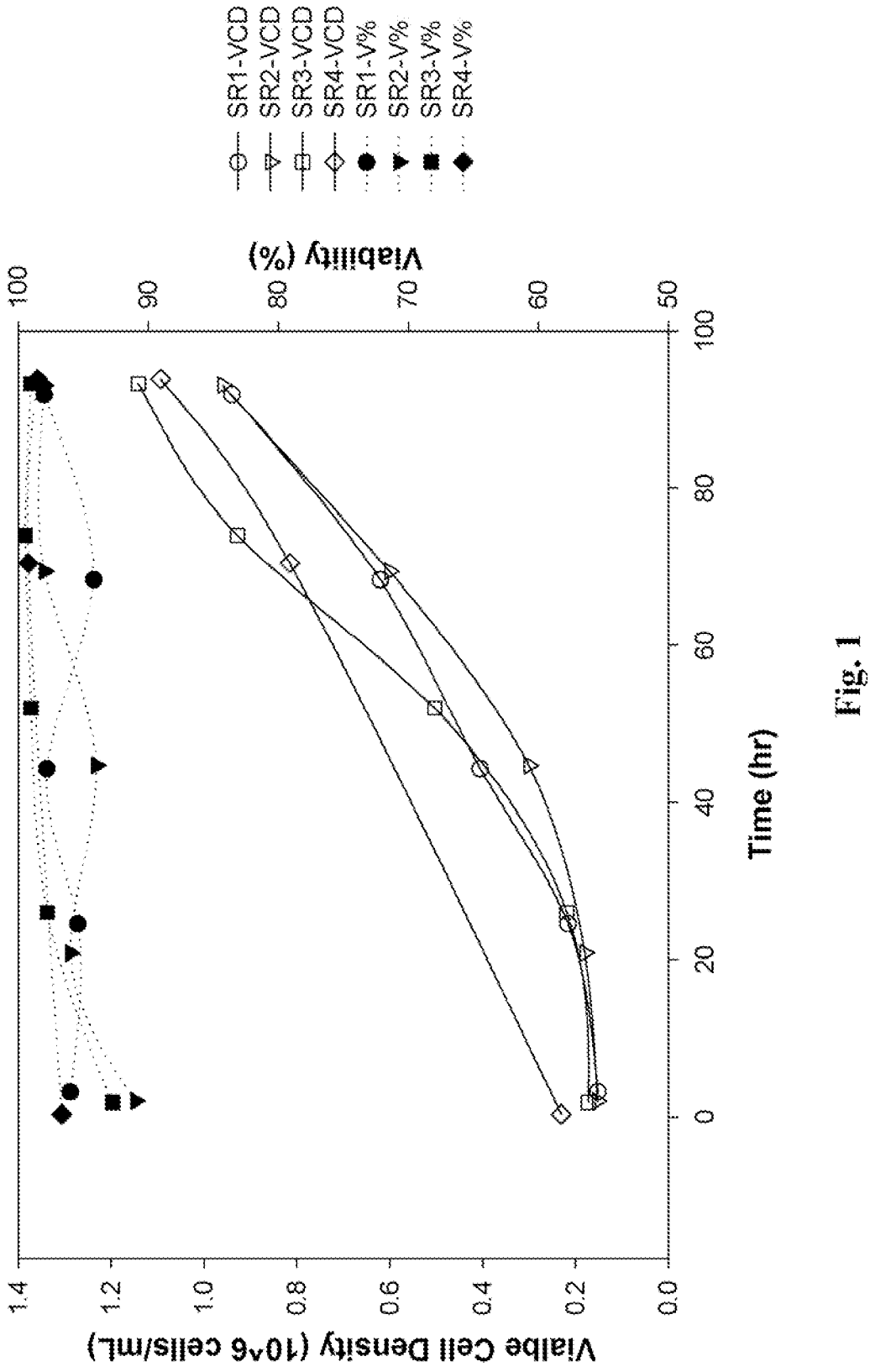

SR1-VCD
SR2-VCD
SR3-VCD
SR4-VCD
SR1-V%
SR2-V%
SR3-V%
SR4-V%
Viable Cell Density (10^6 cells/mL)
Viability (%)
Time (hr)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
50
60
70
80
90
100
0
20
40
60
80
100

Fig. 1

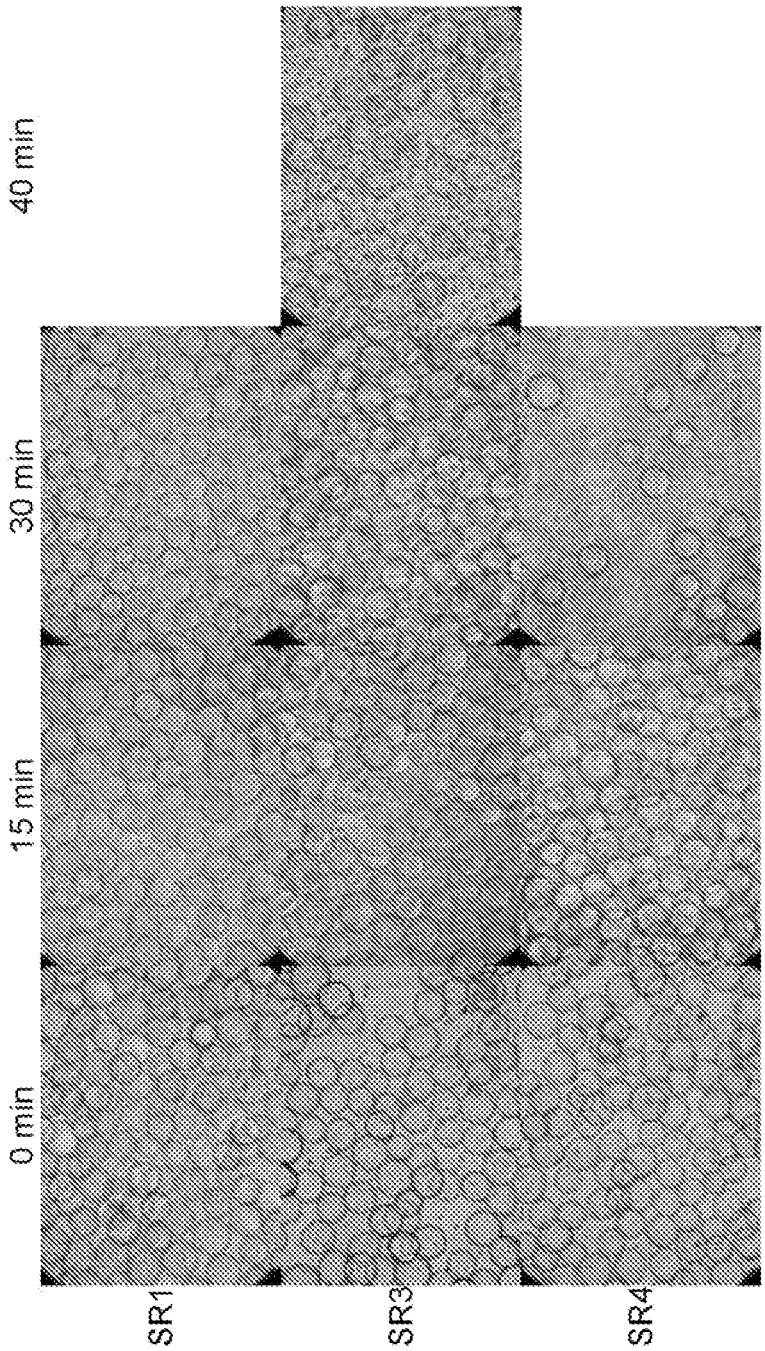


Fig. 2

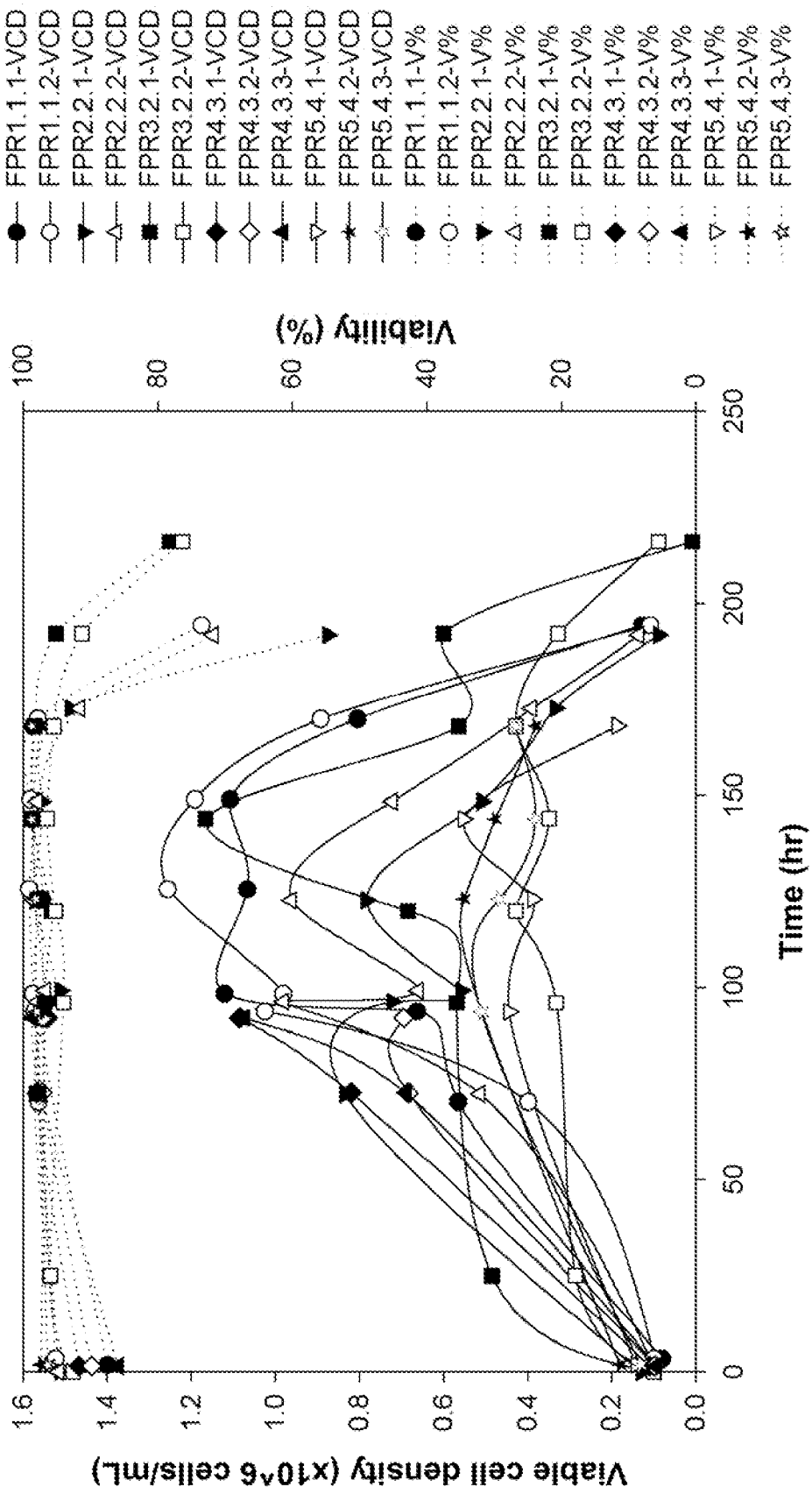

FPR1.1.1-VCD
FPR1.1.2-VCD
FPR2.2.1-VCD
FPR2.2.2-VCD
FPR3.2.1-VCD
FPR3.2.2-VCD
FPR4.3.1-VCD
FPR4.3.2-VCD
FPR4.3.3-VCD
FPR5.4.1-VCD
FPR5.4.2-VCD
FPR5.4.3-VCD
FPR1.1.1-V%
FPR1.1.2-V%
FPR2.2.1-V%
FPR2.2.2-V%
FPR3.2.1-V%
FPR3.2.2-V%
FPR4.3.1-V%
FPR4.3.2-V%
FPR4.3.3-V%
FPR5.4.1-V%
FPR5.4.2-V%
FPR5.4.3-V%
Viable cell density (x10^6 cells/mL)
Viability (%)
Time (hr)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
0
20
40
60
80
100
0
50
100
150
200
250

Fig. 3

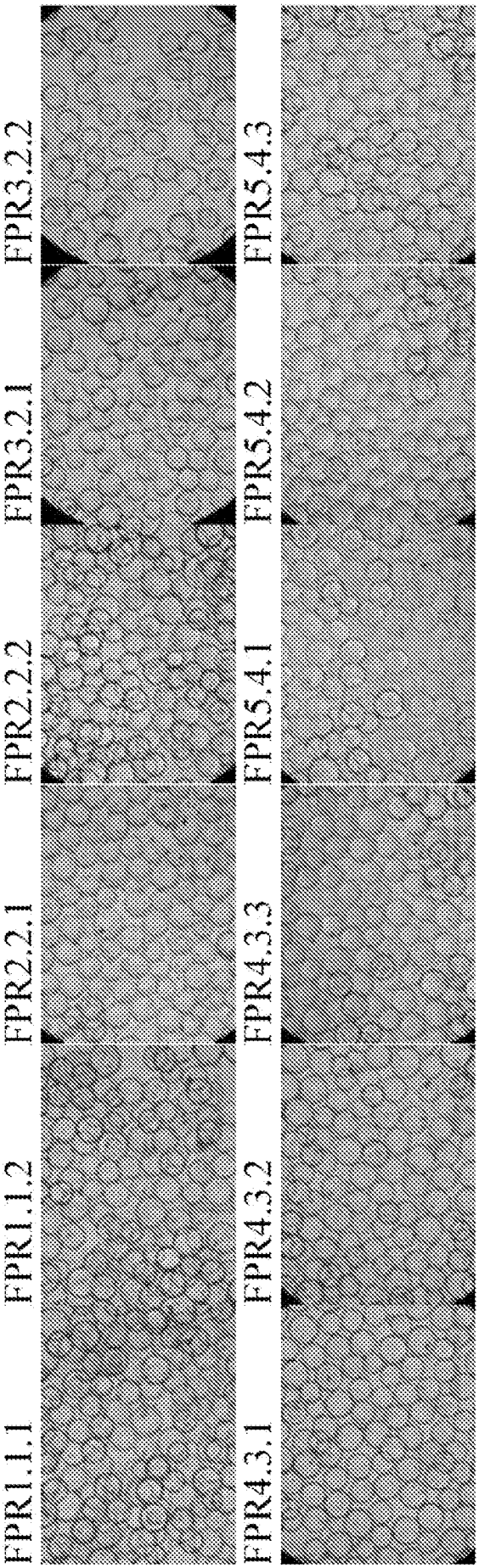

FPR1.1.1
FPR1.1.2
FPR2.2.1
FPR2.2.2
FPR3.2.1
FPR3.2.2
FPR4.3.1
FPR4.3.2
FPR4.3.3
FPR5.4.1
FPR5.4.2
FPR5.4.3

Fig. 4

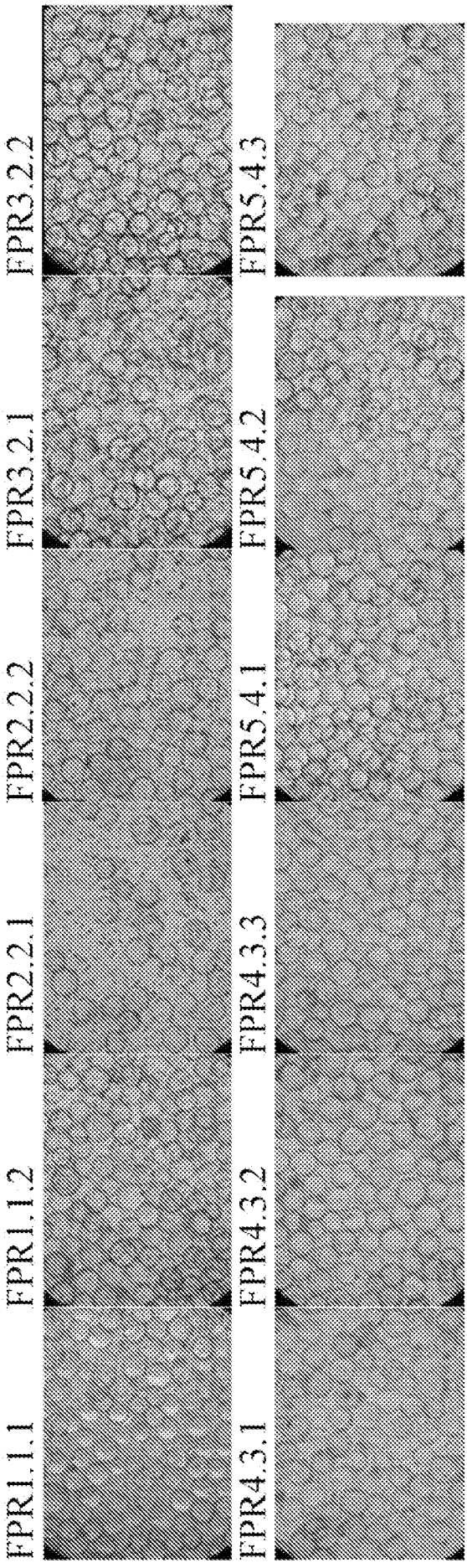

FPR1.1.1
FPR1.1.2
FPR2.2.1
FPR2.2.2
FPR3.2.1
FPR3.2.2
FPR4.3.1
FPR4.3.2
FPR4.3.3
FPR5.4.1
FPR5.4.2
FPR5.4.3

Fig. 5

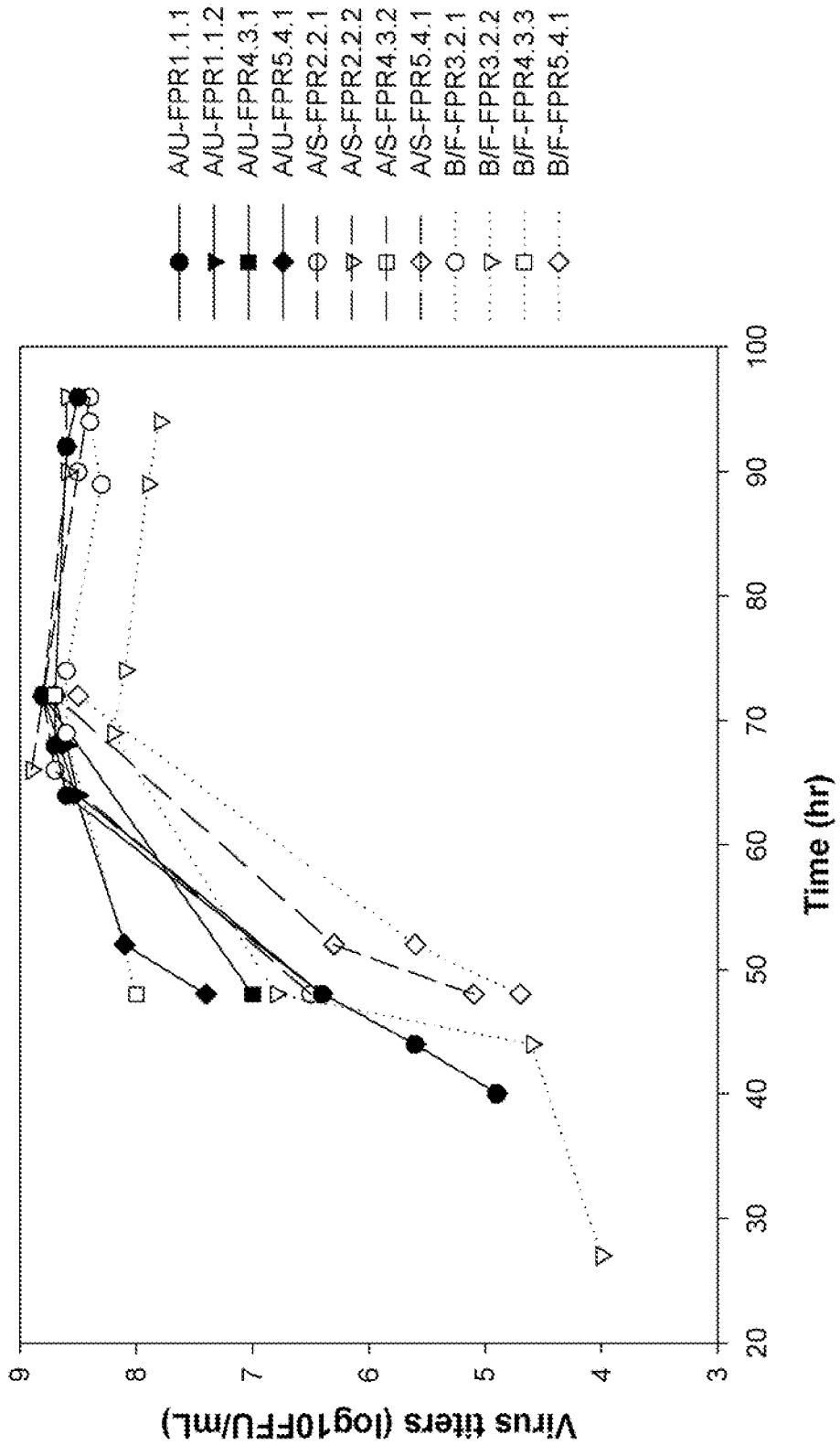

A/U-FPR1.1.1
A/U-FPR1.1.2
A/U-FPR4.3.1
A/U-FPR5.4.1
A/S-FPR2.2.1
A/S-FPR2.2.2
A/S-FPR4.3.2
A/S-FPR5.4.1
B/F-FPR3.2.1
B/F-FPR3.2.2
B/F-FPR4.3.3
B/F-FPR5.4.1
Virus titers (log10FFU/mL)
Time (hr)
9
8
7
6
5
4
3
20
30
40
50
60
70
80
90
100

Fig. 6

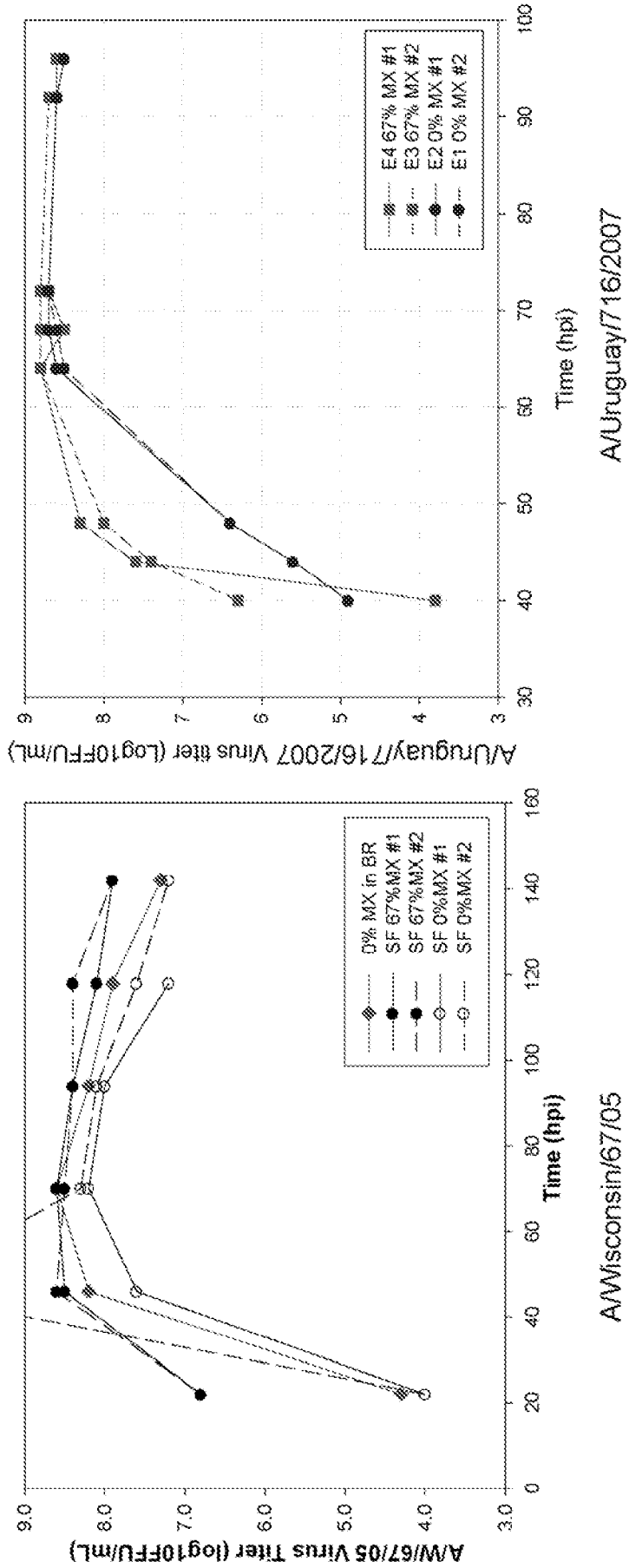
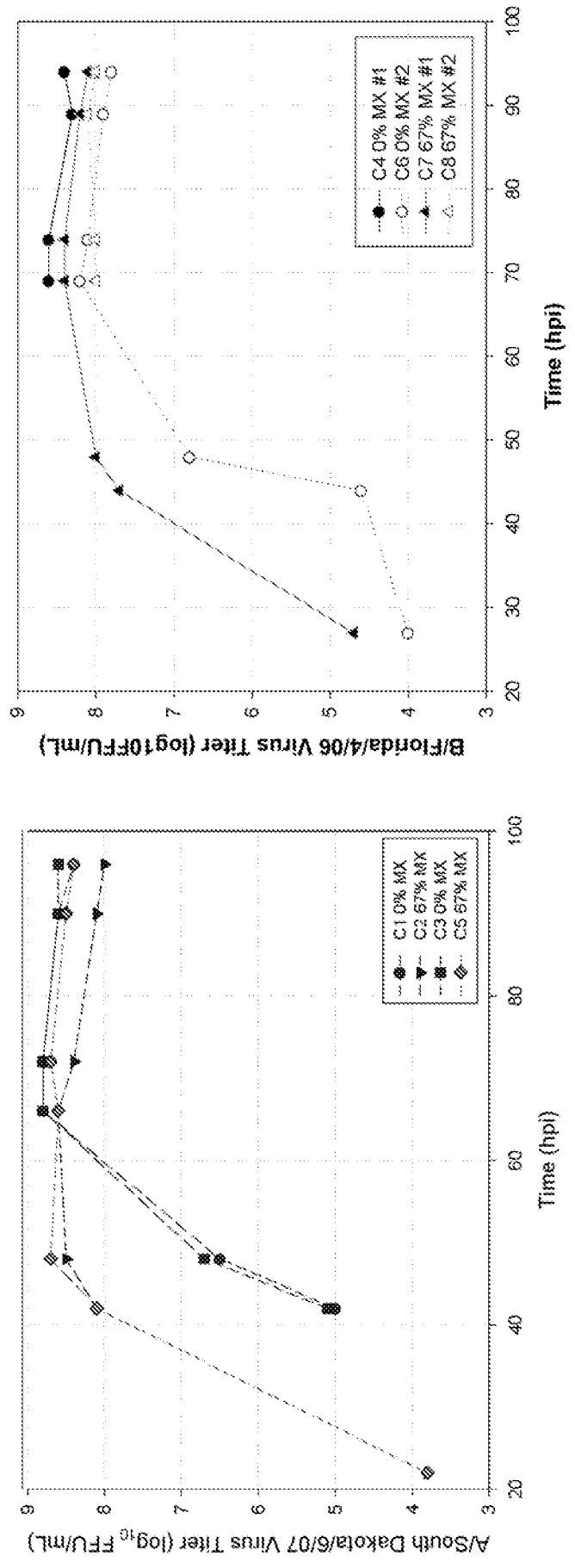

A/Uruguay/716/2007 Virus titer (Log10FFU/mL)
Time (hpi)
E4 67% MX #1
E3 67% MX #2
E2 0% MX #1
E1 0% MX #2
A/Uruguay/716/2007
A/W/67/05 Virus Titer (log10FFU/mL)
Time (hpi)
0% MX in BR
SF 67%MX #1
SF 67%MX #2
SF 0%MX #1
SF 0%MX #2
A/Wisconsin/67/05

Fig. 7A

Sheet 9/#



B/Florida/4/06

A/South Dakota/6/07

Fig. 7B

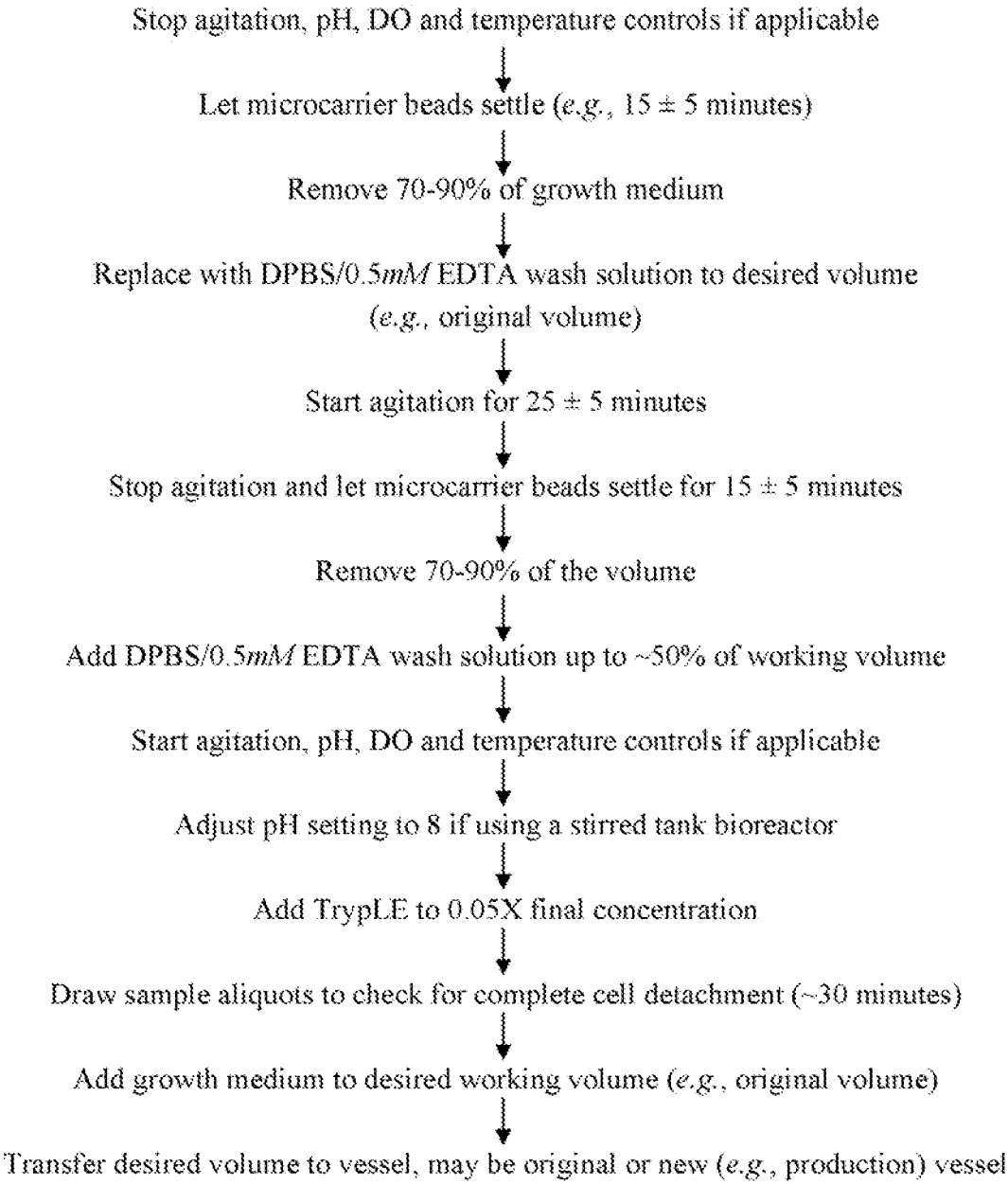

Stop agitation, pH, DO and temperature controls if applicable
Let microcarrier beads settle (*e.g.*, 15 ± 5 minutes)
Remove 70-90% of growth medium
Replace with DPBS/0.5*mM* EDTA wash solution to desired volume (*e.g.*, original volume)
Start agitation for 25 ± 5 minutes
Stop agitation and let microcarrier beads settle for 15 ± 5 minutes
Remove 70-90% of the volume
Add DPBS/0.5*mM* EDTA wash solution up to ~50% of working volume
Start agitation, pH, DO and temperature controls if applicable
Adjust pH setting to 8 if using a stirred tank bioreactor
Add TrypLE to 0.05X final concentration
Draw sample aliquots to check for complete cell detachment (~30 minutes)
Add growth medium to desired working volume (*e.g.*, original volume)
Transfer desired volume to vessel, may be original or new (*e.g.*, production) vessel

Fig. 8A

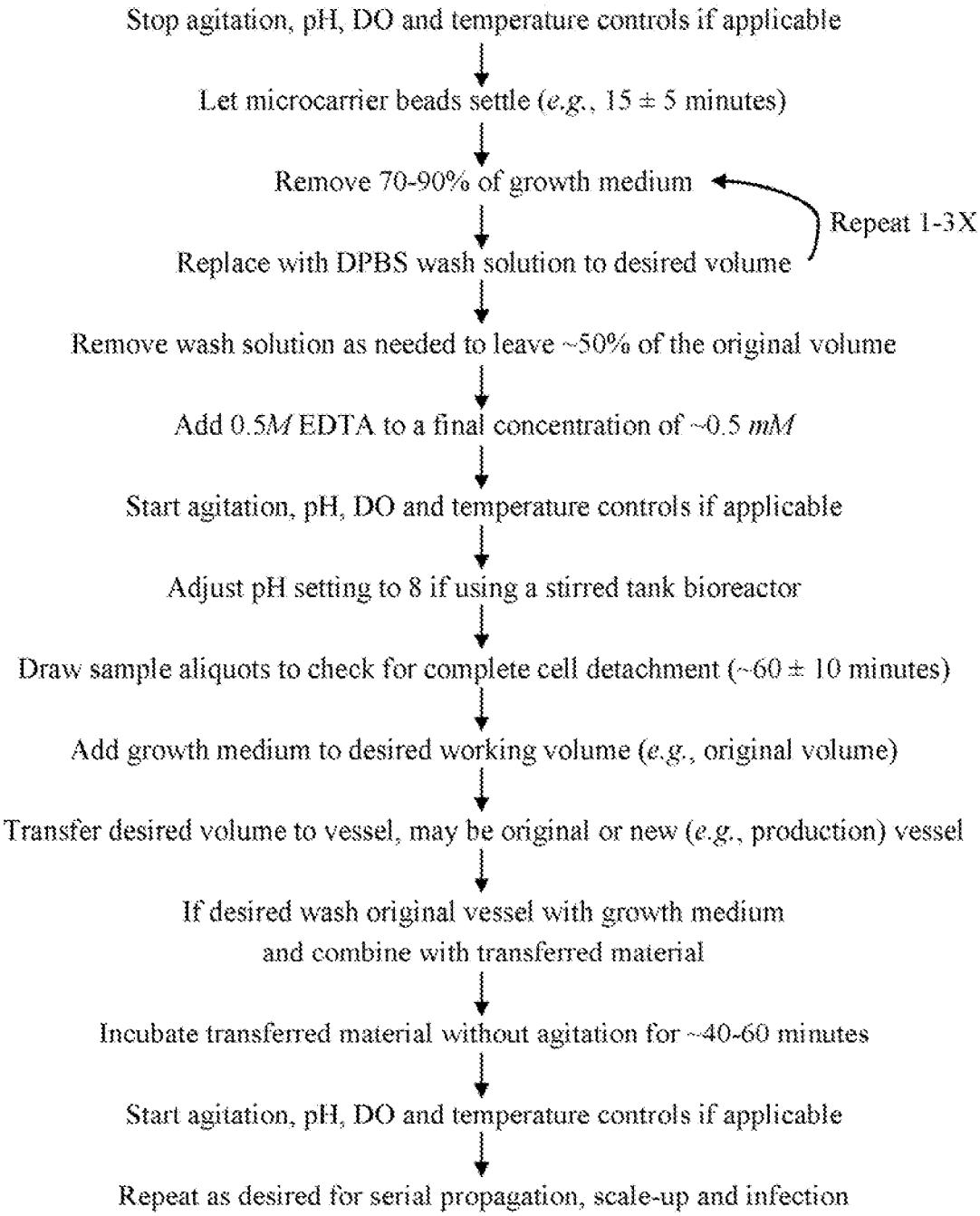
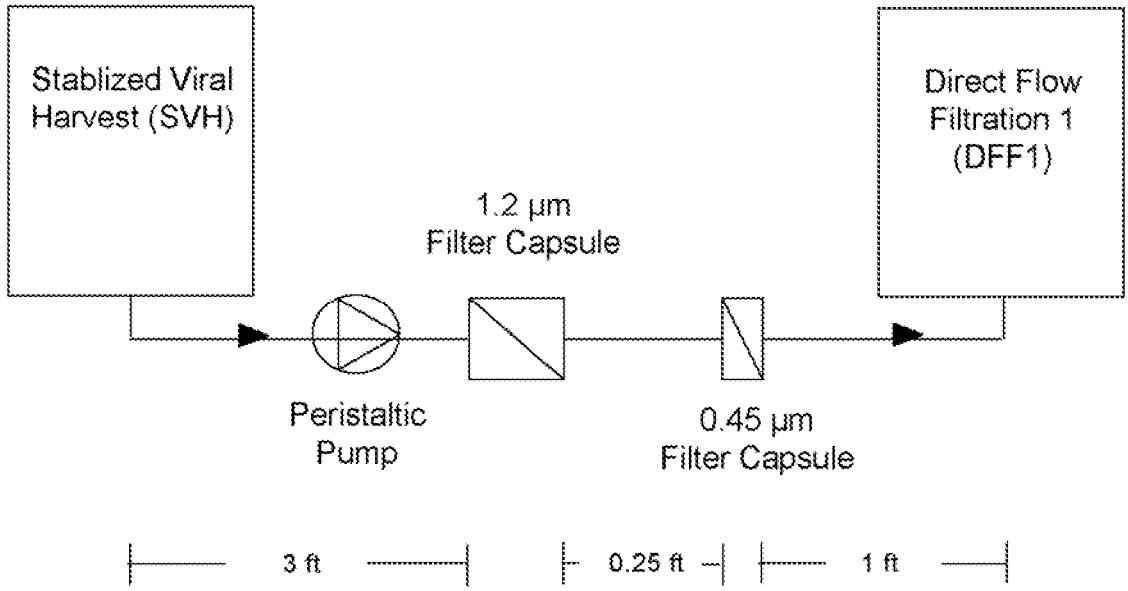

Stop agitation, pH, DO and temperature controls if applicable
Let microcarrier beads settle (e.g., 15 ± 5 minutes)
Remove 70-90% of growth medium
Repeat 1-3X
Replace with DPBS wash solution to desired volume
Remove wash solution as needed to leave ~50% of the original volume
Add 0.5M EDTA to a final concentration of ~0.5 mM
Start agitation, pH, DO and temperature controls if applicable
Adjust pH setting to 8 if using a stirred tank bioreactor
Draw sample aliquots to check for complete cell detachment (~60 ± 10 minutes)
Add growth medium to desired working volume (e.g., original volume)
Transfer desired volume to vessel, may be original or new (e.g., production) vessel
If desired wash original vessel with growth medium
and combine with transferred material
Incubate transferred material without agitation for ~40-60 minutes
Start agitation, pH, DO and temperature controls if applicable
Repeat as desired for serial propagation, scale-up and infection

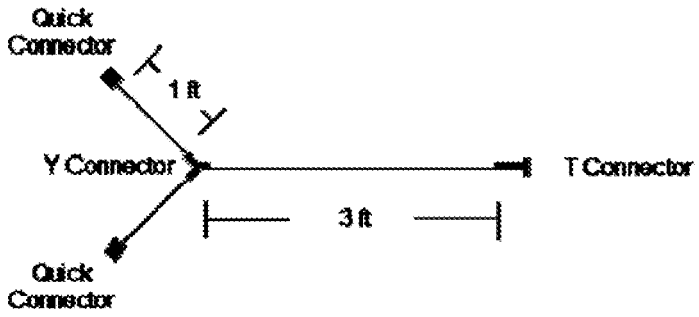
Fig. 8B

Direct Flow Filtration 1 (DFF1) Filter Rig Drawing

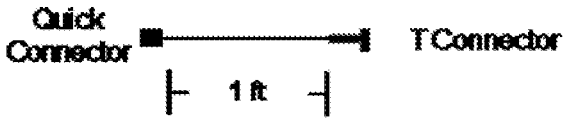


GE Healthcare Uniflux skid Tubing Rigs Drawings

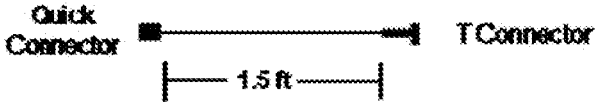
UNIFLUX Sample/Buffer Feed Tubing Rig



UNIFLUX Feed Drain Tubing Rig



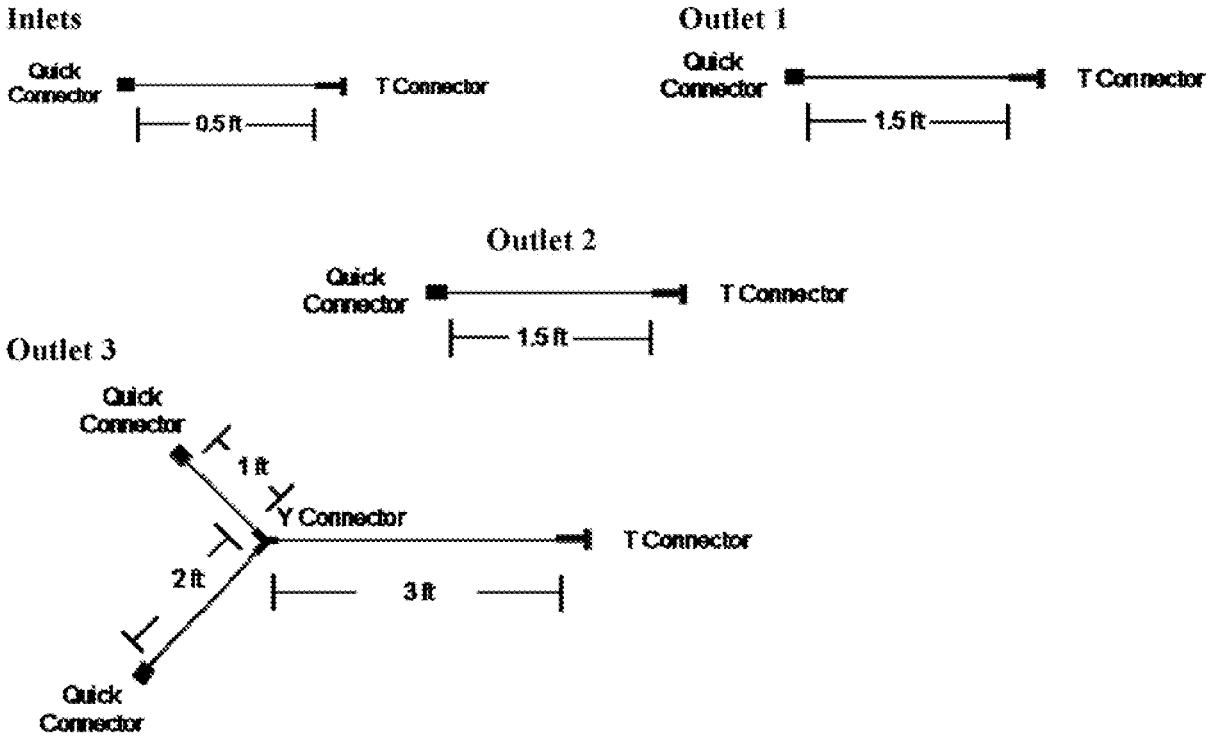
UNIFLUX Permeate Tubing Rig



NOTE: All tubing rigs were prepared with 3/8" id silicone tubings.

Fig. 9A

GE Healthcare AKTA Process skid Tubing Rigs Drawings



NOTE: All tubing rigs were prepared with 3/8" id silicone tubings.

Direct Flow Filtration 2 (DFF2) Filter Rig Drawing

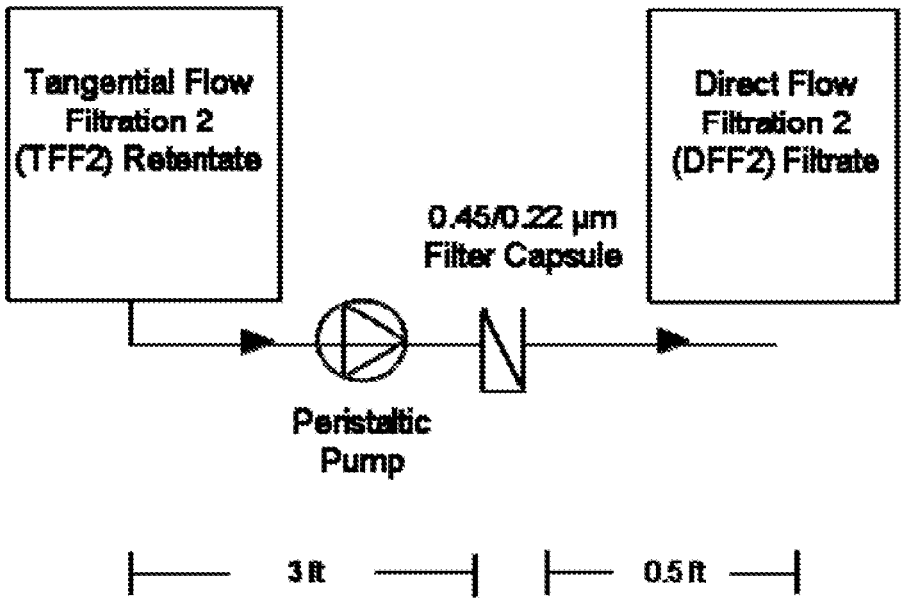


Fig. 9B

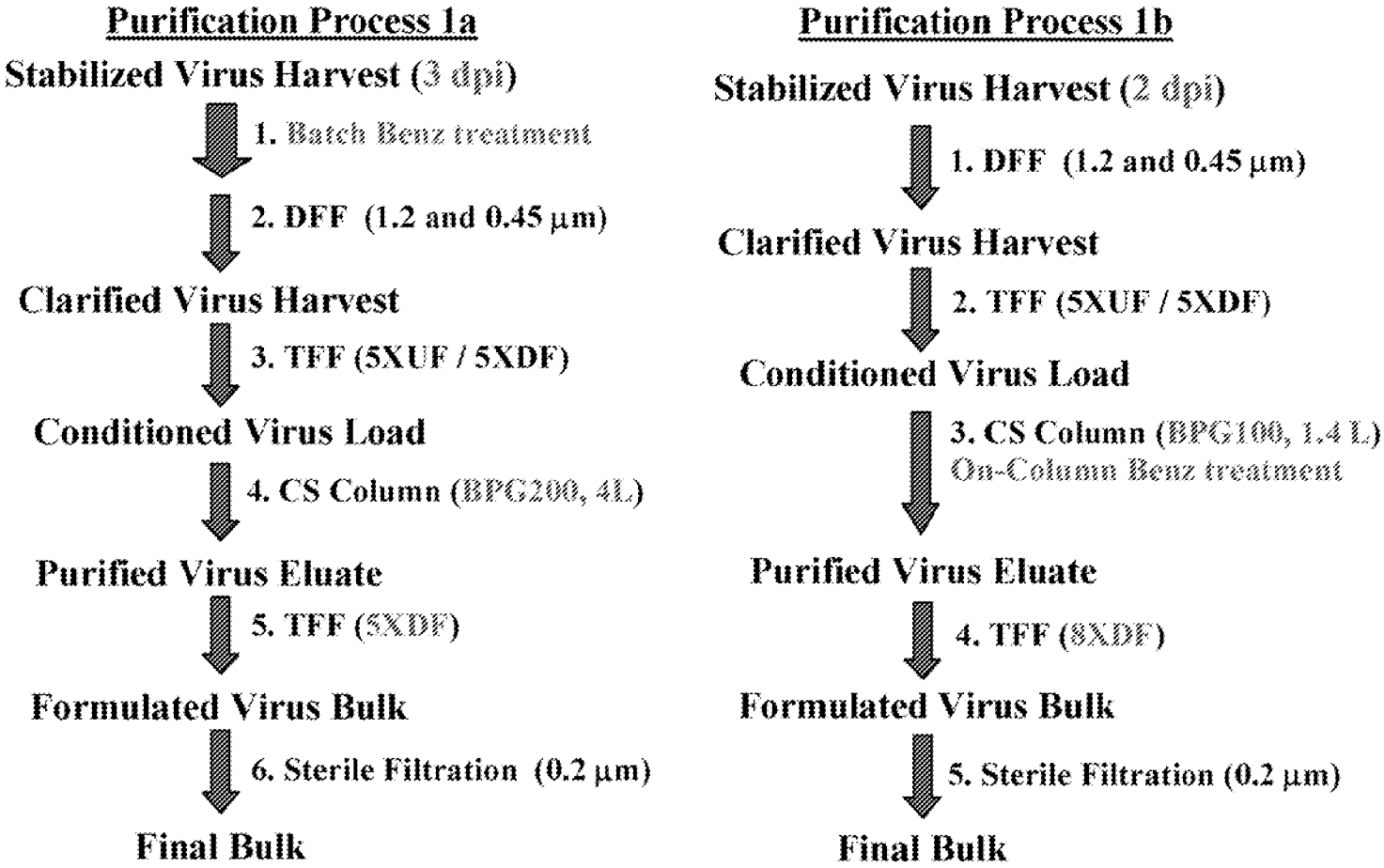

Purification Process 1a
Stabilized Virus Harvest (3 dpi)
1. Batch Benz treatment
2. DFF (1.2 and 0.45 μm)
Clarified Virus Harvest
3. TFF (5XUF / 5XDF)
Conditioned Virus Load
4. CS Column (BPG200, 4L)
Purified Virus Eluate
5. TFF (5XDF)
Formulated Virus Bulk
6. Sterile Filtration (0.2 μm)
Final Bulk
Purification Process 1b
Stabilized Virus Harvest (2 dpi)
1. DFF (1.2 and 0.45 μm)
Clarified Virus Harvest
2. TFF (5XUF / 5XDF)
Conditioned Virus Load
3. CS Column (BPG100, 1.4 L)
On-Column Benz treatment
Purified Virus Eluate
4. TFF (8XDF)
Formulated Virus Bulk
5. Sterile Filtration (0.2 μm)
Final Bulk

Fig. 10A

Modified Purification Process (Optional changes are underlined)
Media Containing Virus (2-3 dpi)
1. COUPLED STEPS
Harvest virus (ATF)
DFF (1.2 and 0.45 µm)
TFF (5X-10XUF / 5XDF; operation using constant Flux or TMP <20 psi)
Conditioned Virus Load
2. CS Column (BPG100, 1.4 L)
On-Column Benz treatment
Purified Virus Eluate
3. TFF (2XUF / 8XDF)
Formulated Virus Bulk
4. Sterile Filtration (0.2 µm)
Final Bulk

Fig. 10B

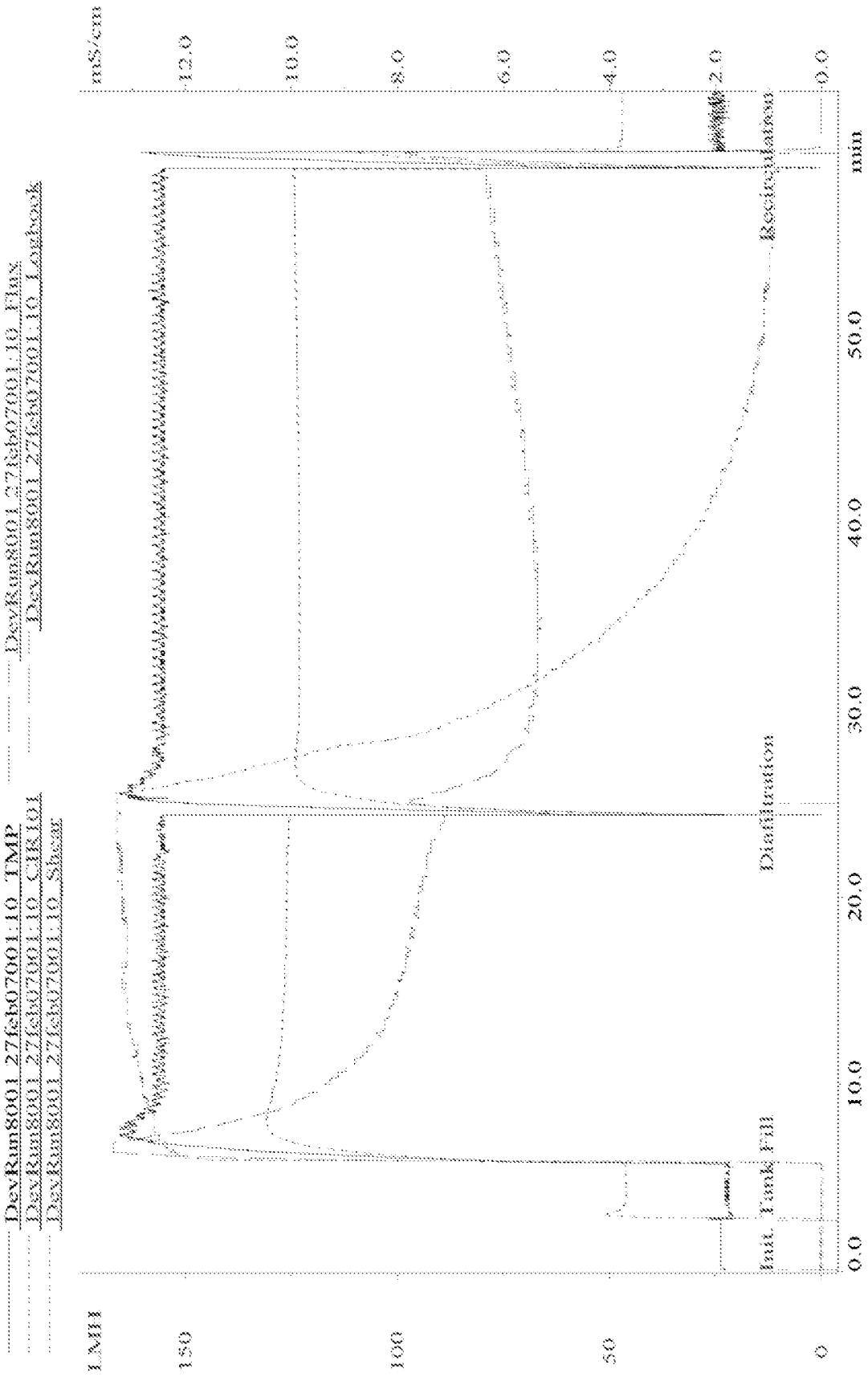

DevRun8001_27feb07001_10_TMP
DevRun8001_27feb07001_10_CIR101
DevRun8001_27feb07001_10_Shear
DevRun8001_27feb07001_10_Flux
DevRun8001_27feb07001_10_Leakback
kMH
150
100
50
0
mS/cm
12.0
10.0
8.0
6.0
4.0
2.0
0.0
0.0
10.0
20.0
30.0
40.0
50.0
min
Init. Tank Fill
Dialization
Recirculation

Fig. 11

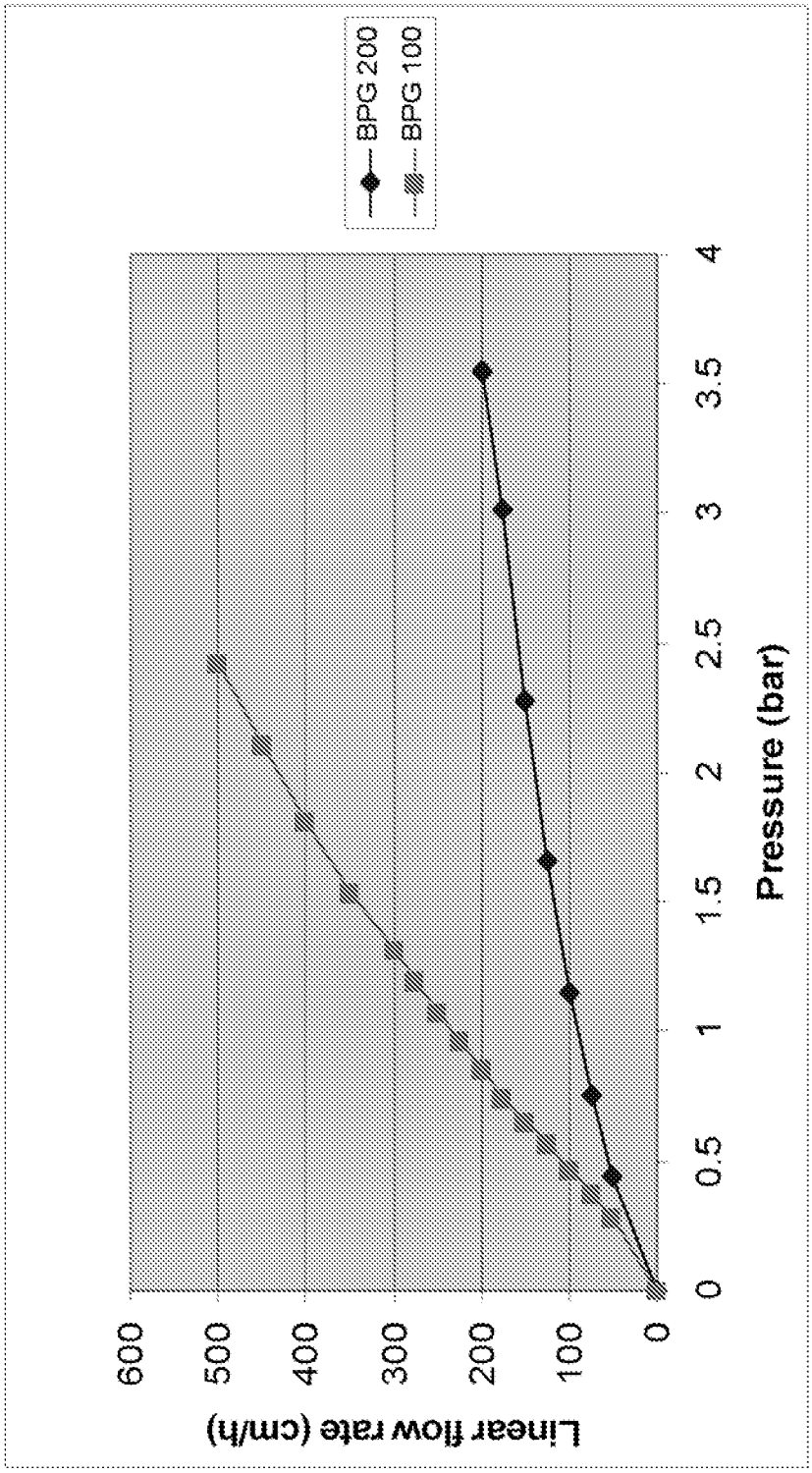

BPG 200
BPG 100
Linear flow rate (cm/h)
600
500
400
300
200
100
0
0
0.5
1
1.5
2
2.5
3
3.5
4
Pressure (bar)

Fig. 12

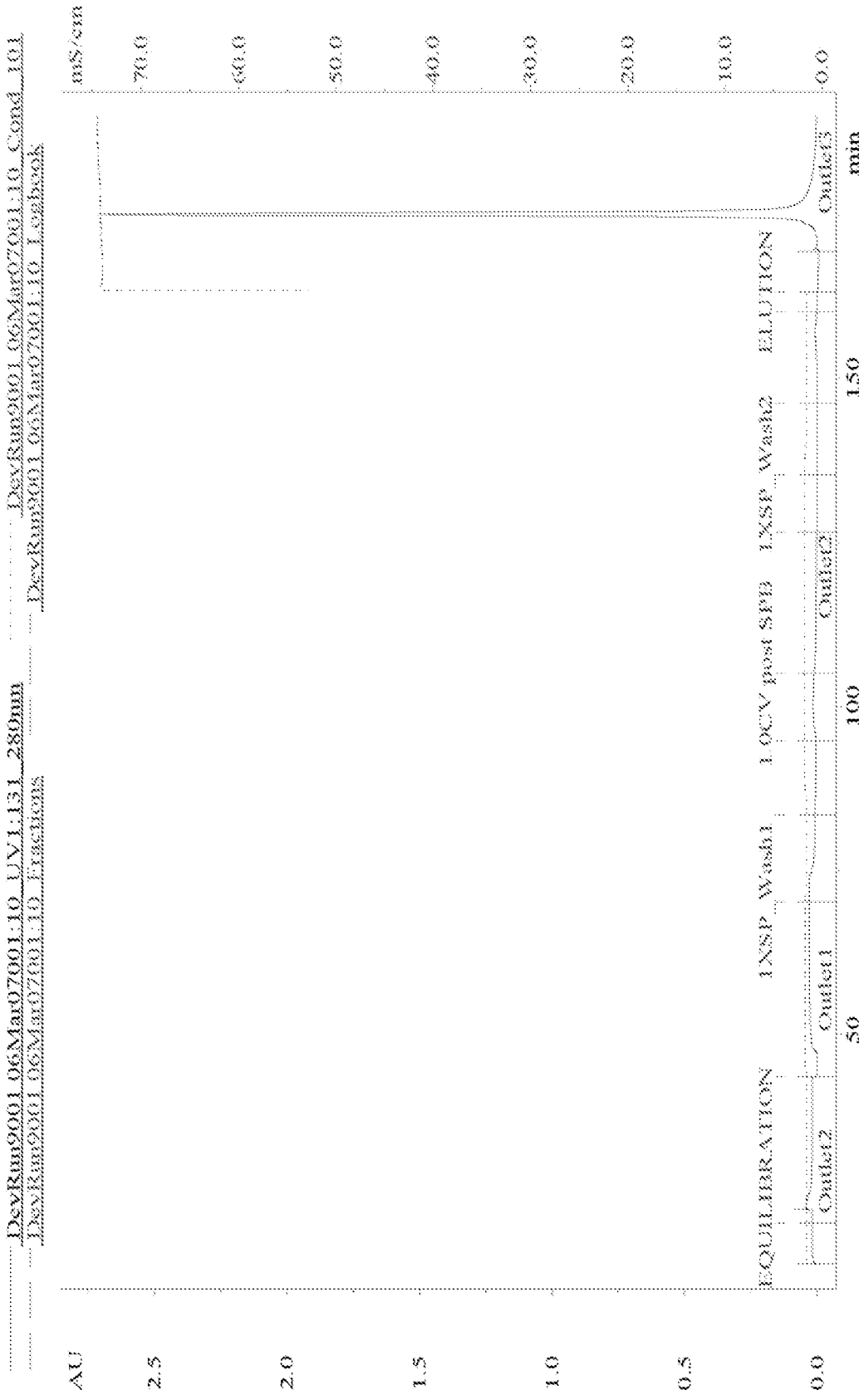

DevRun9001_06Mar07001_10_UV1_131_280nm
DevRun9001_06Mar07001_10_Fractions
DevRun9001_06Mar07001_10_Cond_101
DevRun9001_06Mar07001_10_Lookback
AU
2.5
2.0
1.5
1.0
0.5
0.0
mS/cm
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
EQUILIBRATION
Outlet2
1NSP_Wash1
Outlet1
1 OCV post SPB
Outlet2
1%SP_Wash2
ELUTION
Outlet3
50
100
150
min

Fig. 13

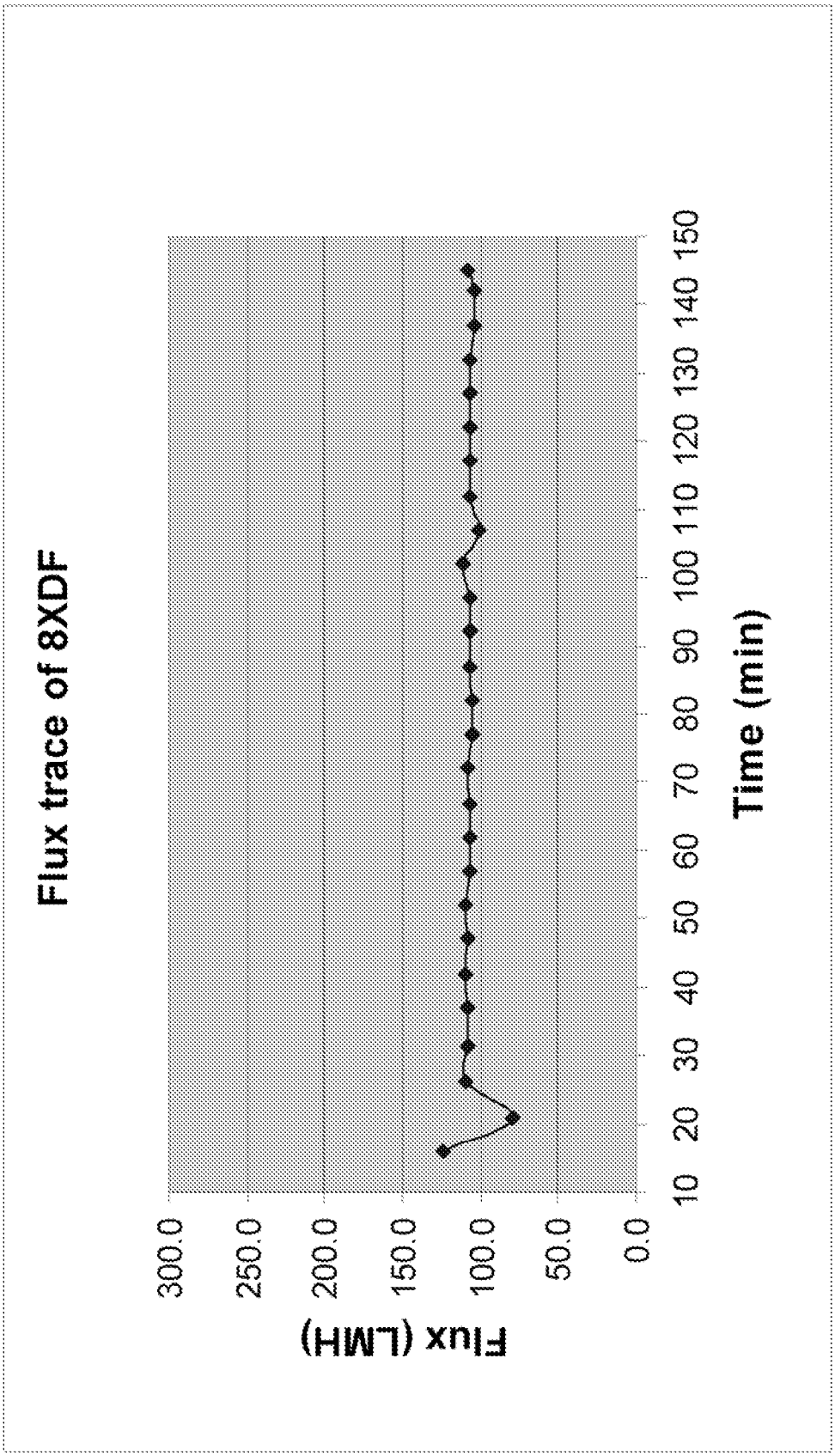

Flux trace of 8XDF
Flux (LMH)
300.0
250.0
200.0
150.0
100.0
50.0
0.0
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150
Time (min)

Fig. 14

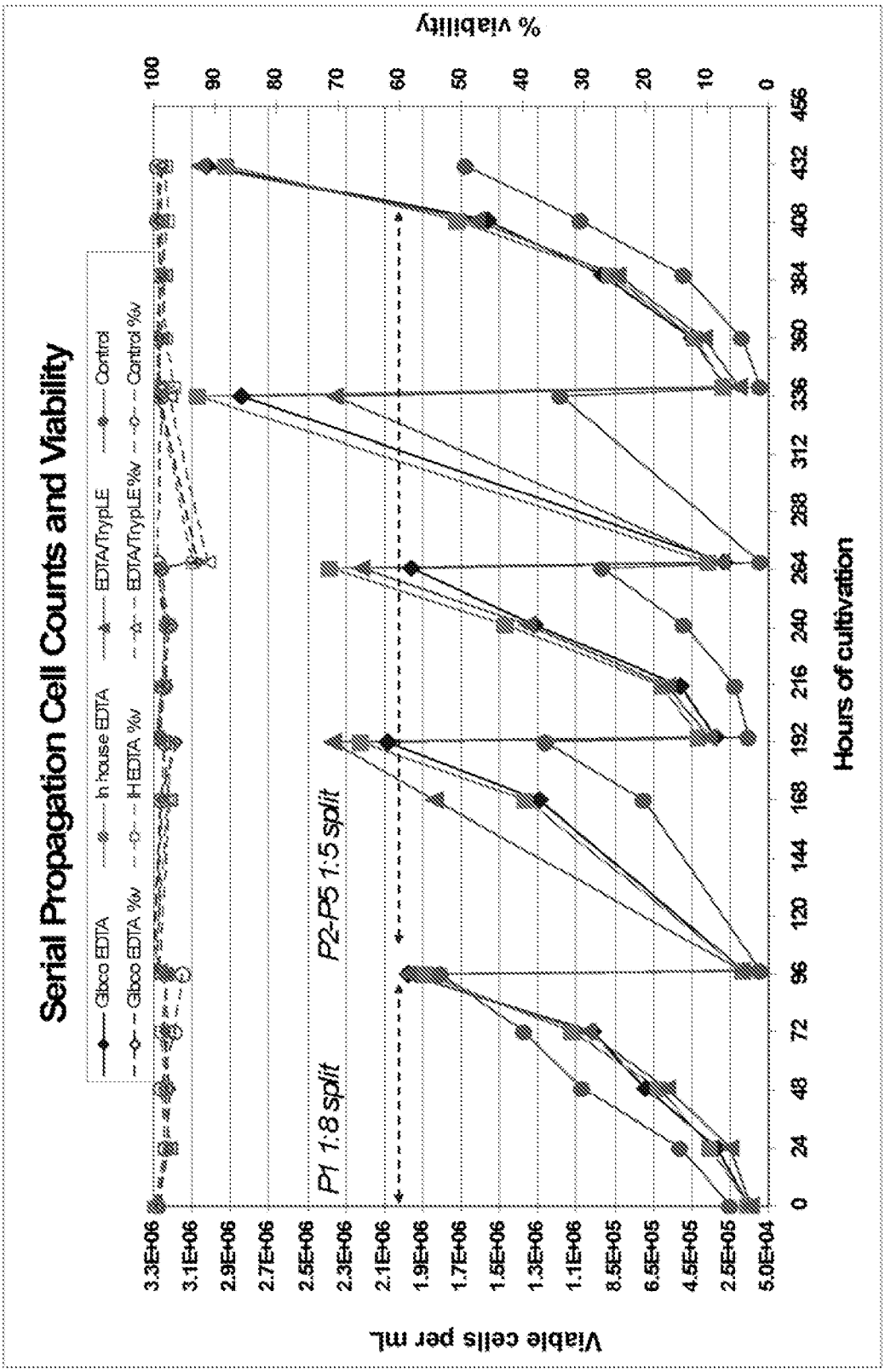
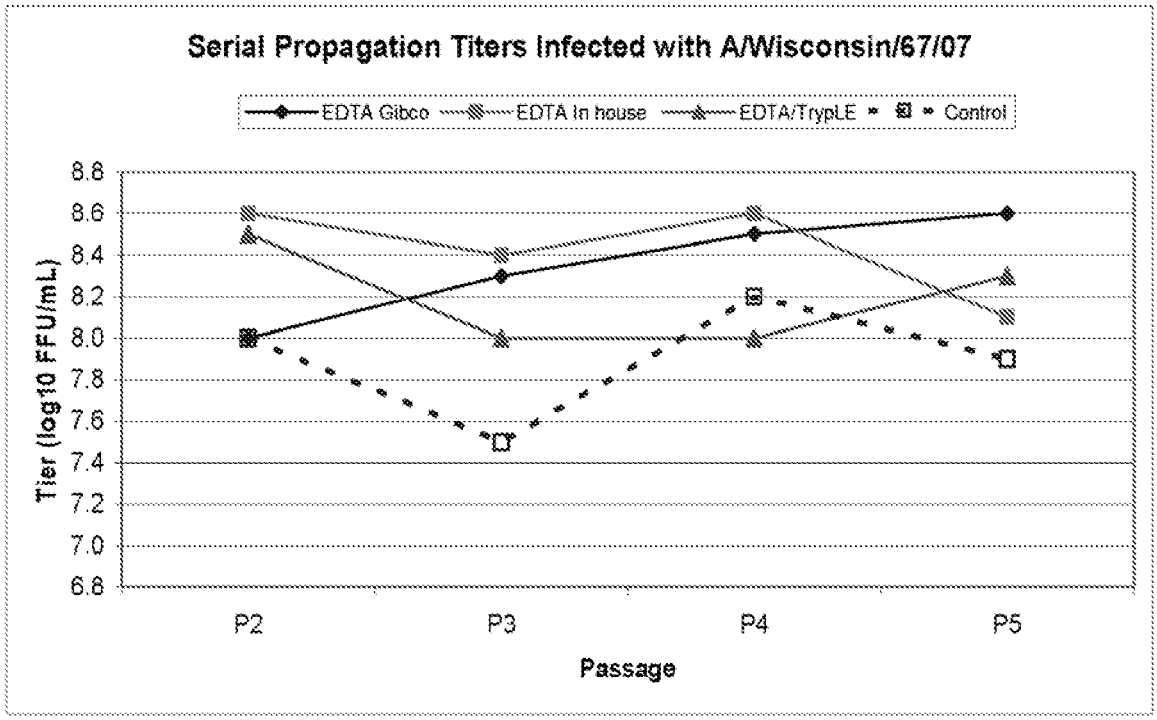

Serial Propagation Cell Counts and Viability
Gibco EDTA
In house EDTA
EDTA/TrypLE
Control
Gibco EDTA %v
IH EDTA %v
EDTA/TrypLE %v
Control %v
P1 1:8 split
P2-P5 1:5 split
Viable cells per mL
% viability
Hours of cultivation
3.3E+06
3.1E+06
2.9E+06
2.7E+06
2.5E+06
2.3E+06
2.1E+06
1.9E+06
1.7E+06
1.5E+06
1.3E+06
1.1E+06
8.5E+05
6.5E+05
4.5E+05
2.5E+05
5.0E+04
100
90
80
70
60
50
40
30
20
10
0
0
24
48
72
96
120
144
168
192
216
240
264
288
312
336
360
384
408
432
456

Fig. 15


Serial Propagation Titers Infected with A/Wisconsin/67/07
EDTA Gibco
EDTA In house
EDTA/TrypLE
Control
Titer (log10 FFU/mL)
8.8
8.6
8.4
8.2
8.0
7.8
7.6
7.4
7.2
7.0
6.8
P2
P3
P4
P5
Passage

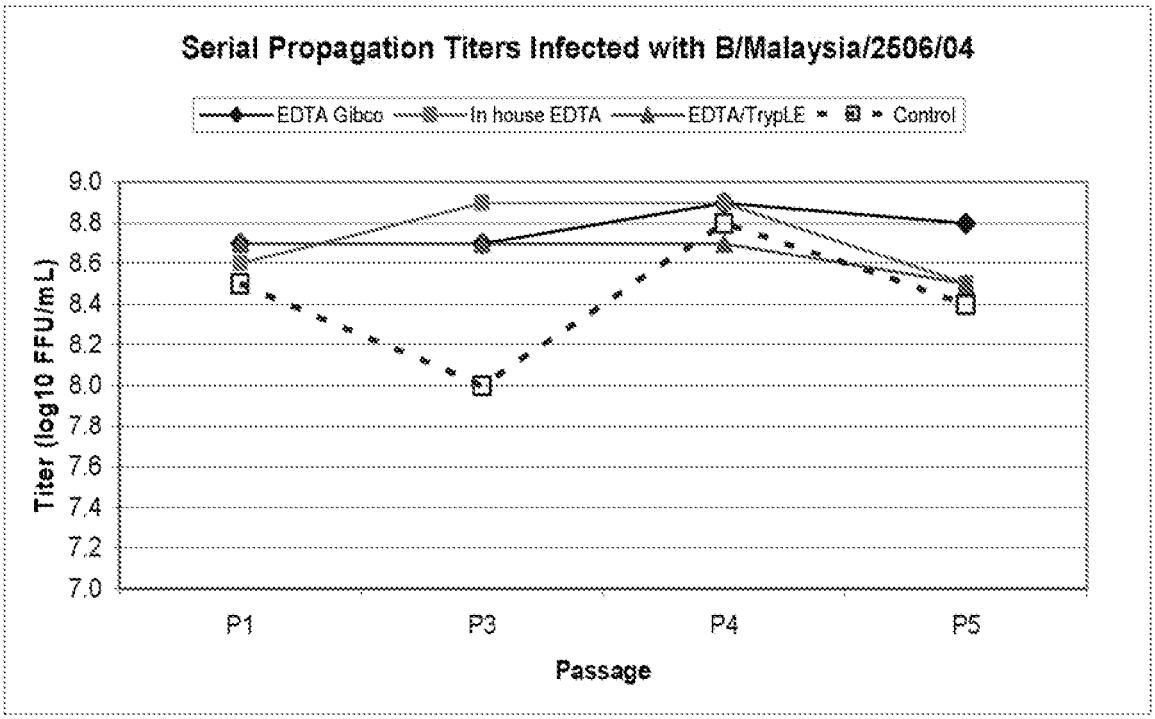

Serial Propagation Titers Infected with B/Malaysia/2606/04
EDTA Gibco
In house EDTA
EDTA/TrypLE
Control
Titer (log10 FFU/mL)
9.0
8.8
8.6
8.4
8.2
8.0
7.8
7.6
7.4
7.2
7.0
P1
P3
P4
P5
Passage

Fig. 16

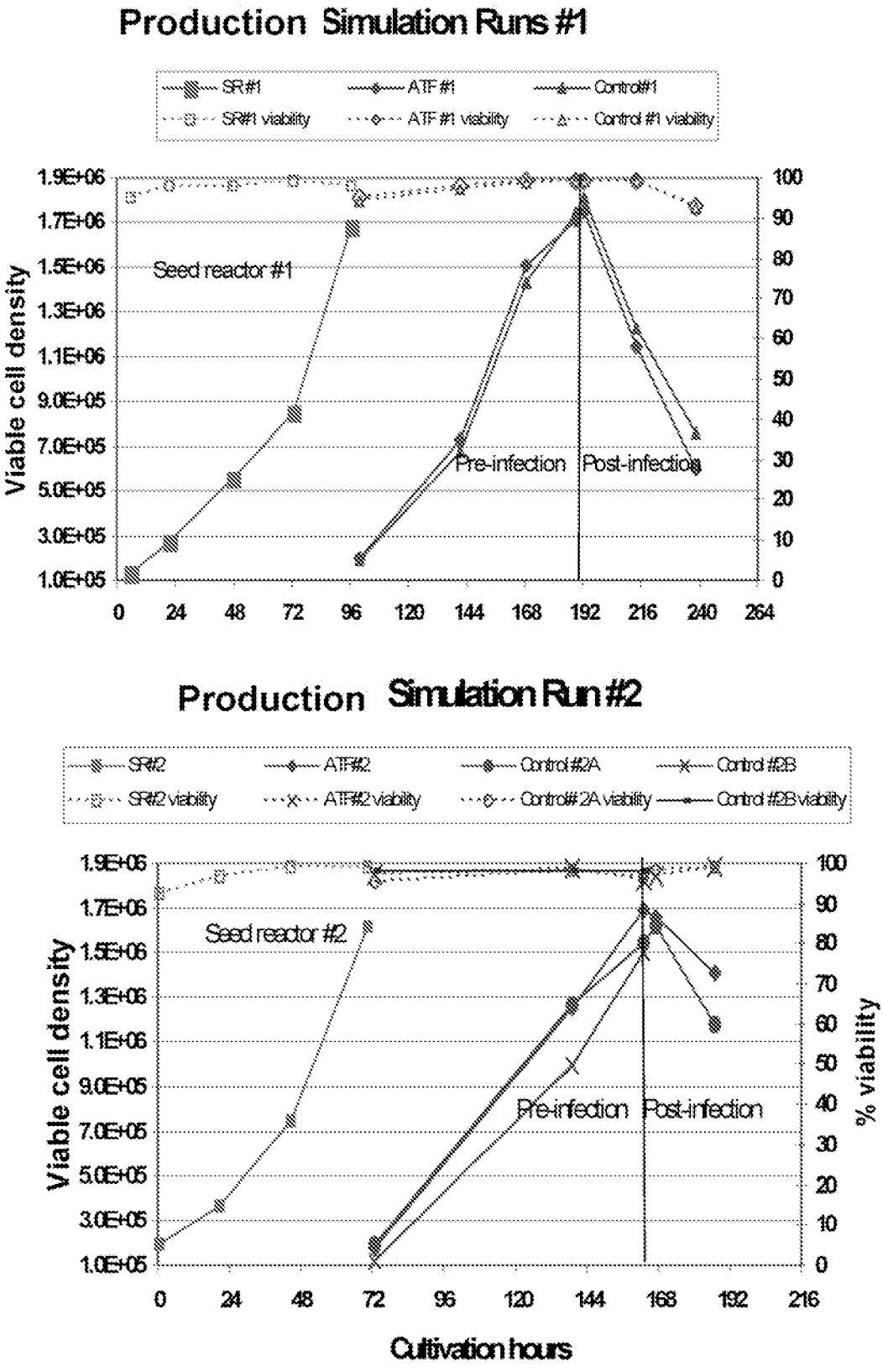
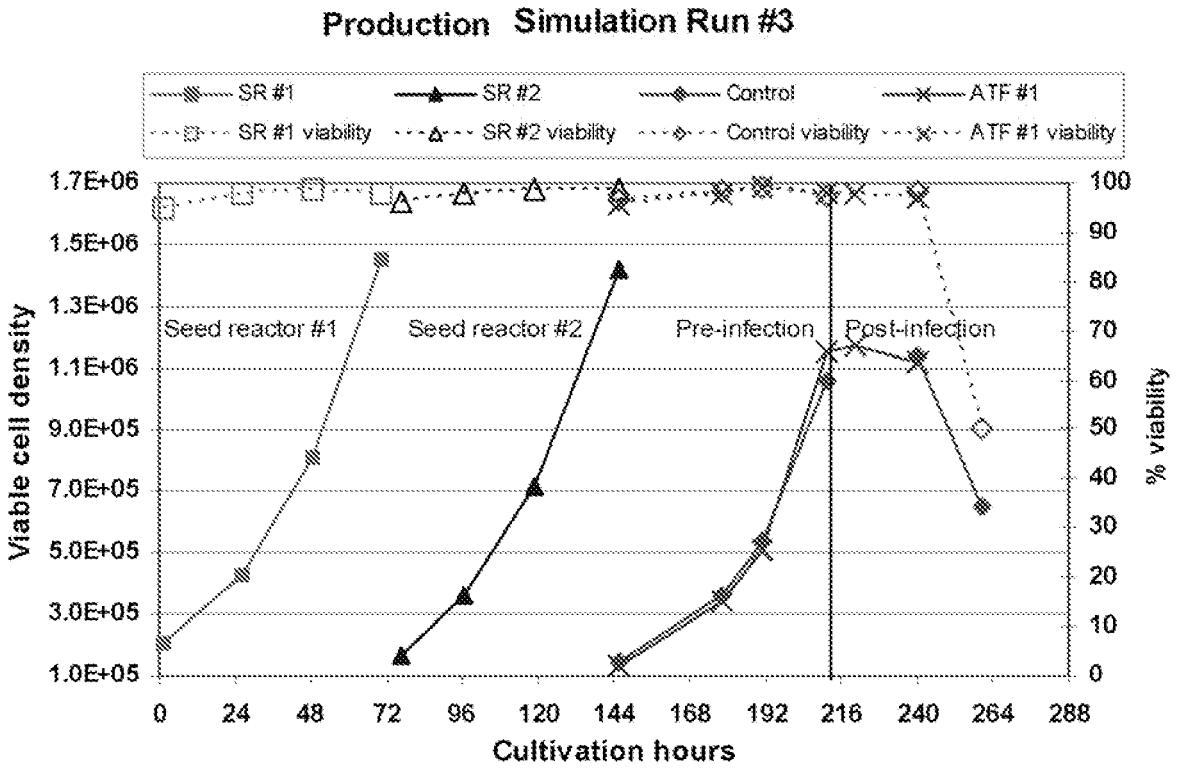

Production Simulation Runs #1
SR#1
ATF #1
Control#1
SR#1 viability
ATF #1 viability
Control #1 viability
Viable cell density
Seed reactor #1
Pre-infection
Post-infection
1.9E+06
1.7E+06
1.5E+06
1.3E+06
1.1E+06
9.0E+05
7.0E+05
5.0E+05
3.0E+05
1.0E+05
100
90
80
70
60
50
40
30
20
10
0
0
24
48
72
96
120
144
168
192
216
240
264
Production Simulation Run #2
SR#2
ATF#2
Control #2A
Control #2B
SR#2 viability
ATF#2 viability
Control# 2A viability
Control #2B viability
Viable cell density
Seed reactor #2
Pre-infection
Post-infection
% viability
Cultivation hours

Fig. 17A


Production Simulation Run #3
SR #1
SR #2
Control
ATF #1
SR #1 viability
SR #2 viability
Control viability
ATF #1 viability
Viable cell density
% viability
Seed reactor #1
Seed reactor #2
Pre-infection
Post-infection
Cultivation hours
1.7E+06
1.5E+06
1.3E+06
1.1E+06
9.0E+05
7.0E+05
5.0E+05
3.0E+05
1.0E+05
100
90
80
70
60
50
40
30
20
10
0
0
24
48
72
96
120
144
168
192
216
240
264
288

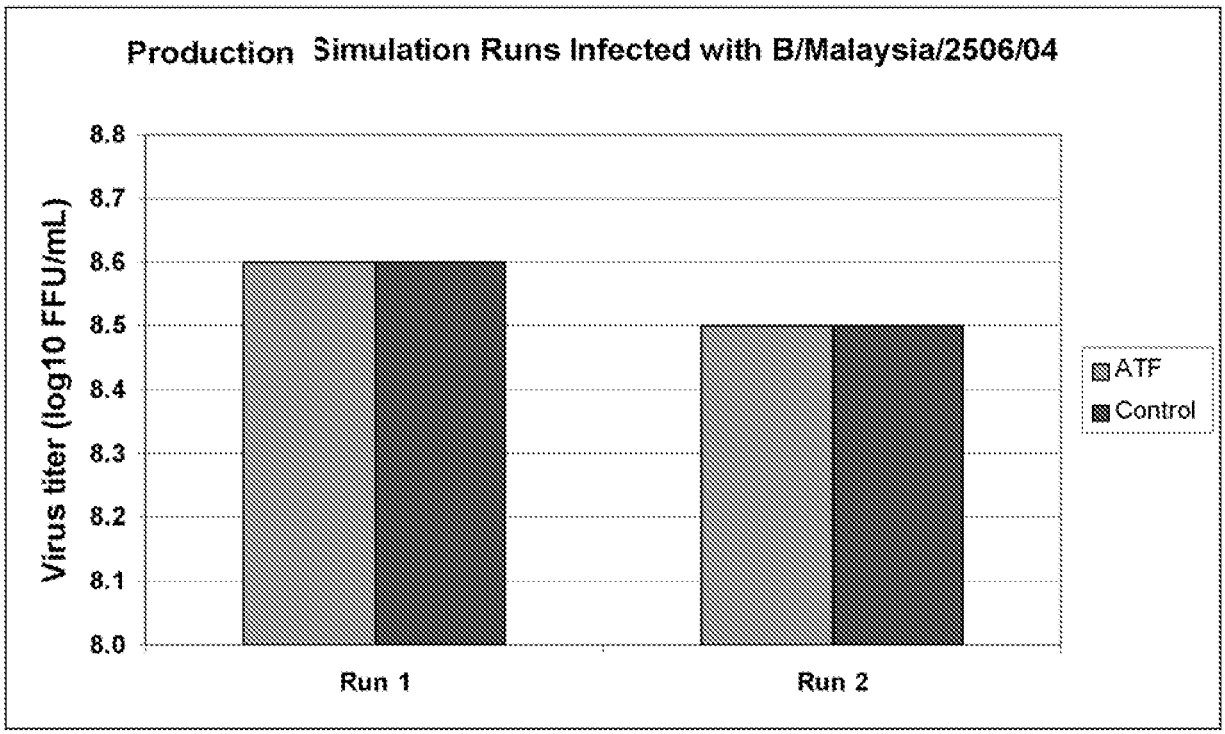

Production Simulation Runs Infected with B/Malaysia/2506/04
Virus titer (log10 FFU/mL)
8.8
8.7
8.6
8.5
8.4
8.3
8.2
8.1
8.0
Run 1
Run 2
ATF
Control

Fig. 17B

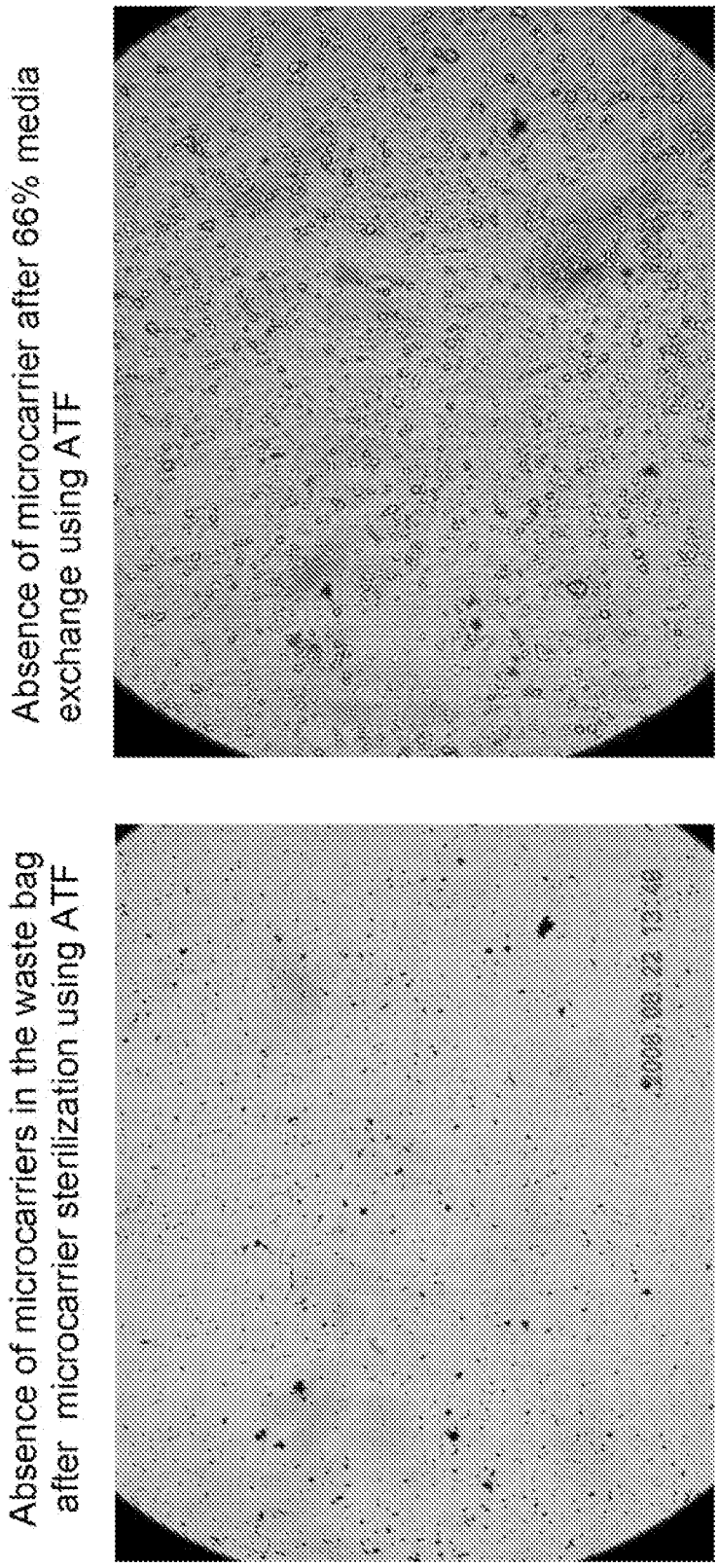

Absence of microcarrier after 66% media exchange using ATF
Absence of microcarriers in the waste bag after microcarrier sterilization using ATF

Fig. 18

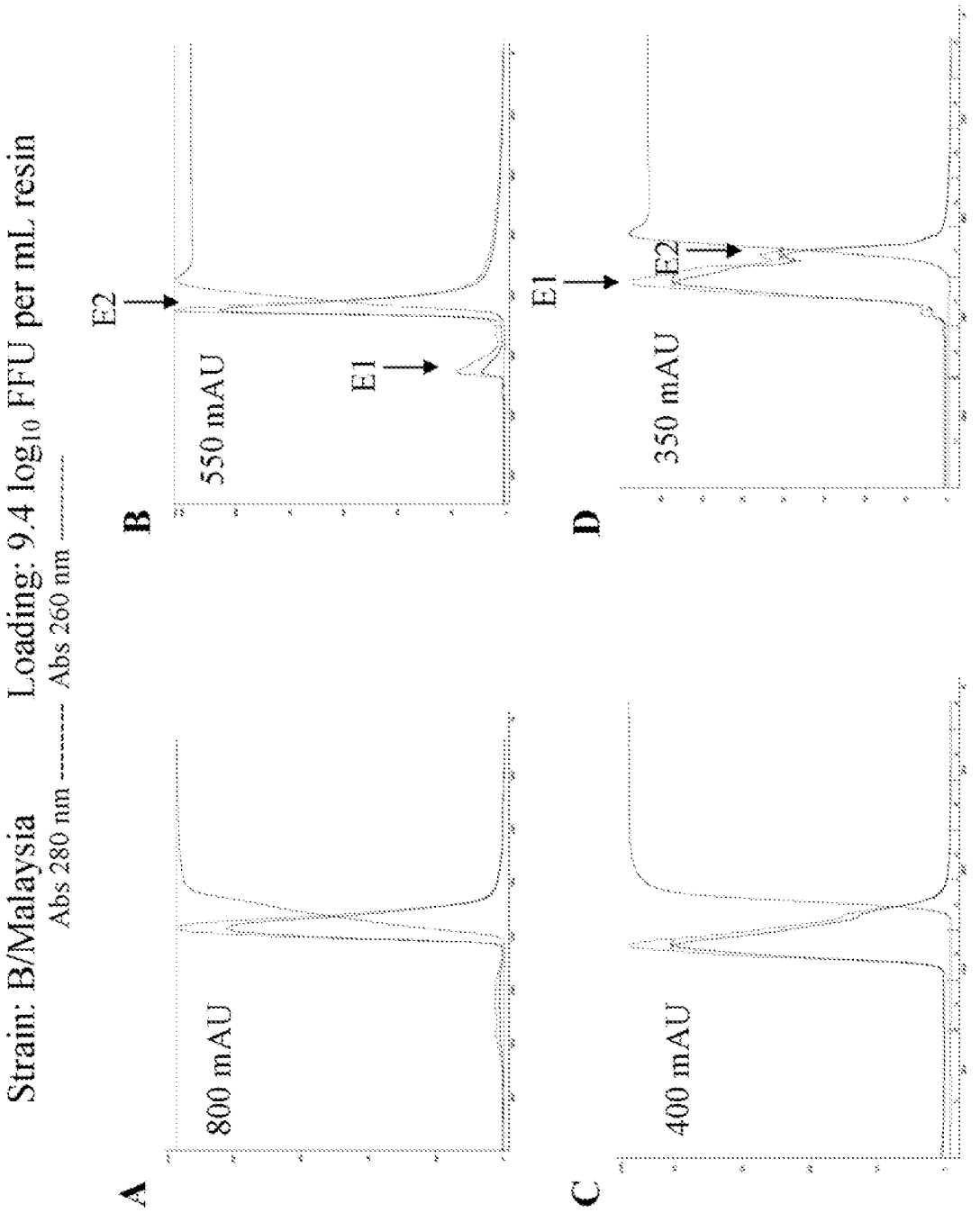

Strain: B/Malaysia
Loading: 9.4 log10 FFU per mL resin
Abs 280 nm ------- Abs 260 nm ---------
A
800 mAU
B
550 mAU
E2
E1
C
400 mAU
D
350 mAU
E1
E2

Fig. 19

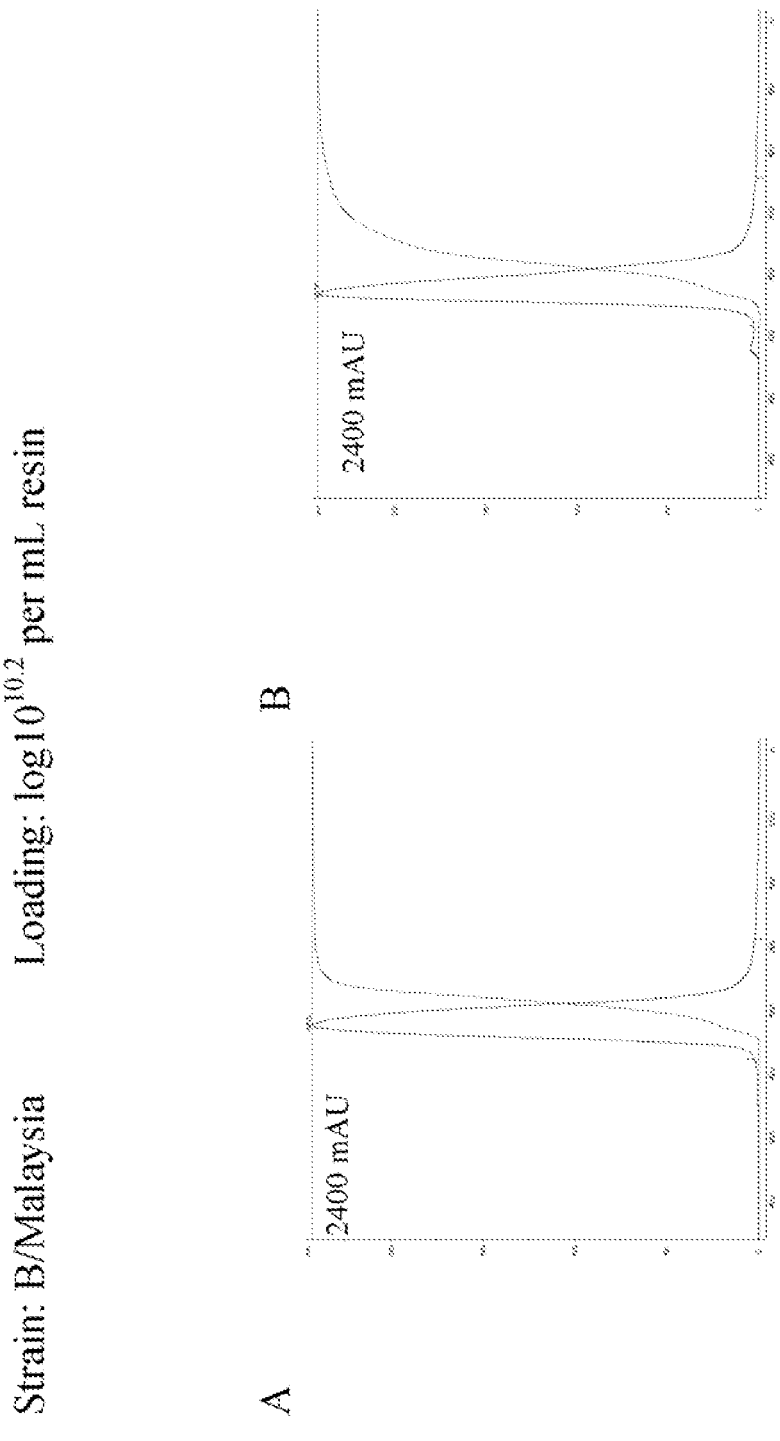

Strain: B/Malaysia
Loading: log10^10.2 per mL resin
A
2400 mAU
B
2400 mAU

Fig. 20

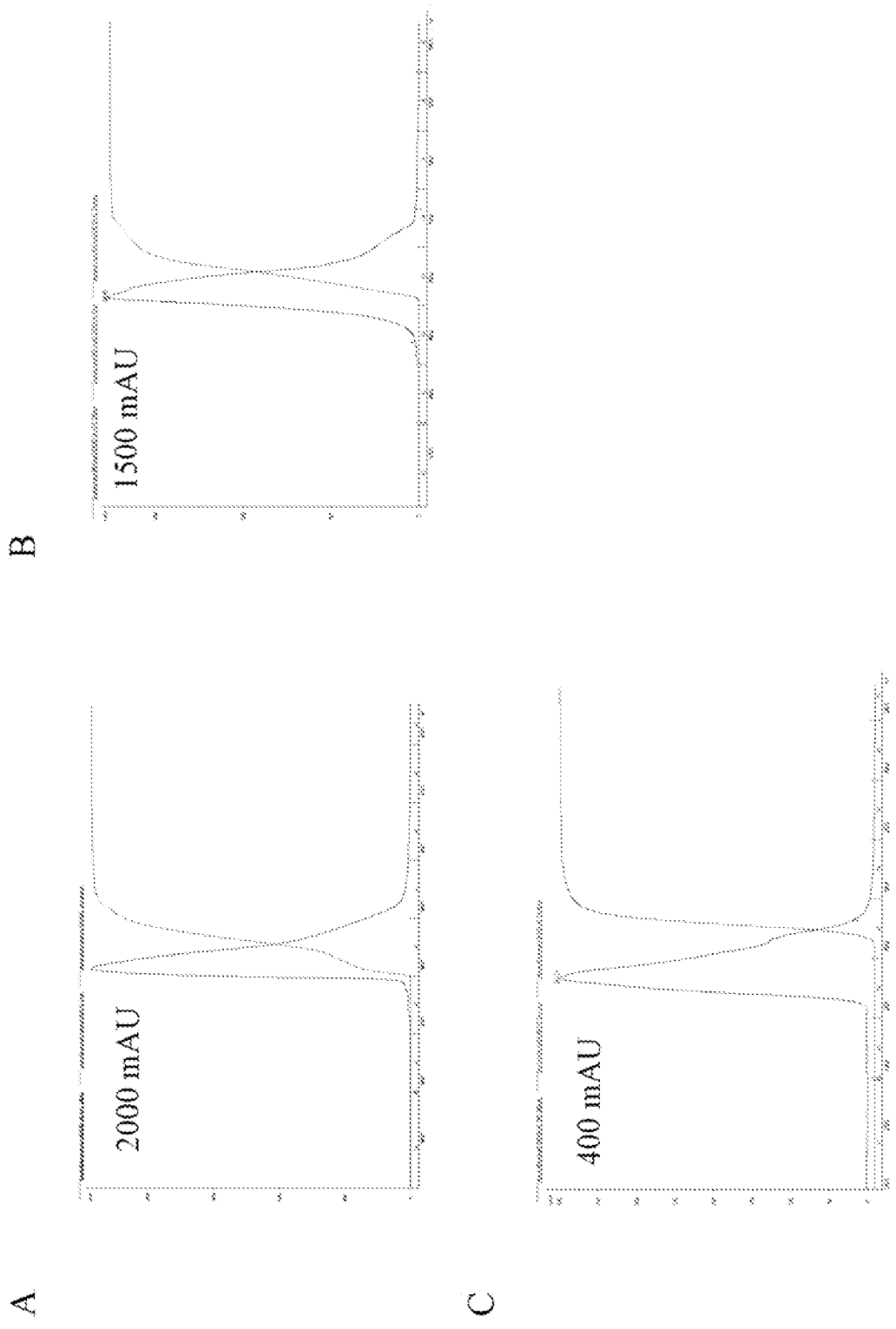


Fig. 21

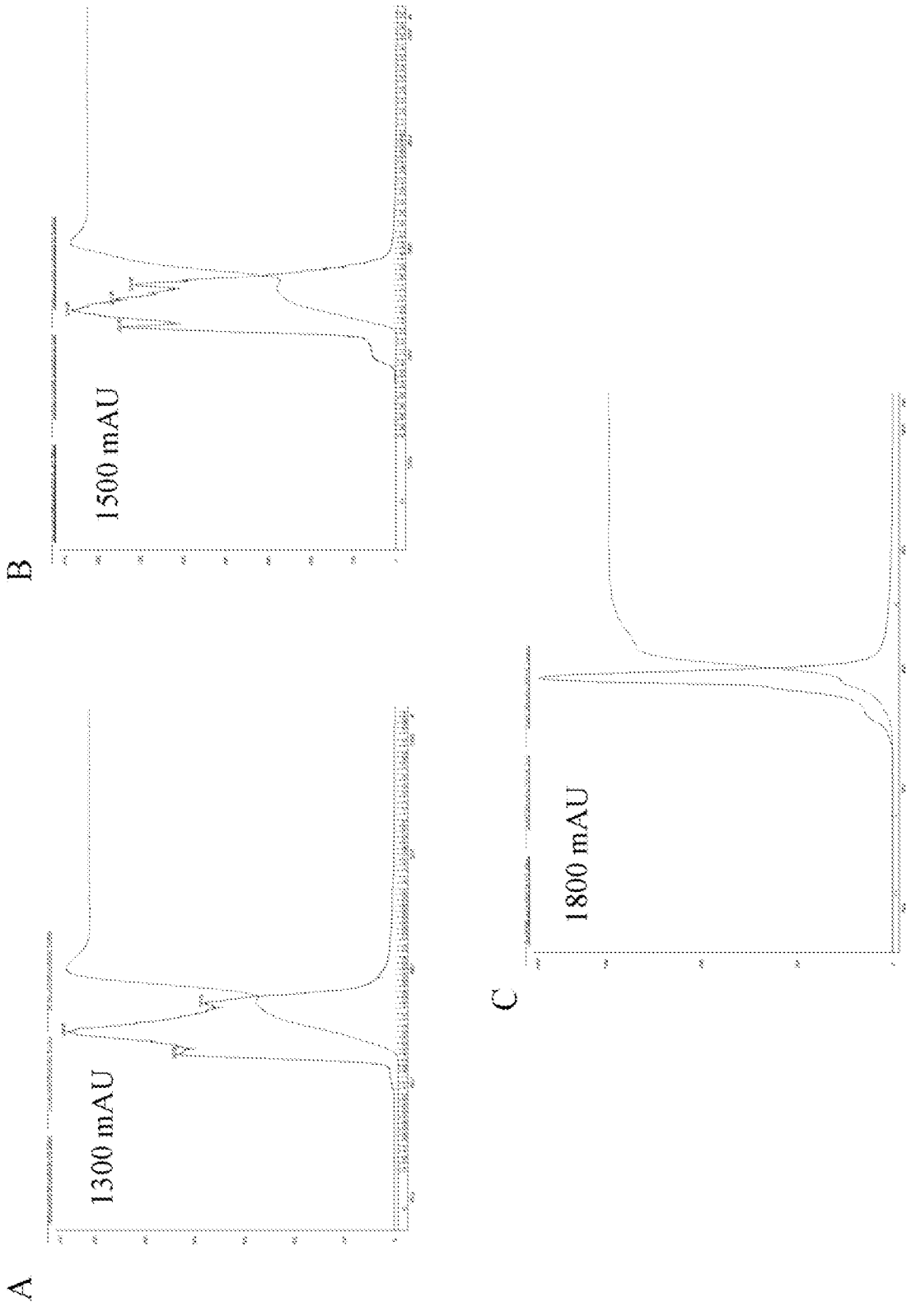


Fig. 22

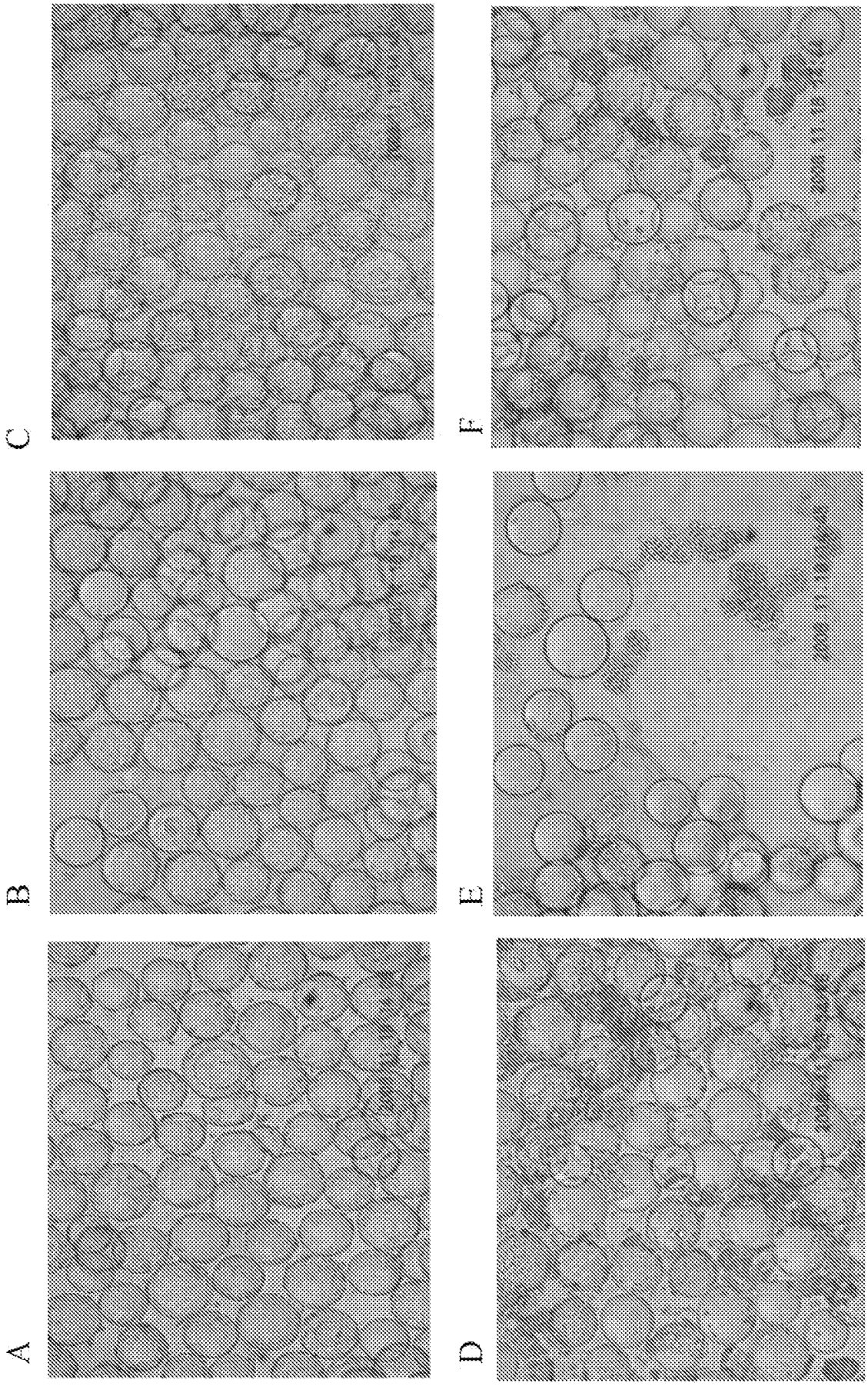


Fig. 23

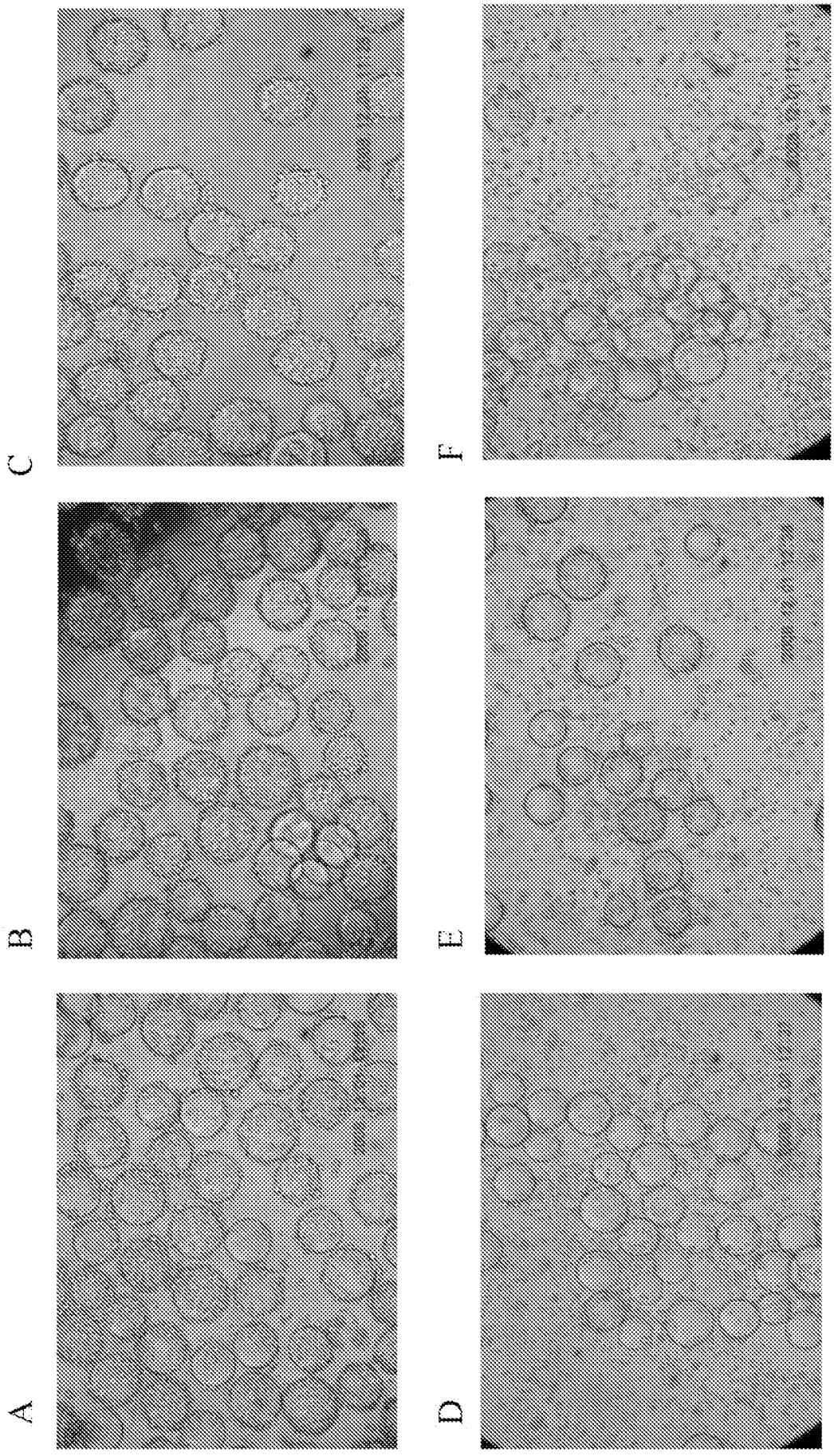


Fig. 24