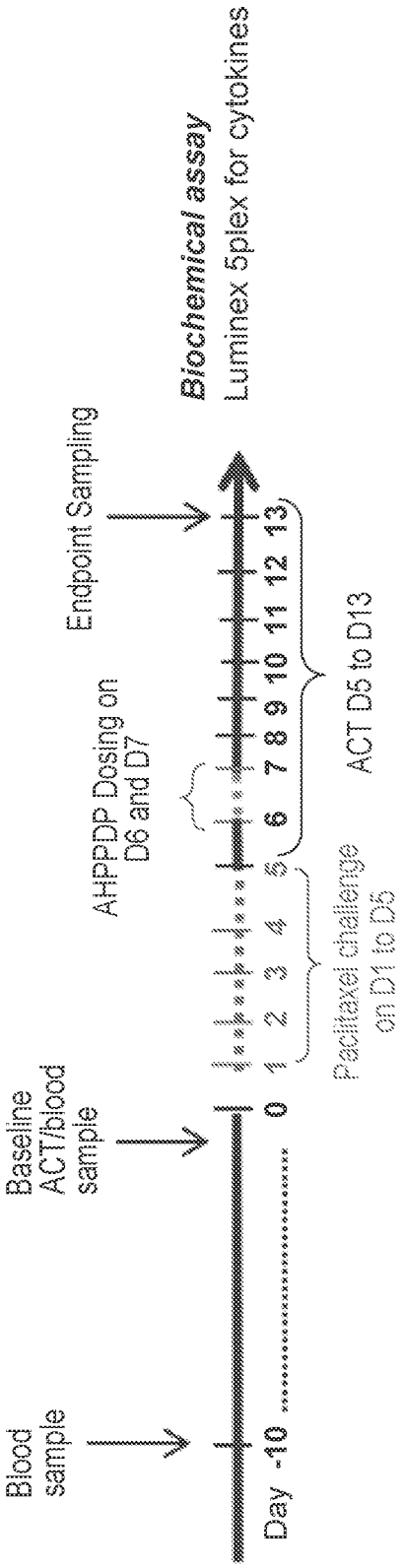
Blood sample
Baseline ACT/blood sample
AHPPDP Dosing on D6 and D7
Endpoint Sampling
Biochemical assay
Luminex 5plex for cytokines
Day -10 ..... 0 1 2 3 4 5 6 7 8 9 10 11 12 13
Paclitaxel challenge on D1 to D5
ACT D5 to D13

FIG. 14

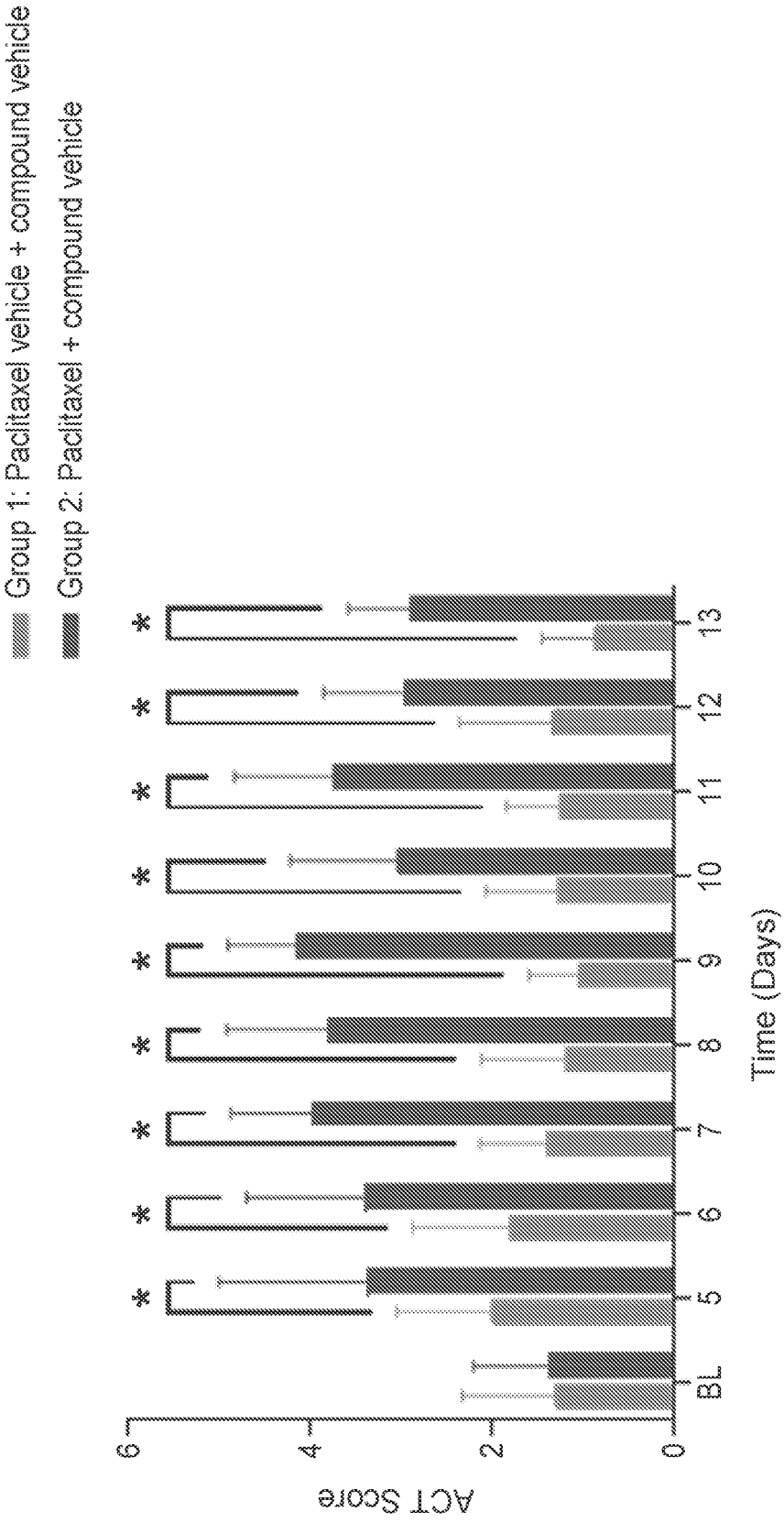
Group 1: Paclitaxel vehicle + compound vehicle
Group 2: Paclitaxel + compound vehicle
ACT Score
0
2
4
6
BL
5
6
7
8
9
10
11
12
13
Time (Days)
*

FIG. 15

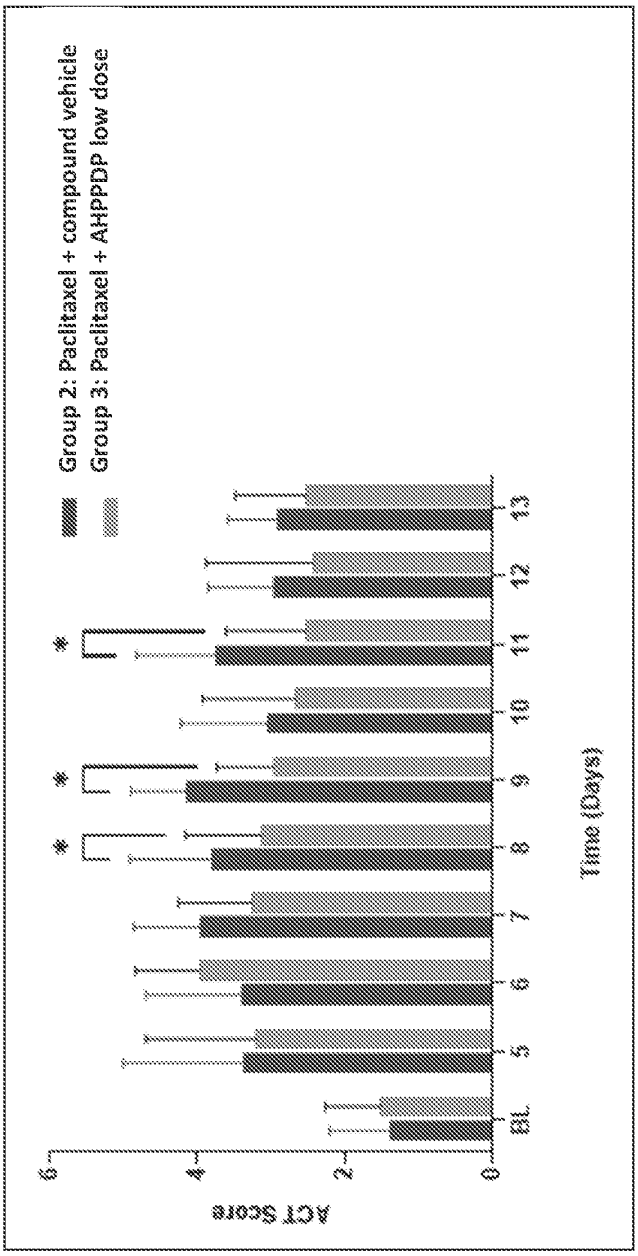
Group 2: Paclitaxel + compound vehicle
Group 3: Paclitaxel + AHPPDP low dose
ACT Score
0
2
4
6
BL
5
6
7
8
9
10
11
12
13
Time (Days)
*
*
*

FIG. 16

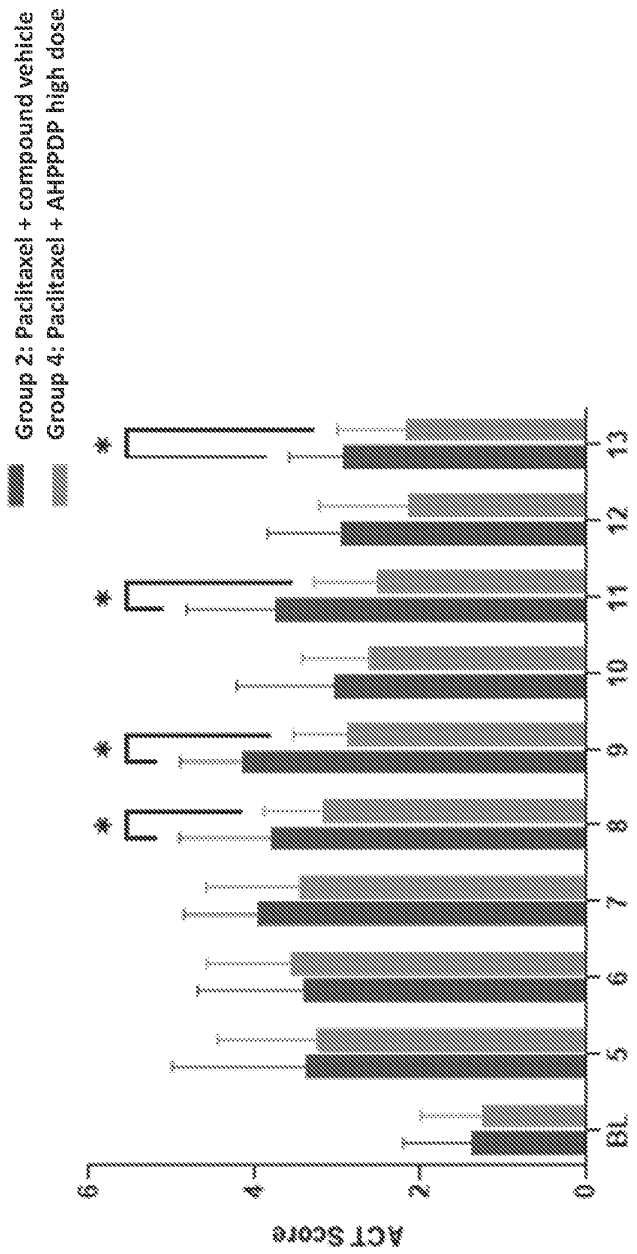
Group 2: Paclitaxel + compound vehicle
Group 4: Paclitaxel + AHPPDP high dose
ACT Score
0
2
4
6
BL
5
6
7
8
9
10
11
12
13
Time (Days)
*
*
*
*

FIG. 17

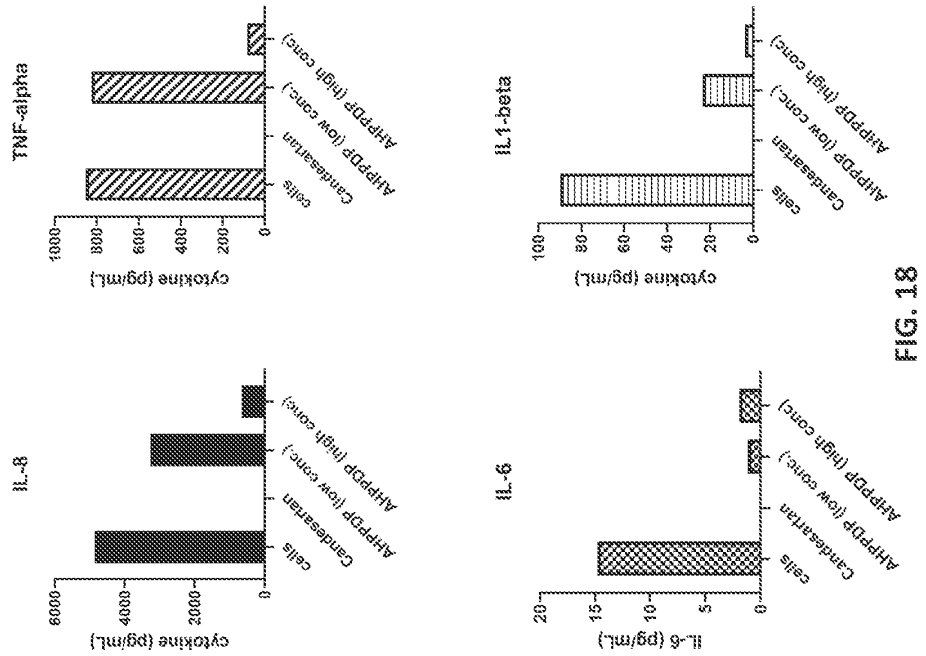
TNF-alpha
cytokine (pg/mL)
0
200
400
600
800
1000
cells
Candesartan
AHPPDP (low conc.)
AHPPDP (high conc.)
IL-8
cytokine (pg/mL)
0
2000
4000
6000
cells
Candesartan
AHPPDP (low conc.)
AHPPDP (high conc.)
IL1-beta
cytokine (pg/mL)
0
20
40
60
80
100
cells
Candesartan
AHPPDP (low conc.)
AHPPDP (high conc.)
IL-6
IL-6 (pg/mL)
0
5
10
15
20
cells
Candesartan
AHPPDP (low conc.)
AHPPDP (high conc.)

FIG. 18

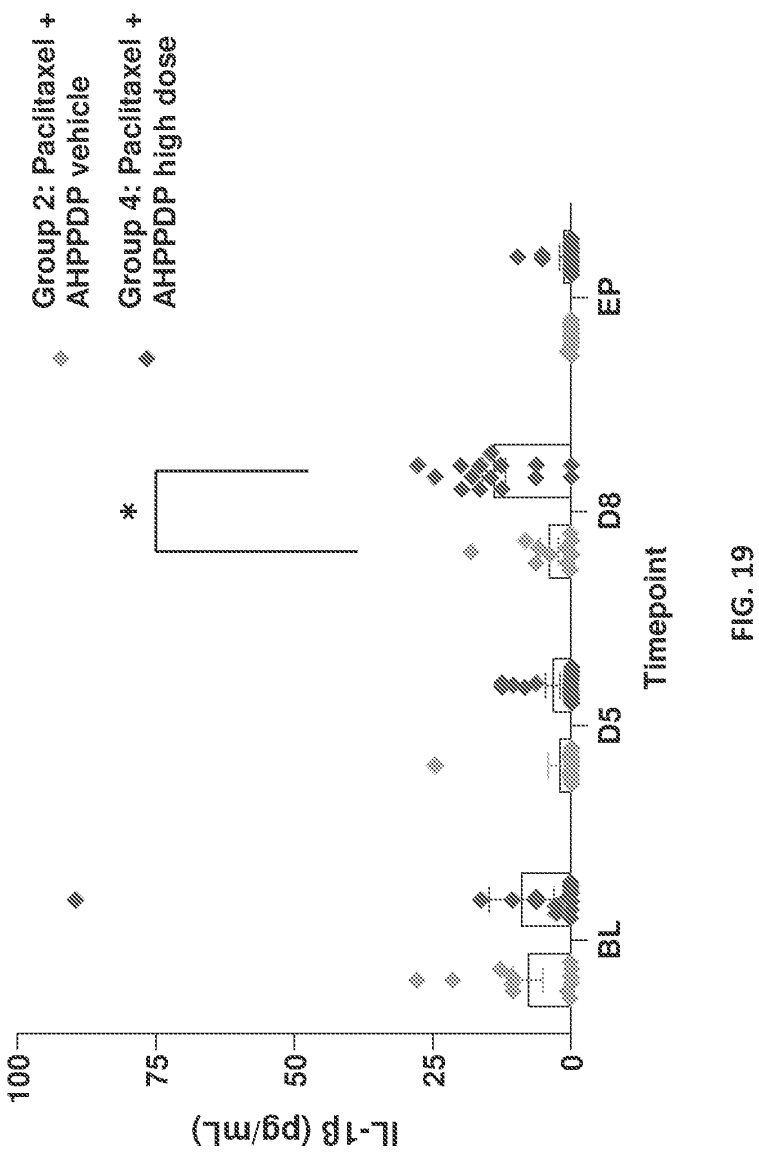
Group 2: Paclitaxel + AHPPDP vehicle
Group 4: Paclitaxel + AHPPDP high dose
*
IL-1β (pg/mL)
100
75
50
25
0
BL
D5
D8
EP
Timepoint

FIG. 19

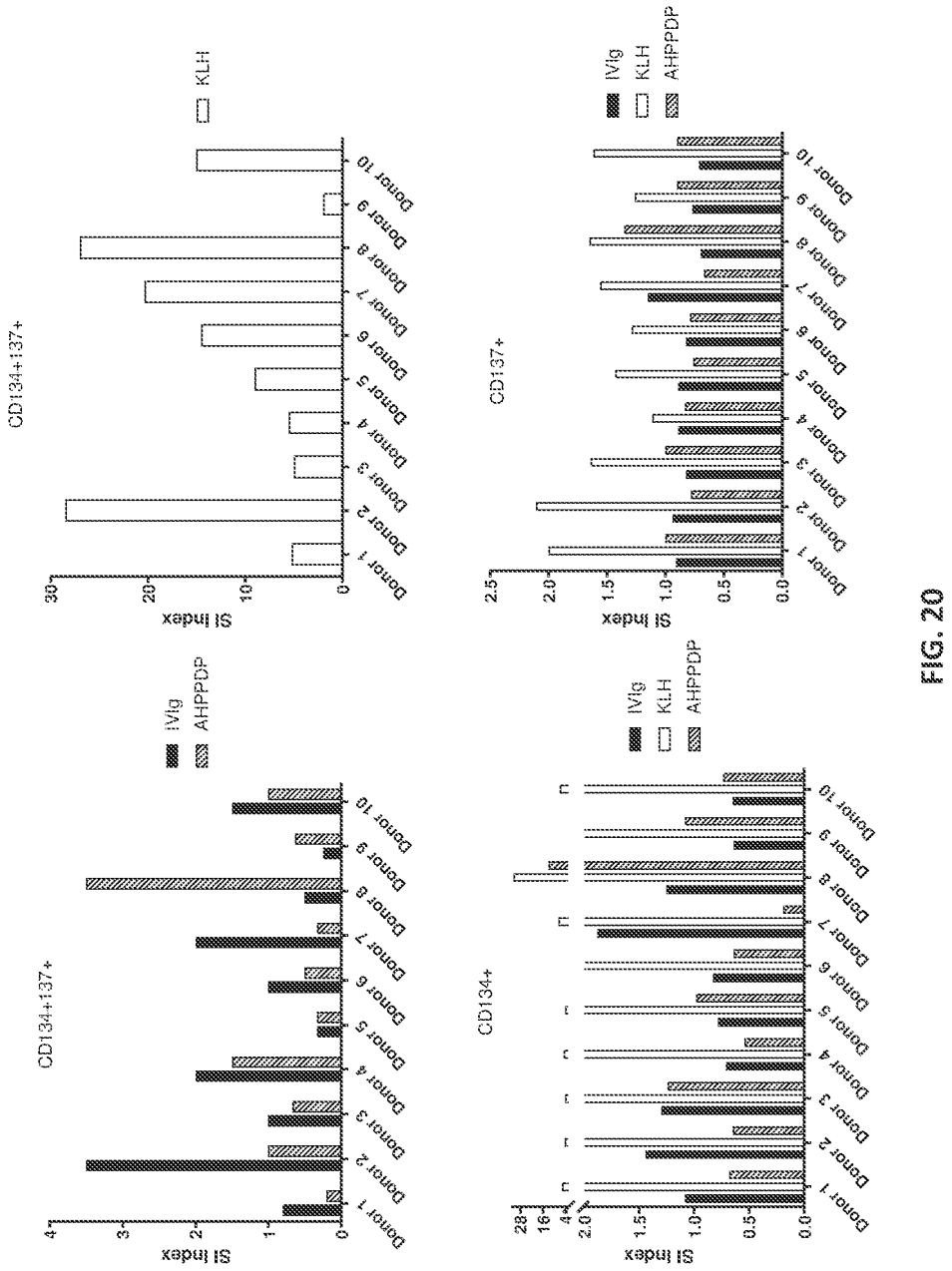
CD134+137+
KLH
SI Index
Donor 1
Donor 2
Donor 3
Donor 4
Donor 5
Donor 6
Donor 7
Donor 8
Donor 9
Donor 10
CD137+
IVIg
KLH
AHPPDP
CD134+137+
IVIg
AHPPDP
CD134+

FIG. 20

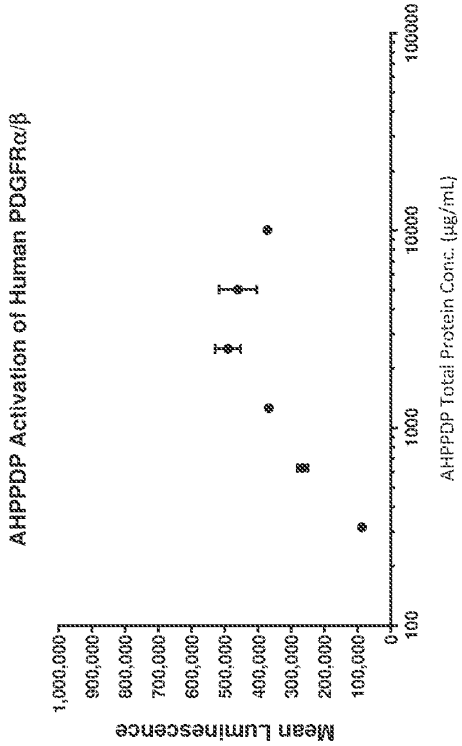

FIG. 21B

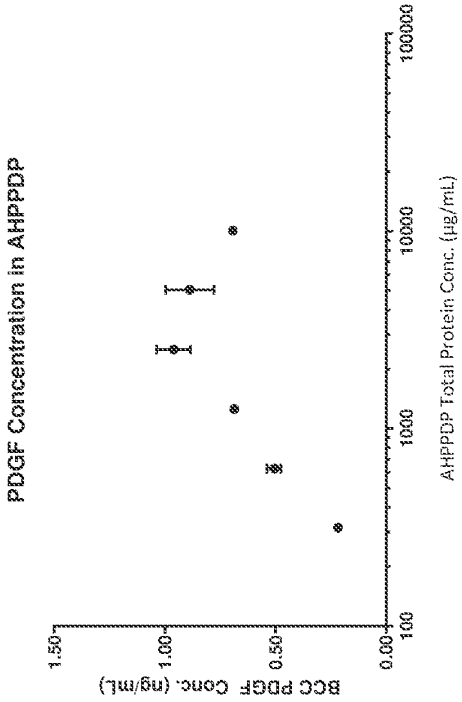

FIG. 21A

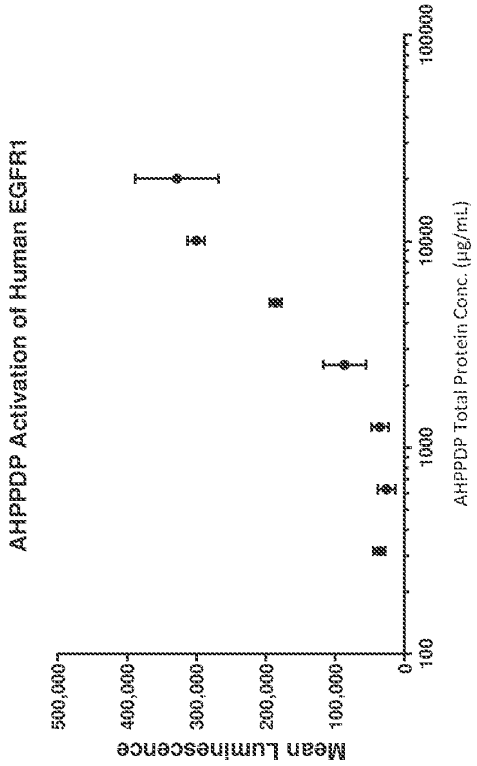

FIG. 21D

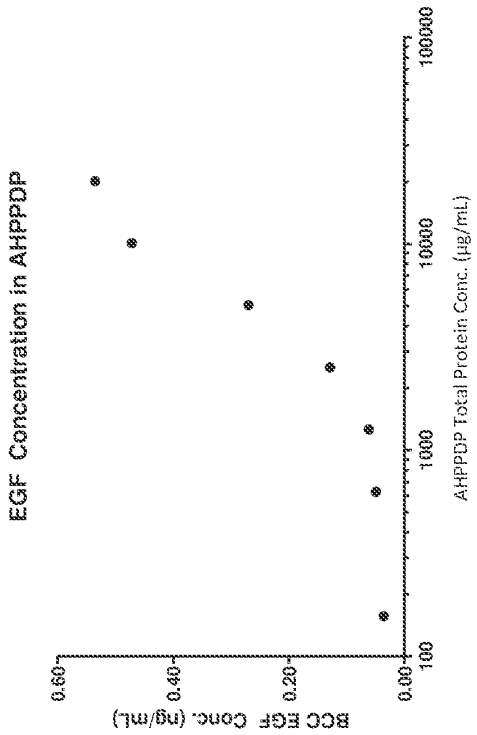

FIG. 21C

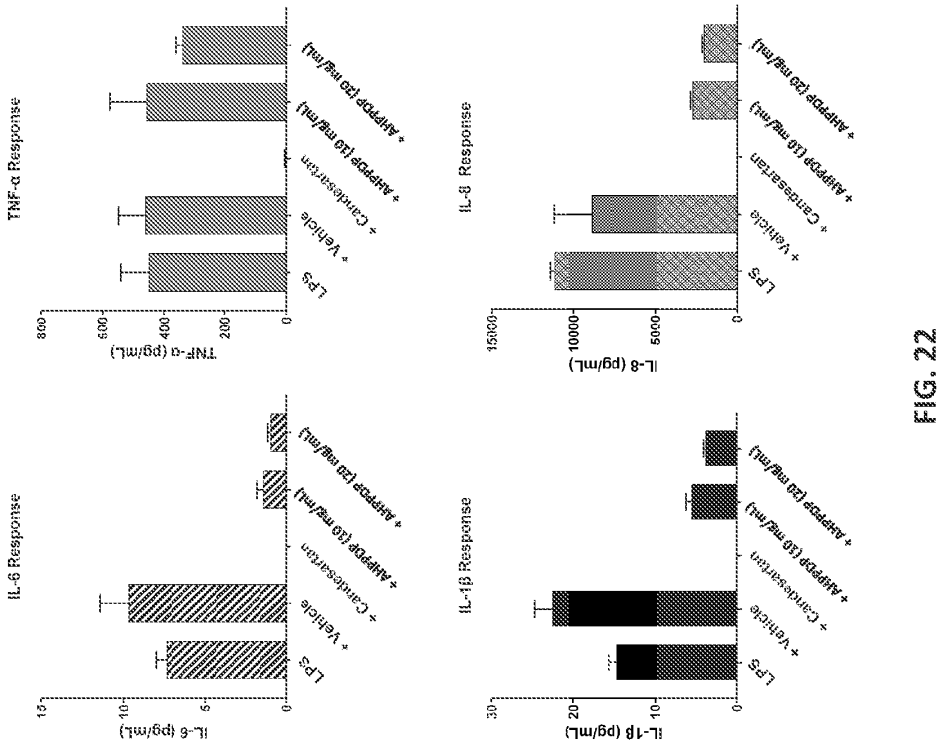
TNF-α Response
TNF-α (pg/mL)
800
600
400
200
0
LPS
+ Vehicle
+ Candesartan
+ AHPPDP (150 mg/mL)
+ AHPPDP (250 mg/mL)
IL-6 Response
IL-6 (pg/mL)
15
10
5
0
LPS
+ Vehicle
+ Candesartan
+ AHPPDP (150 mg/mL)
+ AHPPDP (250 mg/mL)
IL-8 Response
IL-8 (pg/mL)
15000
10000
5000
0
LPS
+ Vehicle
+ Candesartan
+ AHPPDP (150 mg/mL)
+ AHPPDP (250 mg/mL)
IL-1β Response
IL-1β (pg/mL)
30
20
10
0
LPS
+ Vehicle
+ Candesartan
+ AHPPDP (150 mg/mL)
+ AHPPDP (250 mg/mL)

FIG. 22

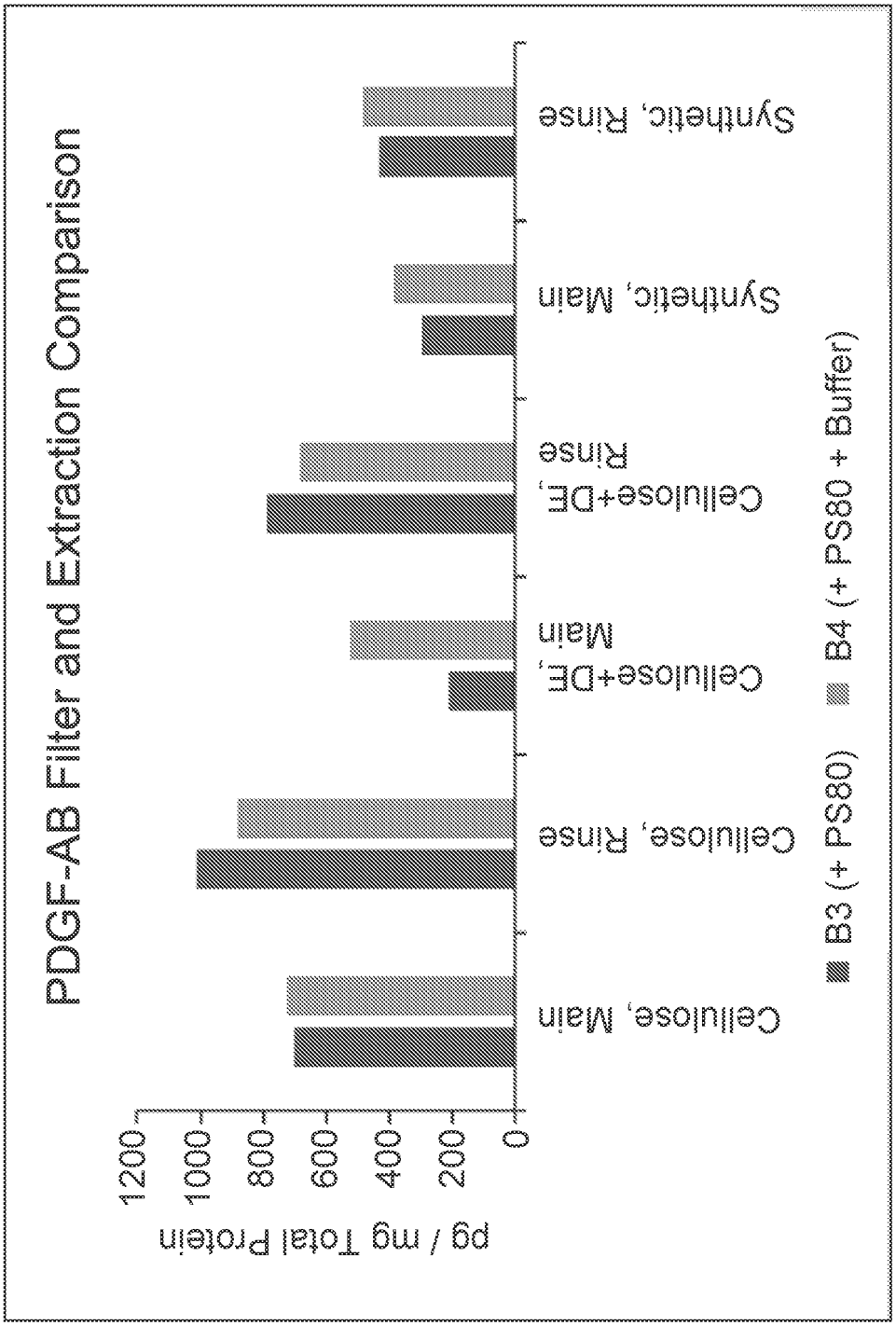
PDGF-AB Filter and Extraction Comparison
pg / mg Total Protein
0
200
400
600
800
1000
1200
Cellulose, Main
Cellulose, Rinse
Cellulose+DE, Main
Cellulose+DE, Rinse
Synthetic, Main
Synthetic, Rinse
B3 (+ PS80)
B4 (+ PS80 + Buffer)

FIG. 23

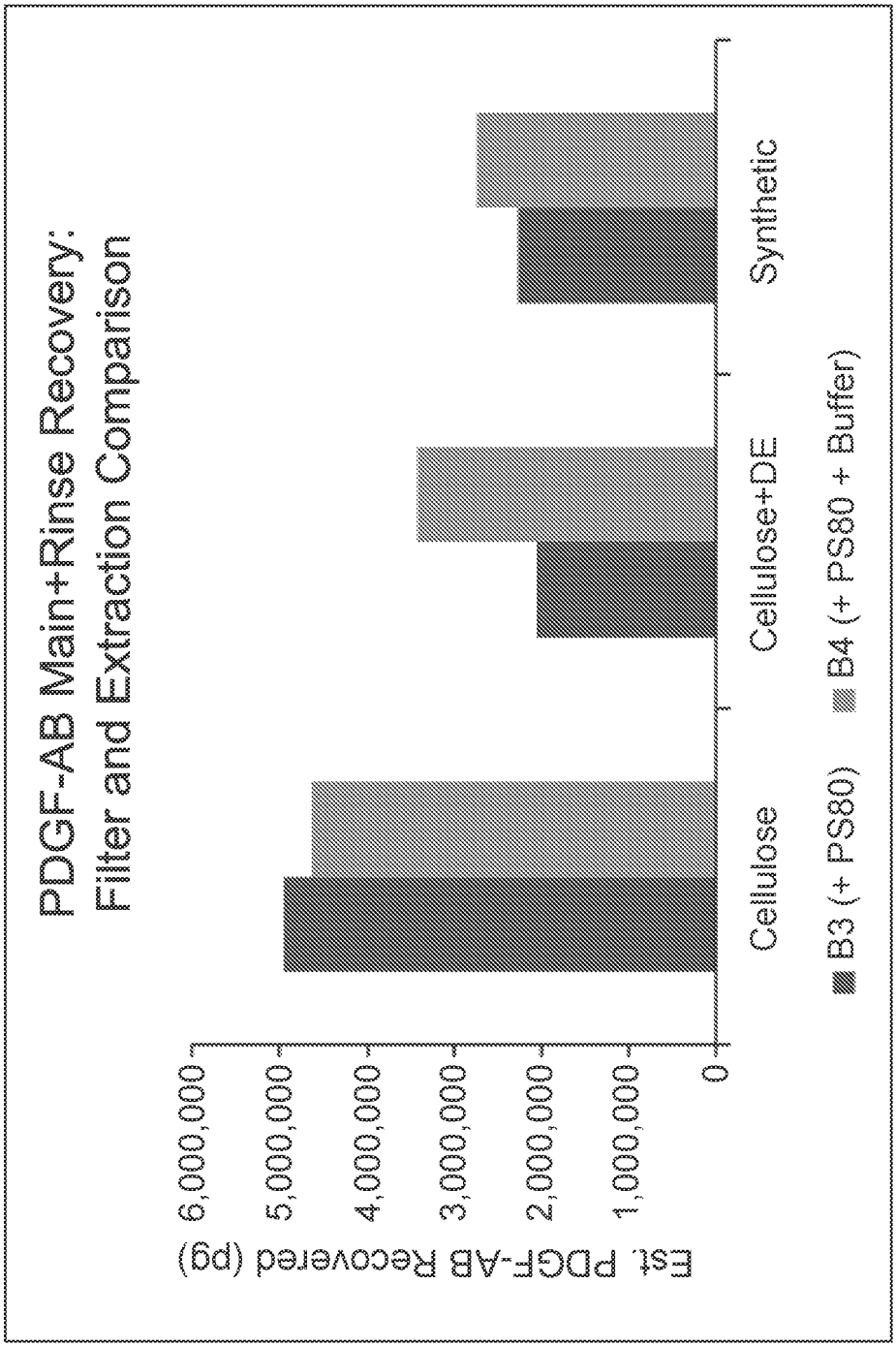
PDGF-AB Main+Rinse Recovery:
Filter and Extraction Comparison
Est. PDGF-AB Recovered (pg)
6,000,000
5,000,000
4,000,000
3,000,000
2,000,000
1,000,000
0
Cellulose
Cellulose+DE
Synthetic
B3 (+ PS80)
B4 (+ PS80 + Buffer)

FIG. 24

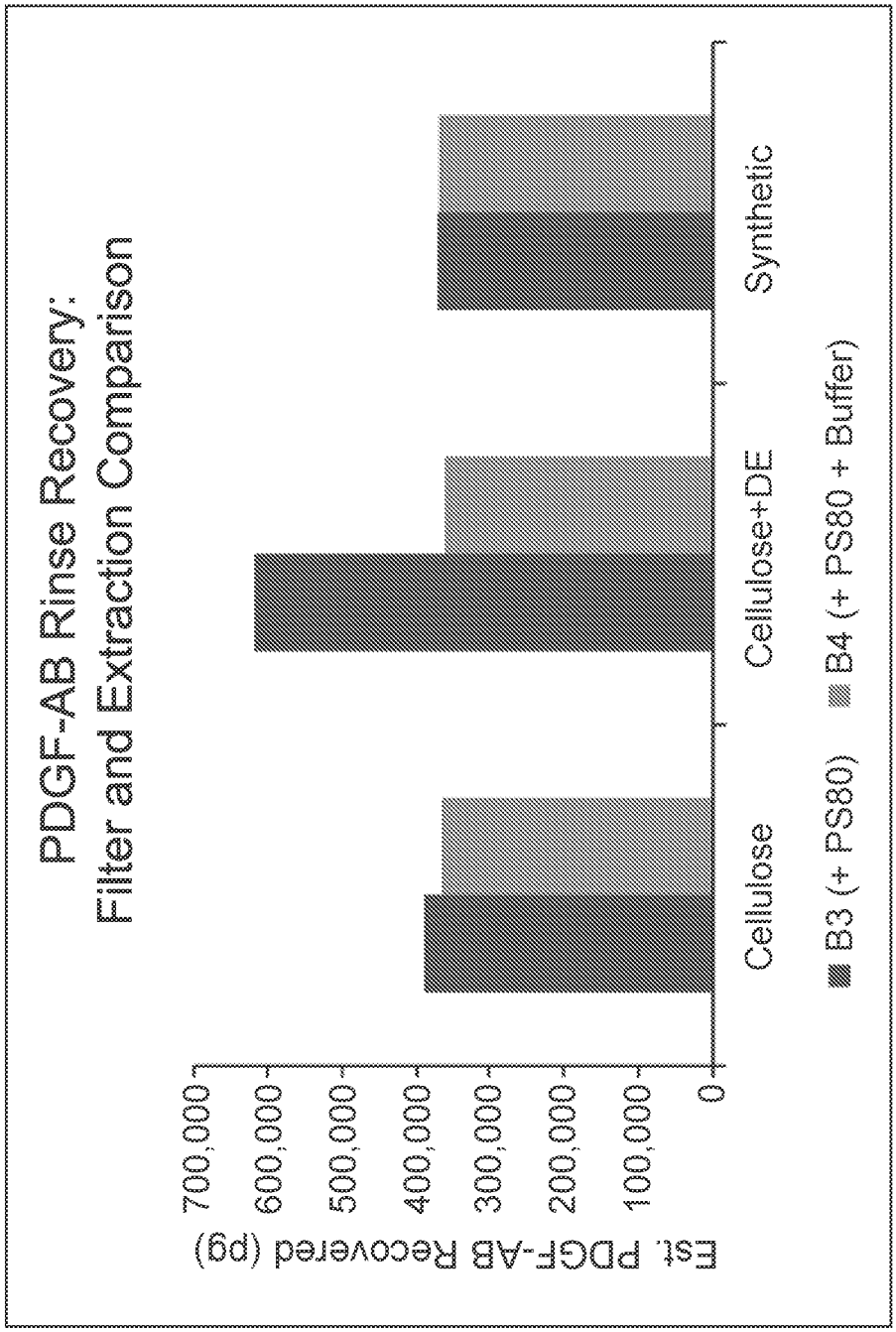
PDGF-AB Rinse Recovery:
Filter and Extraction Comparison
Est. PDGF-AB Recovered (pg)
700,000
600,000
500,000
400,000
300,000
200,000
100,000
0
Cellulose
Cellulose+DE
Synthetic
B3 (+ PS80)
B4 (+ PS80 + Buffer)

FIG. 25

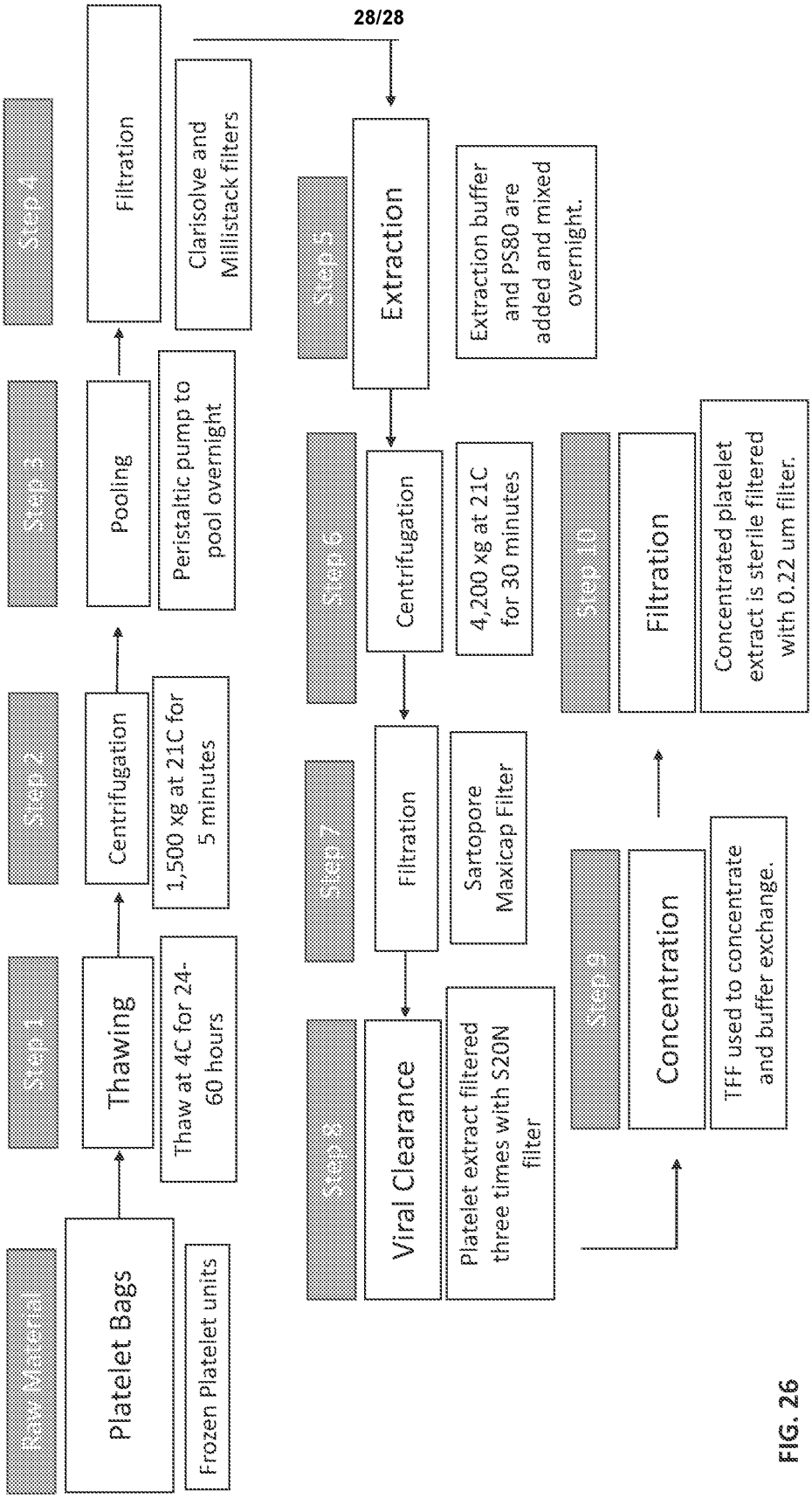
Raw Material
Platelet Bags
Frozen Platelet units
Step 1
Thawing
Thaw at 4C for 24-60 hours
Step 2
Centrifugation
1,500 xg at 21C for 5 minutes
Step 3
Pooling
Peristaltic pump to pool overnight
Step 4
Filtration
Clarisolve and Millistack filters
Step 5
Extraction
Extraction buffer and PS80 are added and mixed overnight.
Step 6
Centrifugation
4,200 xg at 21C for 30 minutes
Step 7
Filtration
Sartopore Maxicap Filter
Step 8
Viral Clearance
Platelet extract filtered three times with S20N filter
Step 9
Concentration
TFF used to concentrate and buffer exchange.
Step 10
Filtration
Concentrated platelet extract is sterile filtered with 0.22 um filter.

FIG. 26