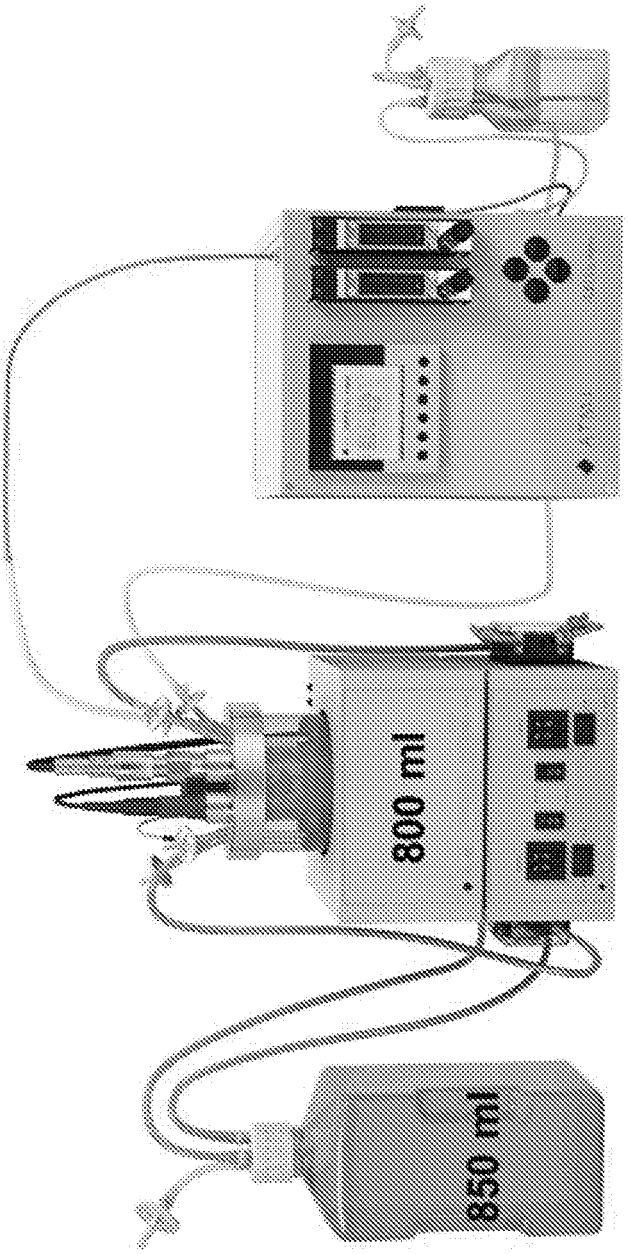
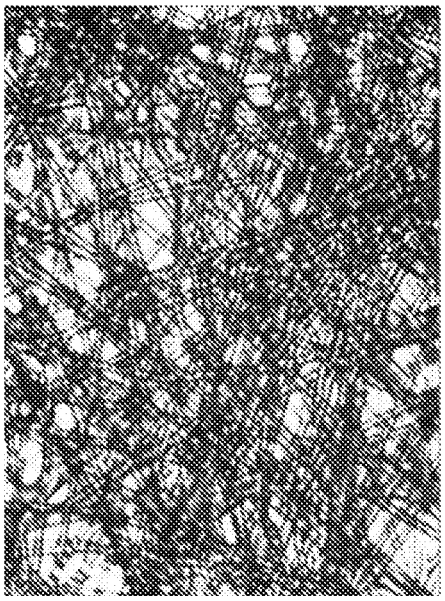


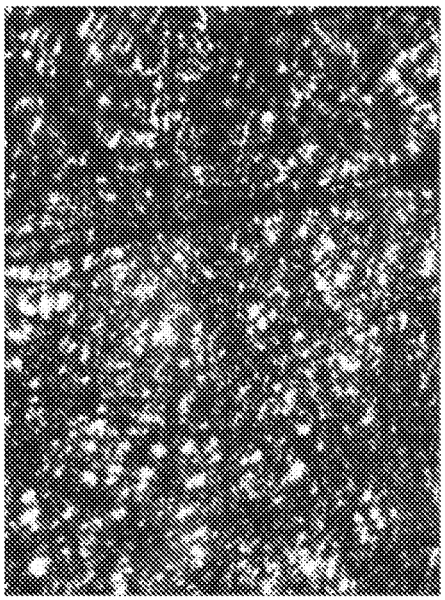
FIG. 1

FIG. 2A



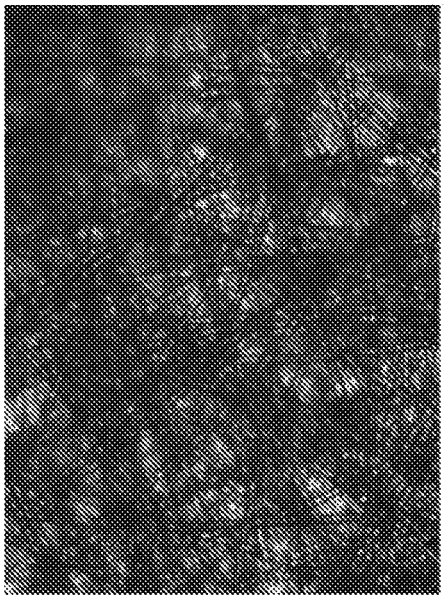
Empty carrier

FIG. 2B



Carrier at Day 3

FIG. 2C



Carrier at Day 6

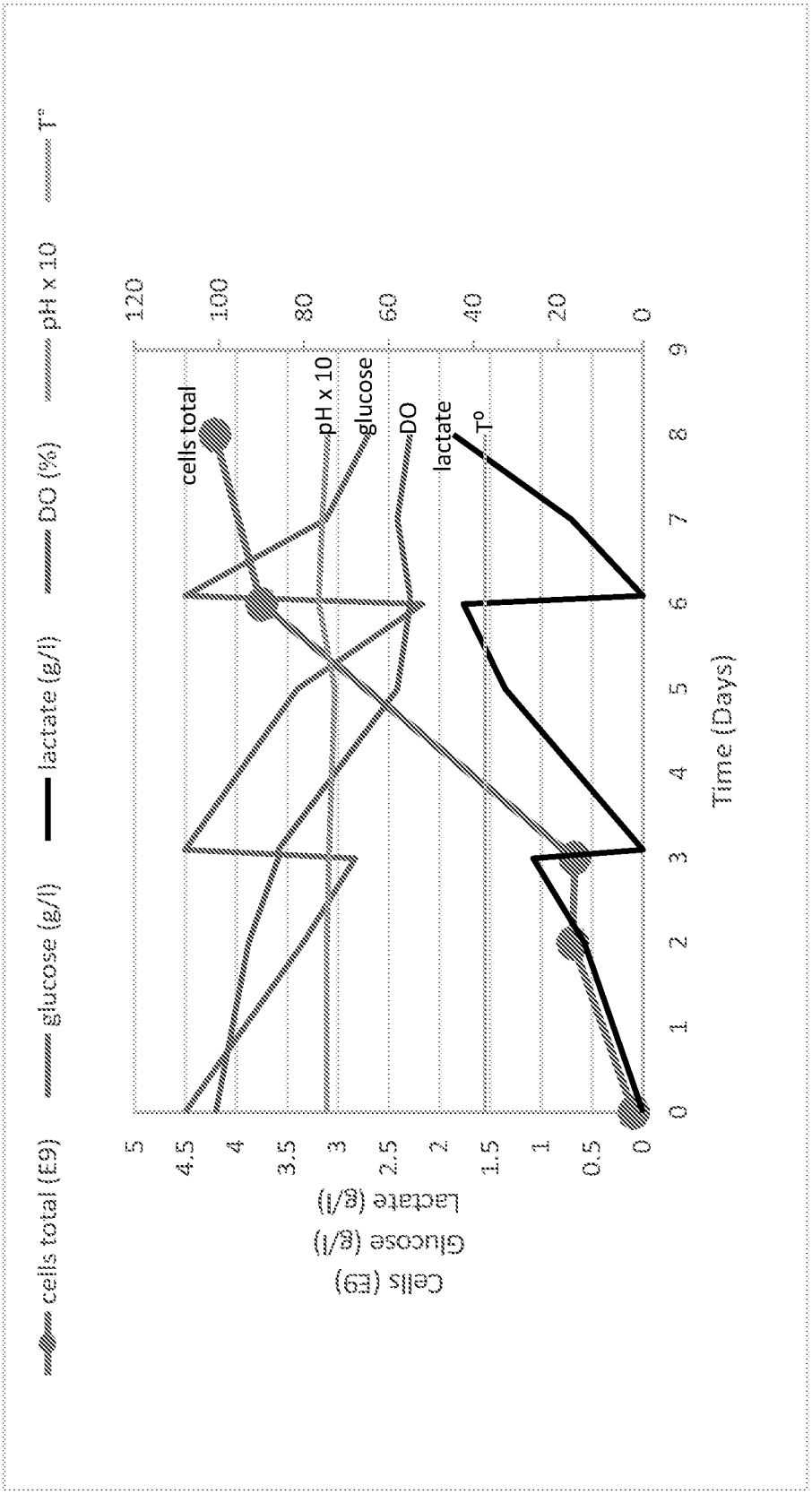

cells total (E9)
glucose (g/l)
lactate (g/l)
DO (%)
pH x 10
T°
Cells (E9)
Glucose (g/l)
lactate (g/l)
Time (Days)
cells total
pH x 10
glucose
DO
lactate
T°

FIG. 3

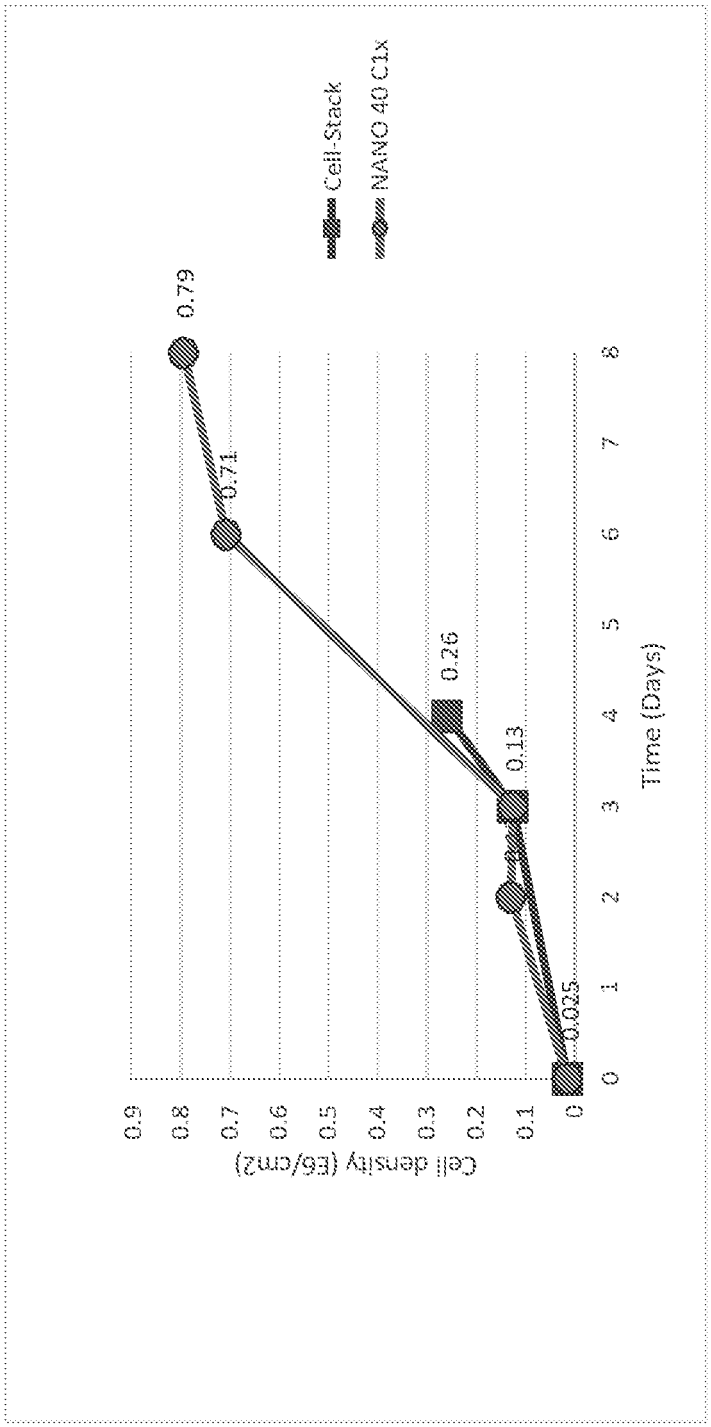

Cell-Stack
NANO 40 C1x
0.79
0.71
0.26
0.13
0.025
Cell density (E6/cm2)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Time (Days)
0
1
2
3
4
5
6
7
8

FIG. 4

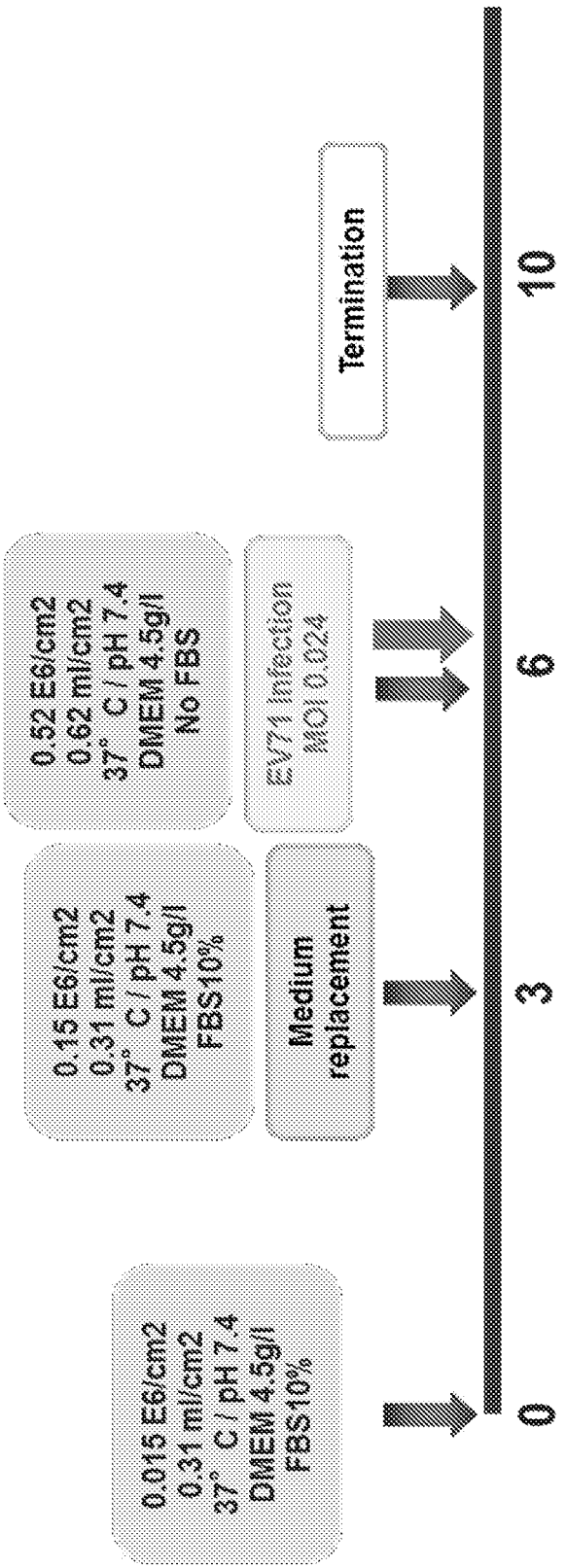

0.015 E6/cm2
0.31 ml/cm2
37° C / pH 7.4
DMEM 4.5g/l
FBS10%
0.15 E6/cm2
0.31 ml/cm2
37° C / pH 7.4
DMEM 4.5g/l
FBS10%
Medium replacement
0.52 E6/cm2
0.62 ml/cm2
37° C / pH 7.4
DMEM 4.5g/l
No FBS
EV71 infection
MOI 0.024
Termination
0
3
6
10

FIG. 5

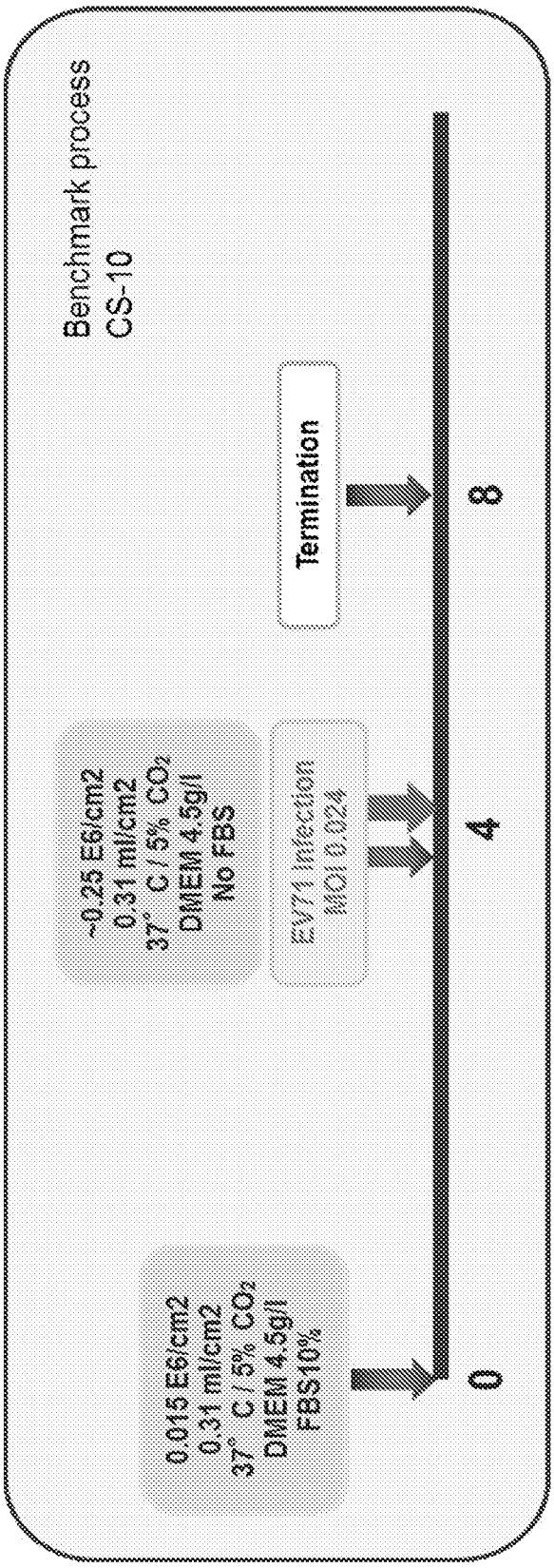

Benchmark process
CS-10
0.015 E6/cm2
0.31 ml/cm2
37° C / 5% CO2
DMEM 4.5g/l
FBS10%
~0.25 E6/cm2
0.31 ml/cm2
37° C / 5% CO2
DMEM 4.5g/l
No FBS
EV71 infection
MOI 0.024
Termination
0
4
8

FIG. 6

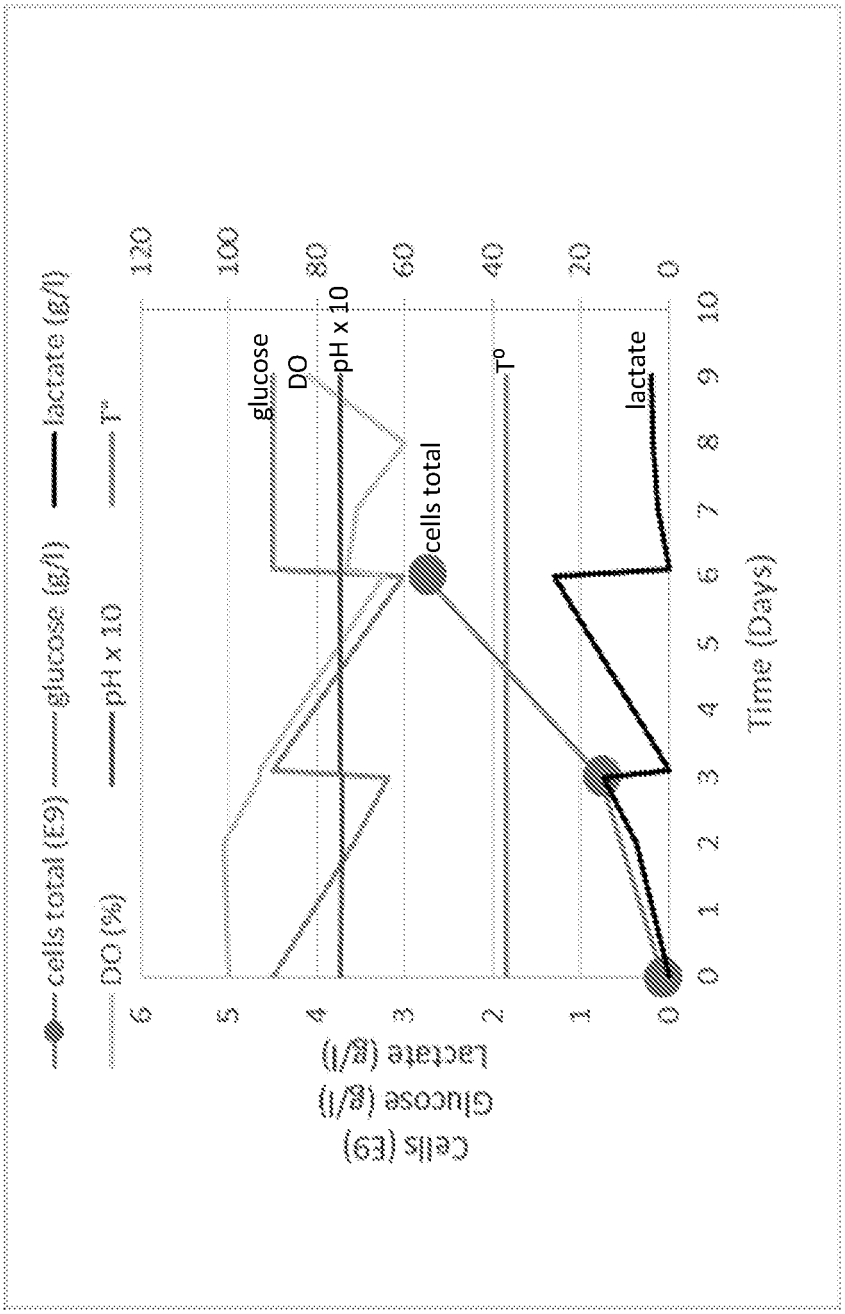

cells total (E9)
glucose (g/l)
lactate (g/l)
DO (%)
pH x 10
T°
glucose
DO
pH x 10
cells total
T°
lactate
Cells (E9)
Glucose (g/l)
lactate (g/l)
Time (Days)
0
1
2
3
4
5
6
0
20
40
60
80
100
120
0
1
2
3
4
5
6
7
8
9
10

FIG. 7

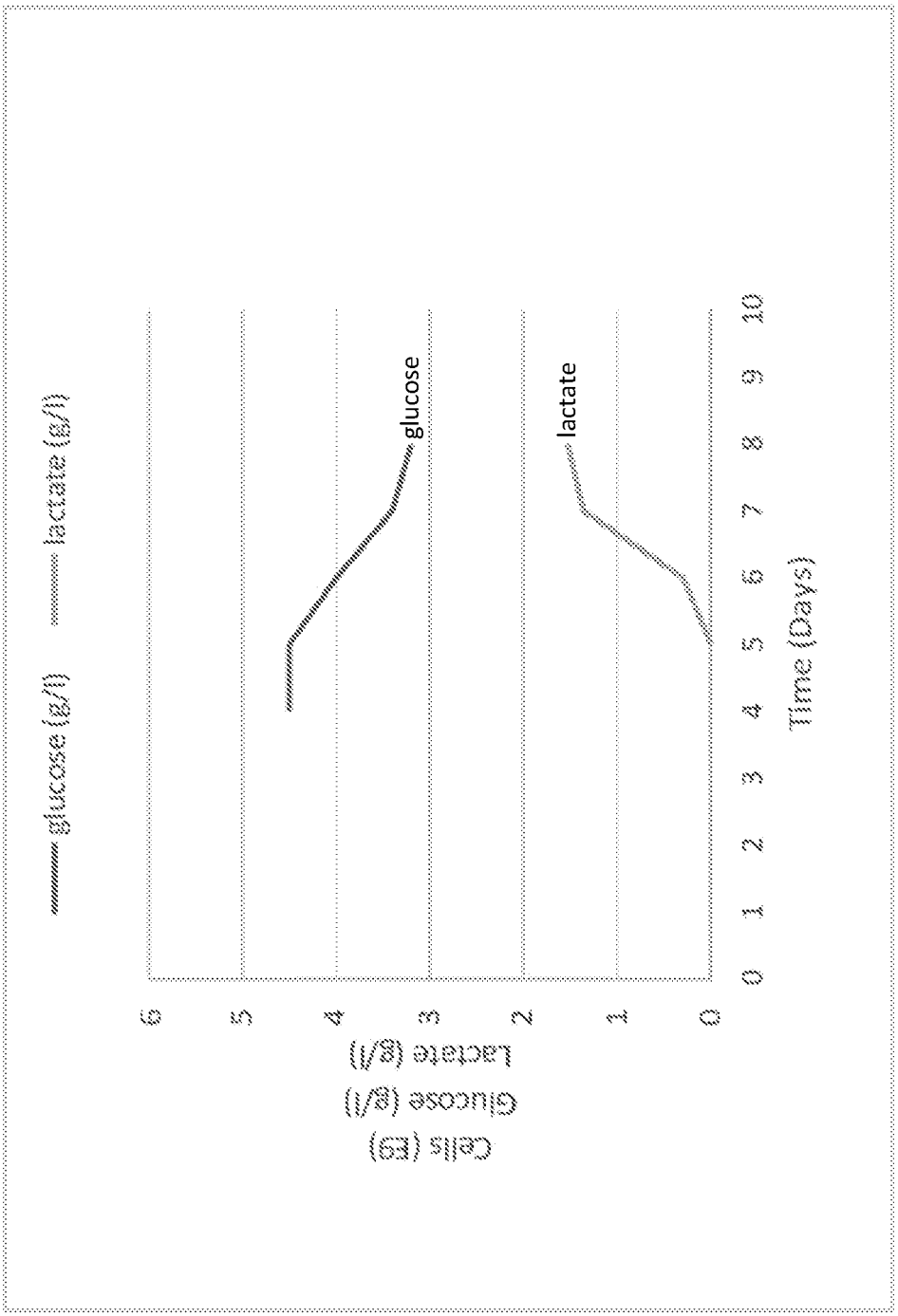

glucose (g/l)
lactate (g/l)
Cells (E9)
Glucose (g/l)
Lactate (g/l)
0
1
2
3
4
5
6
Time (Days)
0
1
2
3
4
5
6
7
8
9
10
glucose
lactate

FIG. 8

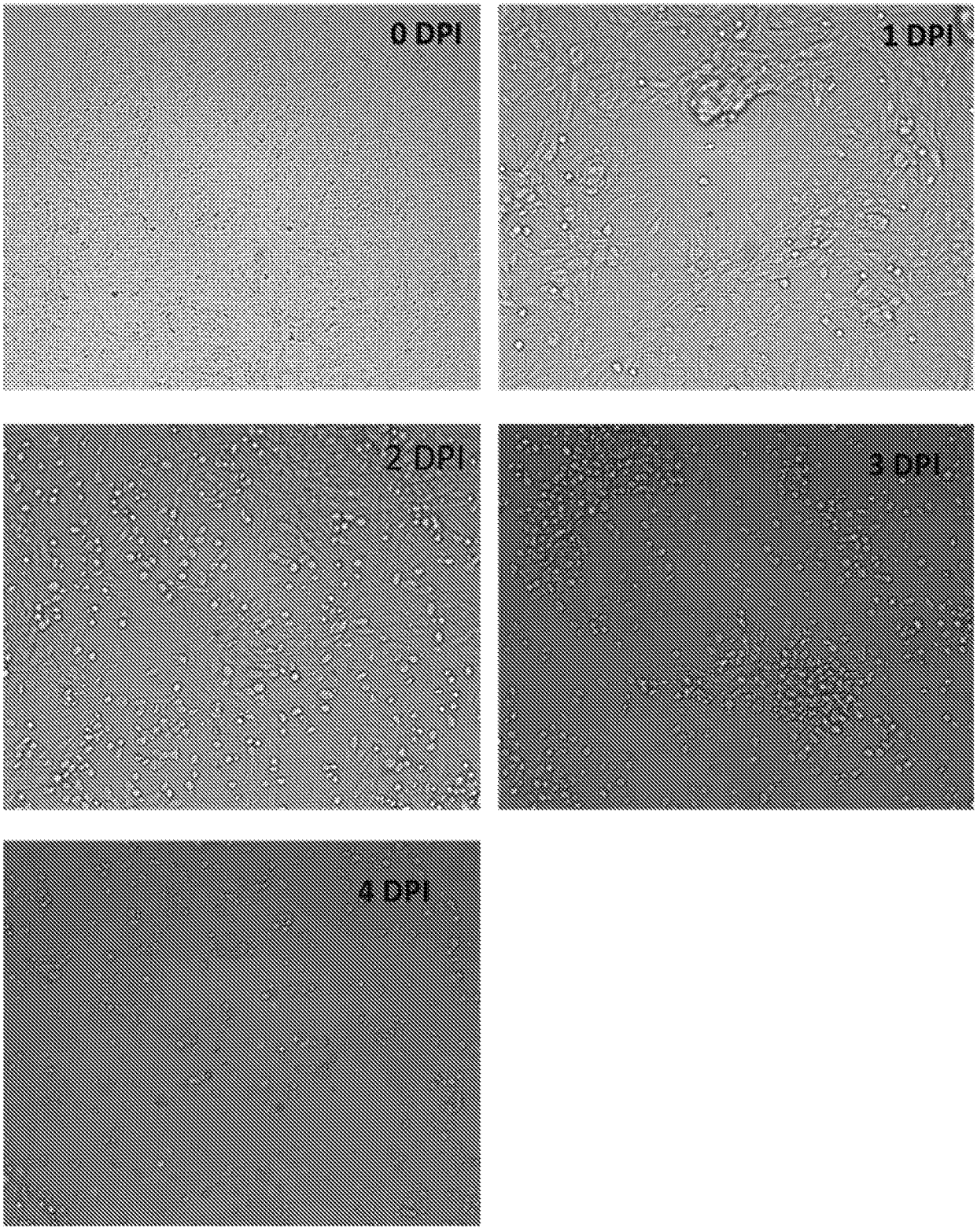


FIG. 9

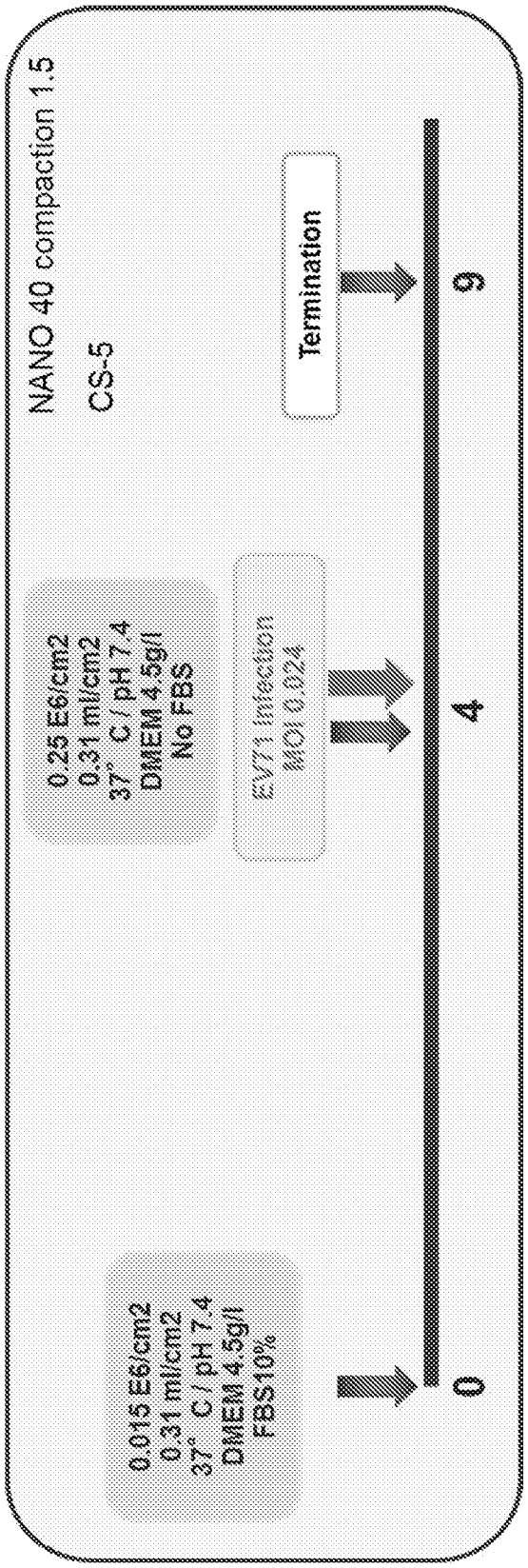

NANO 40 compaction 1.5
CS-5
0.25 E6/cm2
0.31 ml/cm2
37° C / pH 7.4
DMEM 4.5g/l
No FBS
EV71 infection
MOI 0.024
Termination
0.015 E6/cm2
0.31 ml/cm2
37° C / pH 7.4
DMEM 4.5g/l
FBS10%
0
4
9

FIG. 10

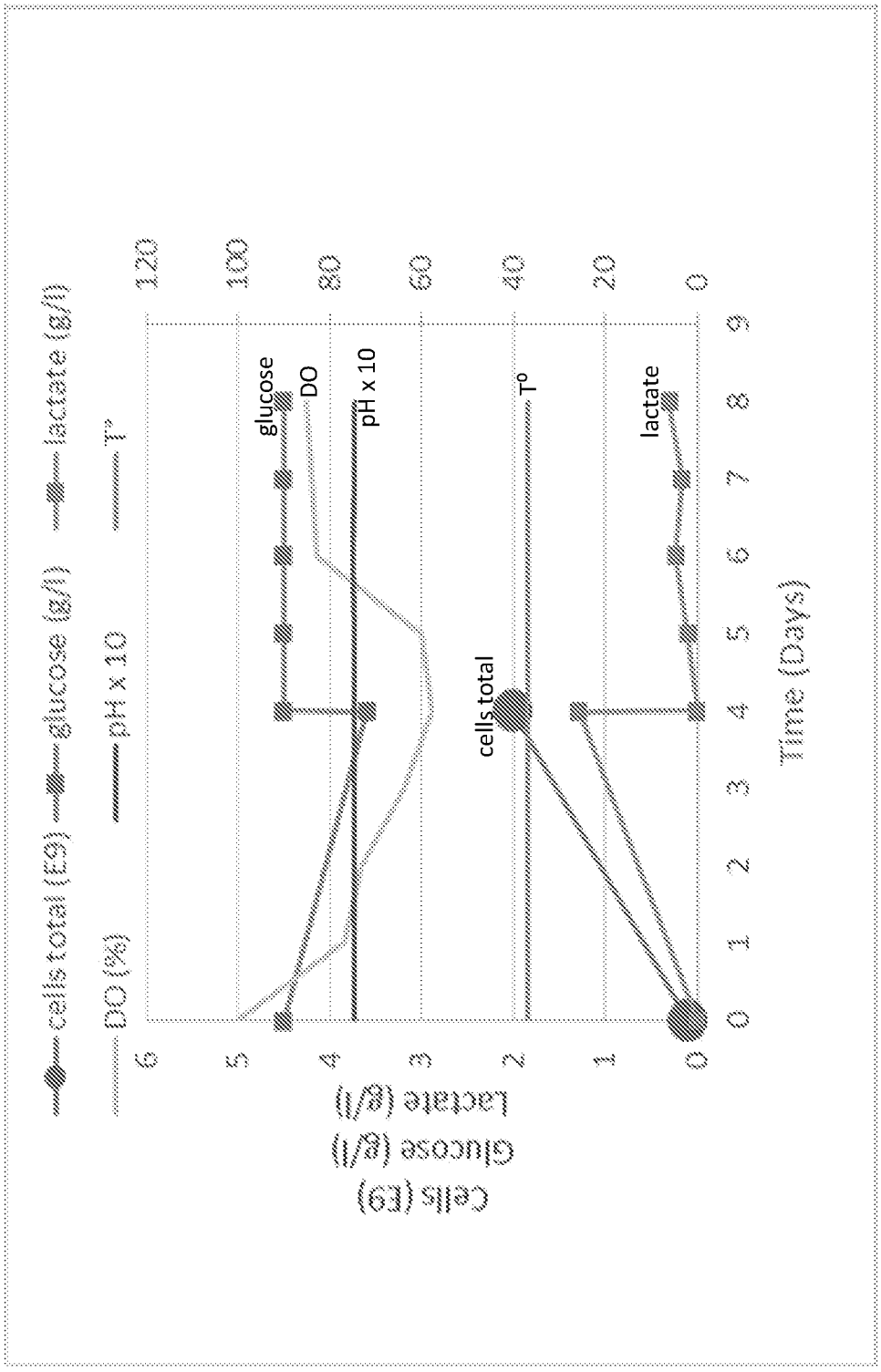

cells total (E9)
glucose (g/l)
lactate (g/l)
DO (%)
pH x 10
T°
cells (E9)
Glucose (g/l)
lactate (g/l)
Time (Days)
glucose
DO
pH x 10
cells total
T°
lactate

FIG. 11

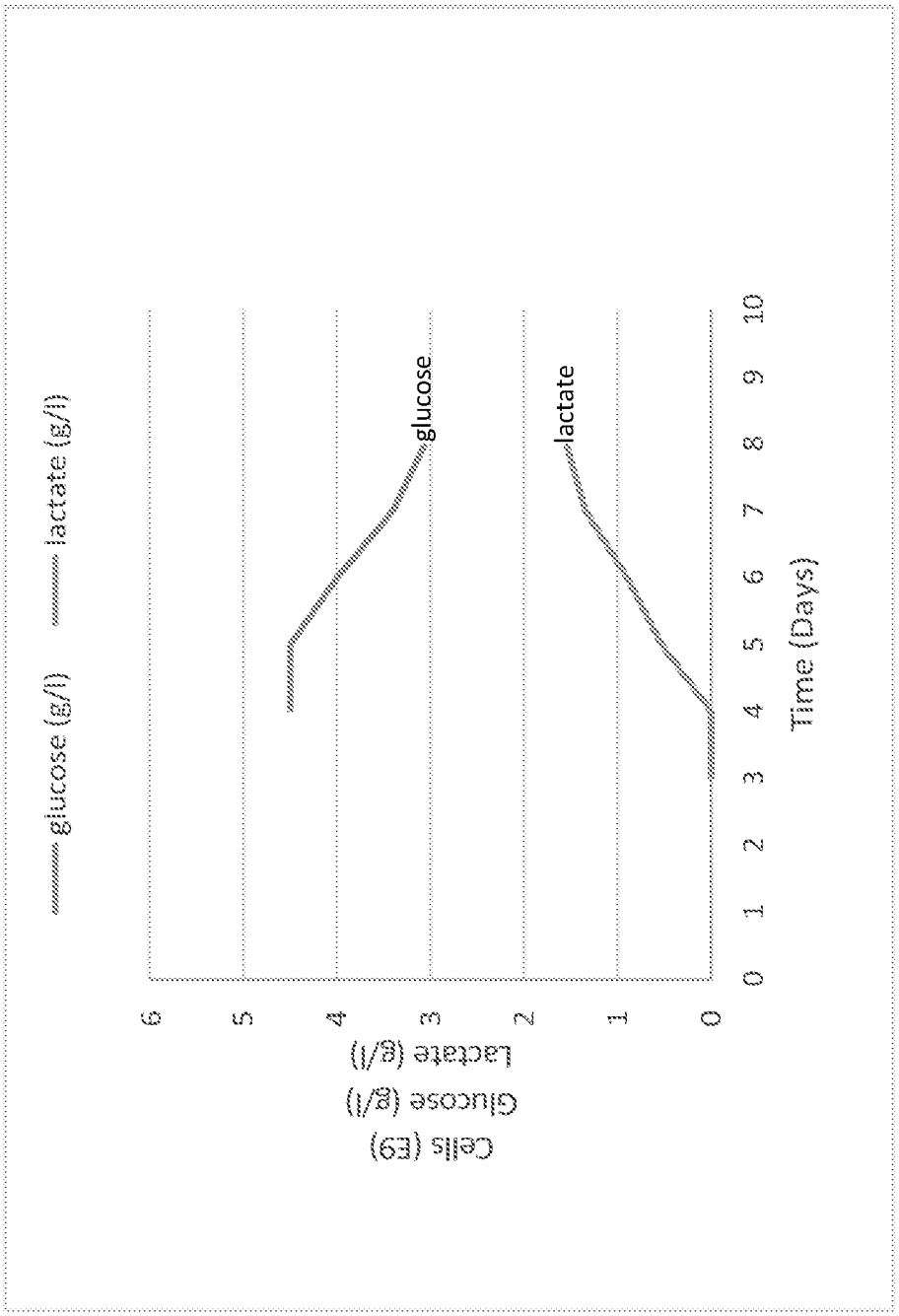

glucose (g/l)
lactate (g/l)
glucose
lactate
Time (Days)
0 1 2 3 4 5 6 7 8 9 10
Cells (E9)
Glucose (g/l)
lactate (g/l)
0 1 2 3 4 5 6

FIG. 12

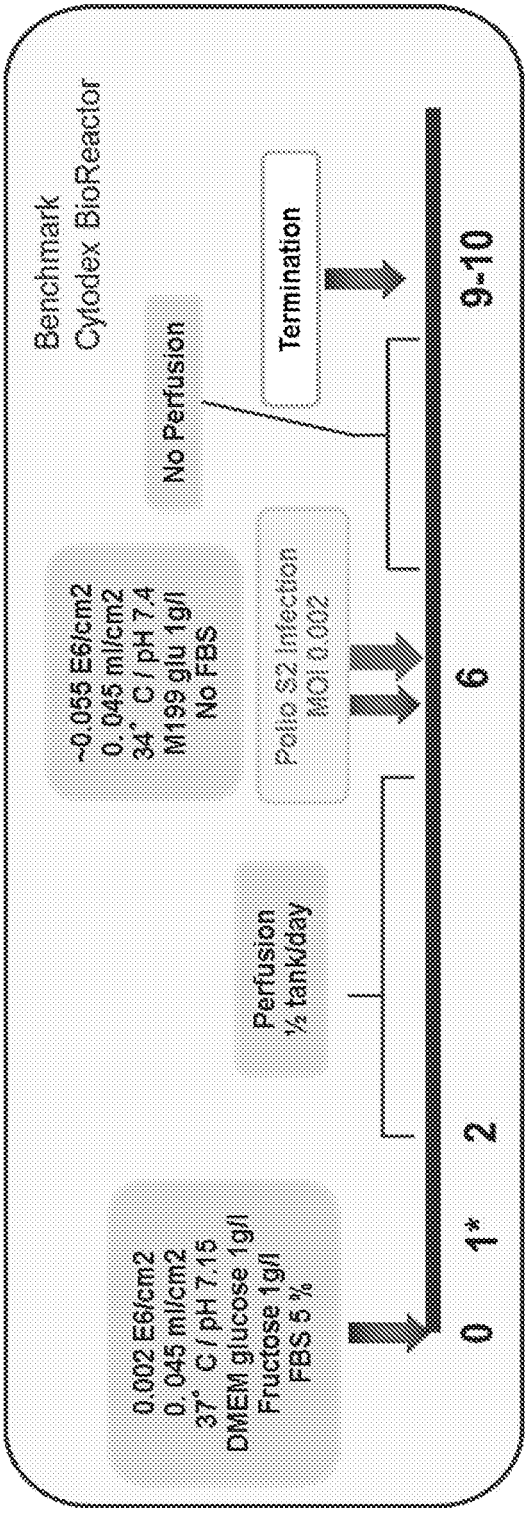

Benchmark
Cytodex BioReactor
0.002 E6/cm2
0.045 ml/cm2
37° C / pH 7.15
DMEM glucose 1g/l
Fructose 1g/l
FBS 5 %
Perfusion
½ tank/day
~0.055 E6/cm2
0.045 ml/cm2
34° C / pH 7.4
M199 glu 1g/l
No FBS
Polio S2 Infection
MOI 0.002
No Perfusion
Termination
0
1*
2
6
9-10

FIG. 13


NANO 40 compaction 1
0.015 E6/cm2
0.31 ml/cm2
37° C / pH 7.4
DMEM 4.5g/l
FBS10%
0.135 E6/cm2
0.31 ml/cm2
37° C / pH 7.4
DMEM 4.5g/l
FBS10%
0.55 E6/cm2
0.62 ml/cm2
34° C / pH 7.4
M199 1g/l
No FBS
Medium replacement
Polio S2 Infection
MOI 0.002
Termination
0
3
6
13

FIG. 14

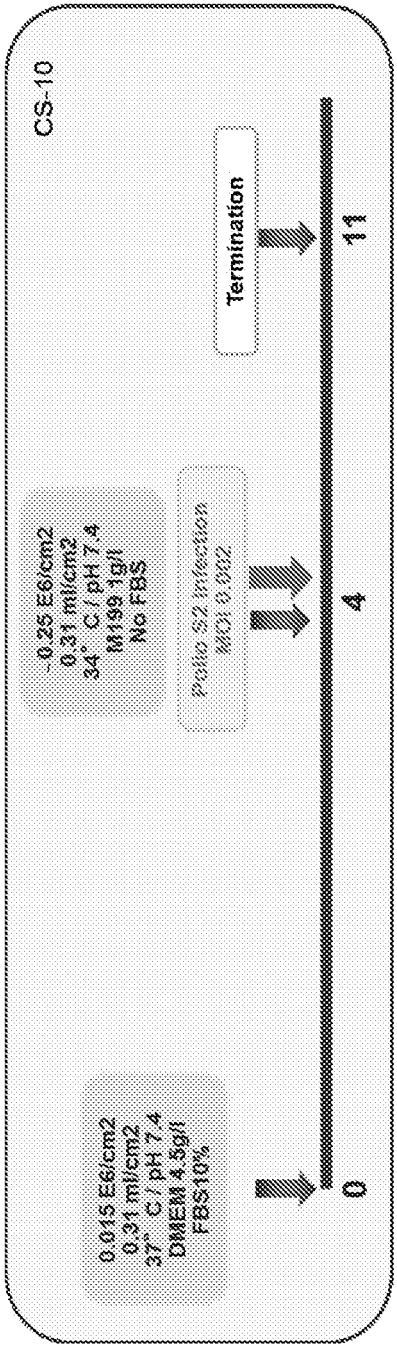

CS-10
0.25 E6/cm2
0.31 ml/cm2
34° C / pH 7.4
M199 1g/l
No FBS
0.015 E6/cm2
0.31 ml/cm2
37° C / pH 7.4
DMEM 4.5g/l
FBS10%
Pelle S2 Infection
MOI 0.002
Termination
0
4
11

FIG. 15

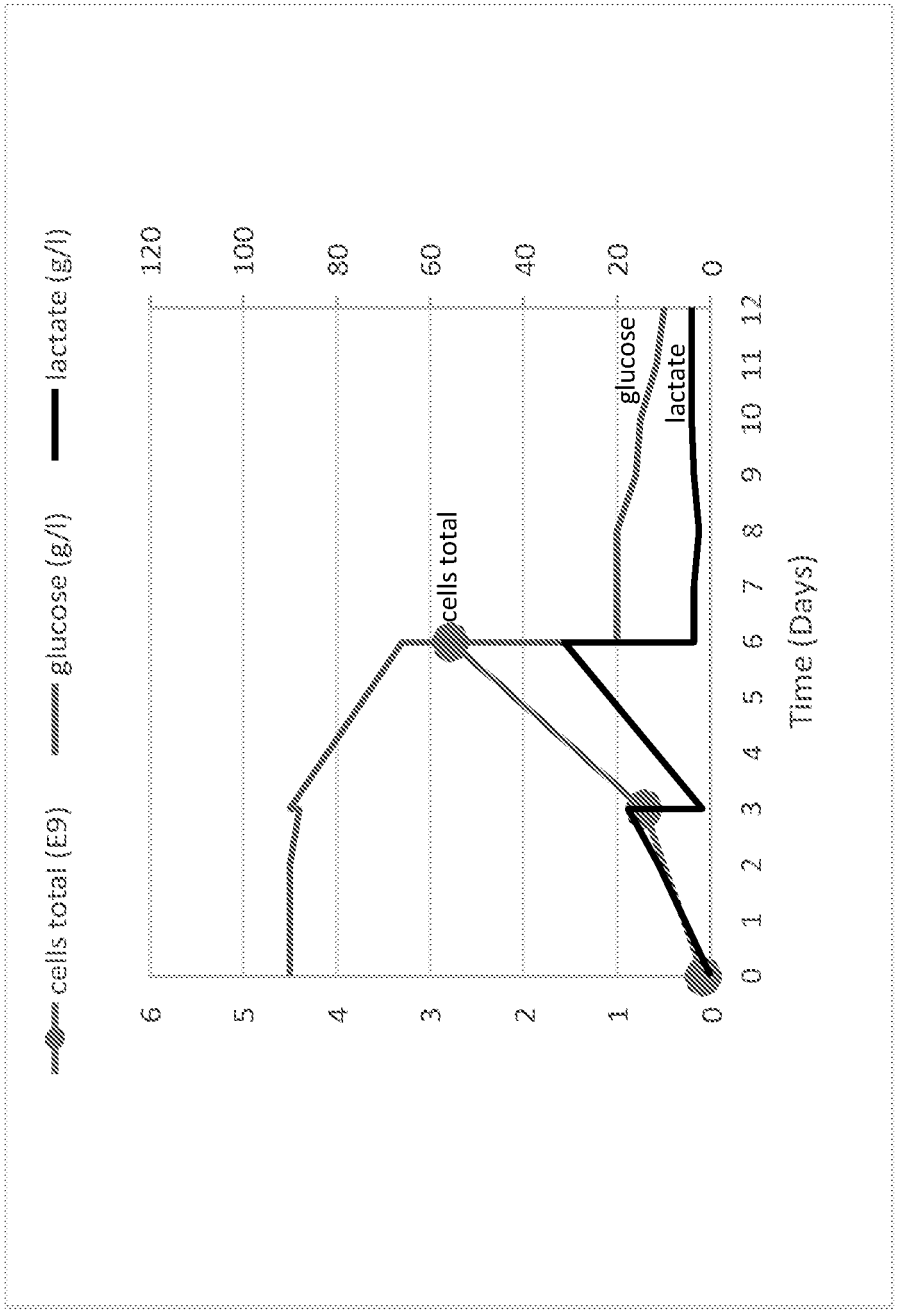

cells total (E9)
glucose (g/l)
lactate (g/l)
120
100
80
60
40
20
0
6
5
4
3
2
1
0
0 1 2 3 4 5 6 7 8 9 10 11 12
Time (Days)
cells total
glucose
lactate

FIG. 16

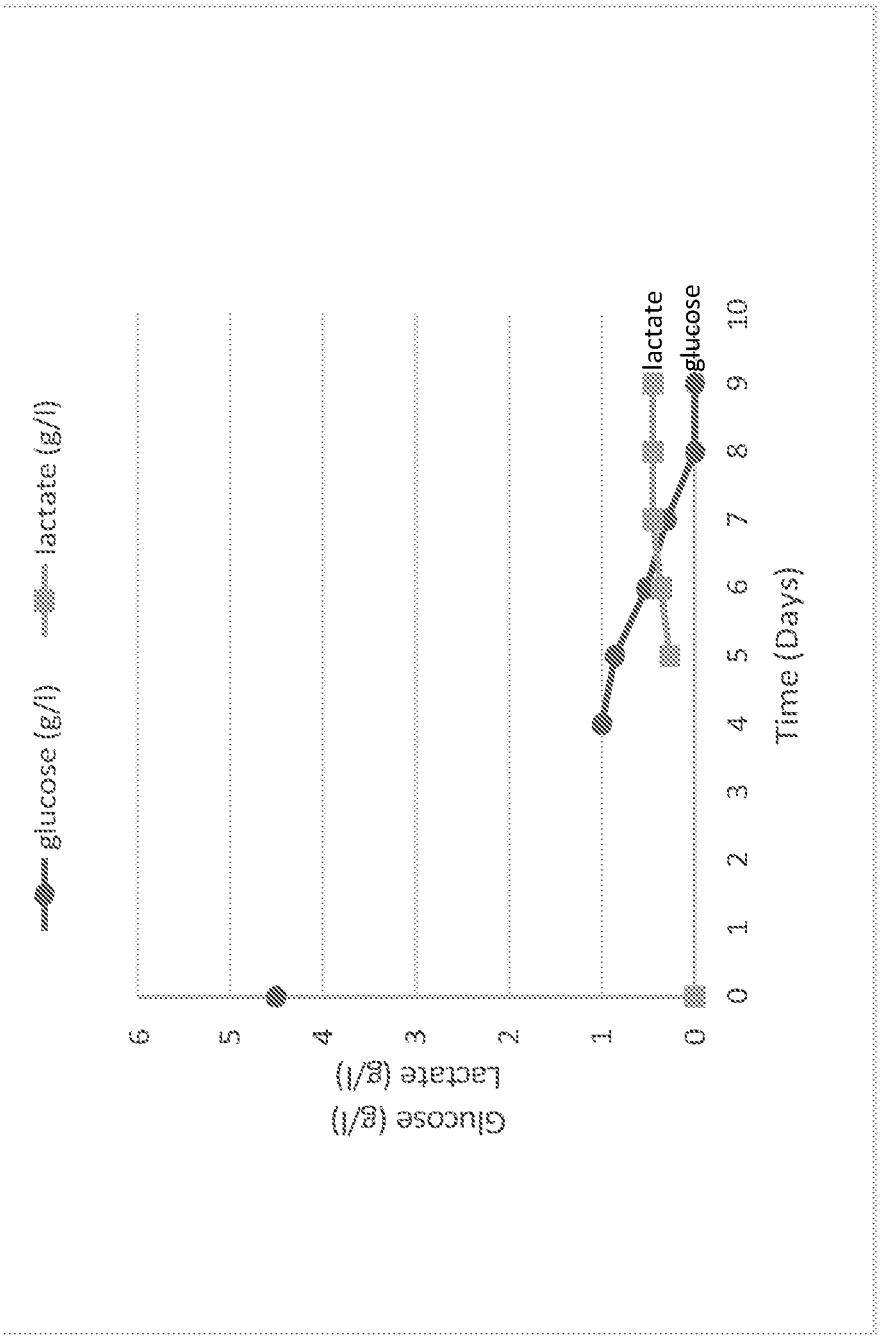

glucose (g/l)
lactate (g/l)
Glucose (g/l)
Lactate (g/l)
Time (Days)
lactate
glucose
0
1
2
3
4
5
6
7
8
9
10

FIG. 17

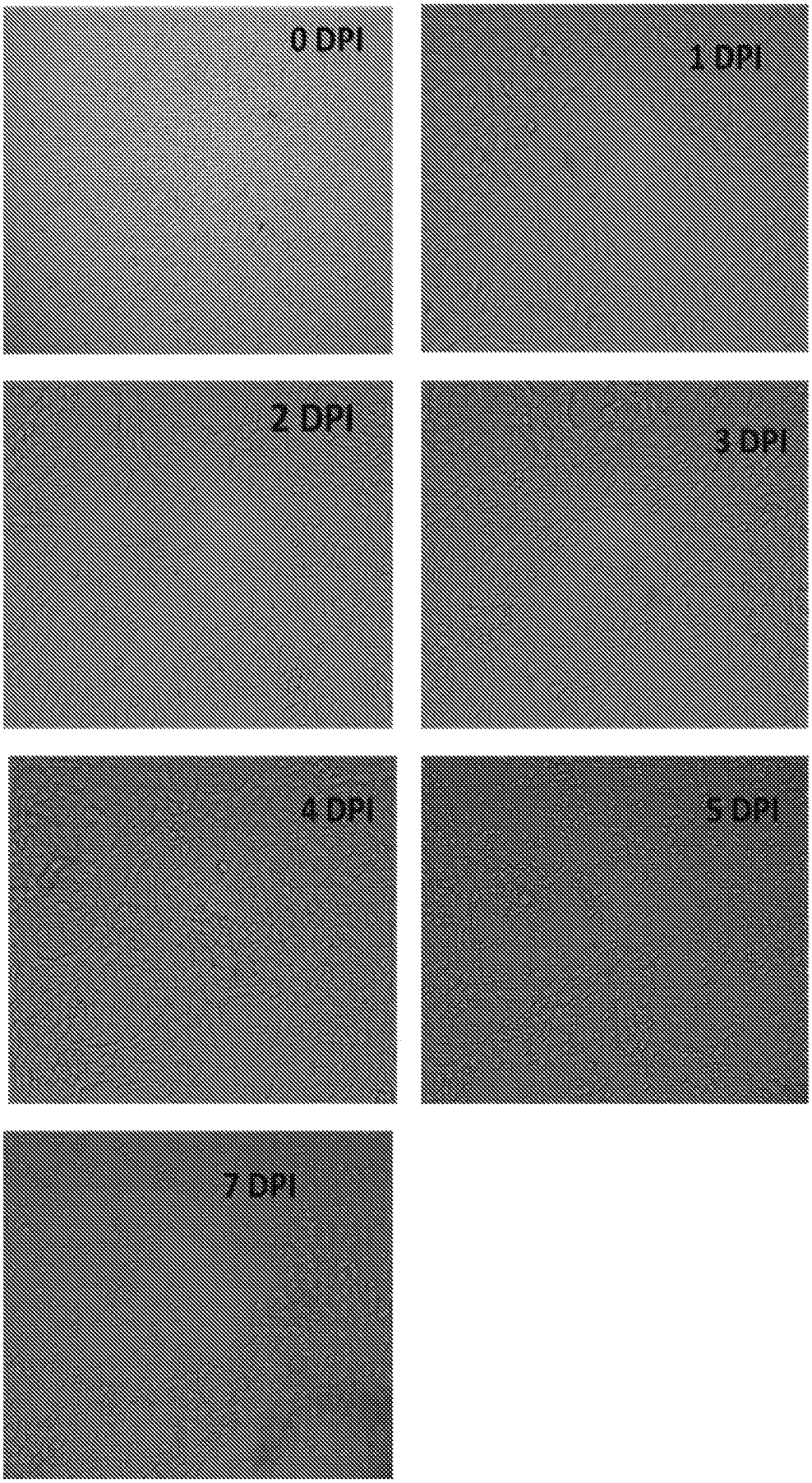


FIG. 18

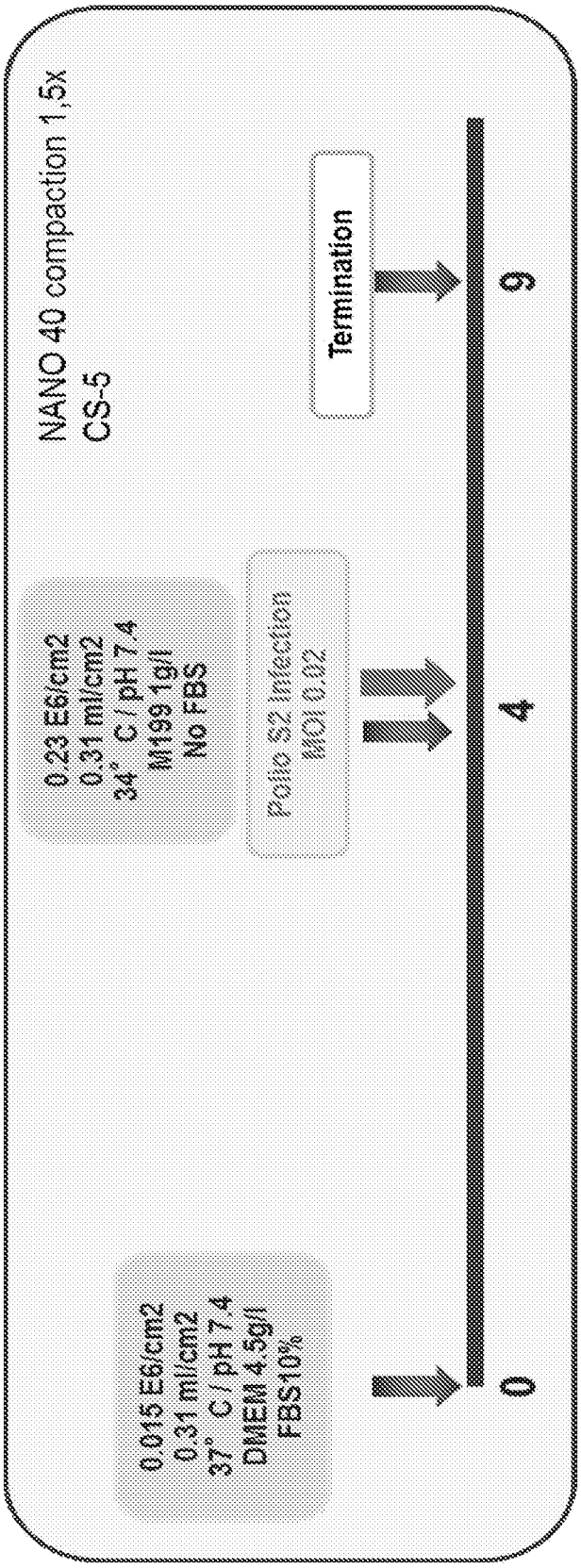

NANO 40 compaction 1,5x
CS-5
0.23 E6/cm2
0.31 ml/cm2
34° C / pH 7.4
M199 1g/l
No FBS
Polio S2 Infection
MOI 0.02
Termination
0.015 E6/cm2
0.31 ml/cm2
37° C / pH 7.4
DMEM 4.5g/l
FBS10%
0
4
9

FIG. 19

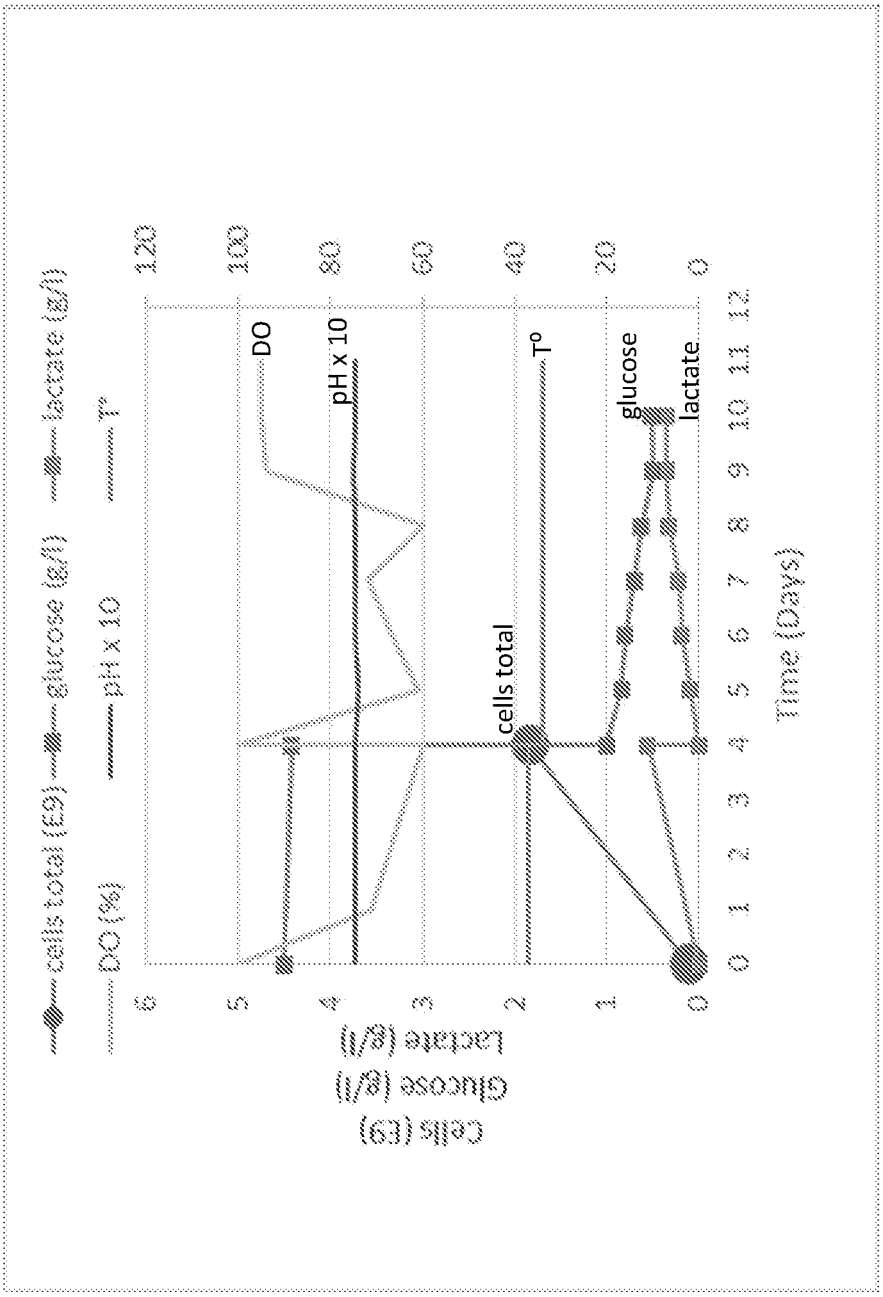

cells total (E9)
glucose (g/l)
lactate (g/l)
DO (%)
pH x 10
T°
Cells (E9)
Glucose (g/l)
Lactate (g/l)
Time (Days)
DO
pH x 10
cells total
T°
glucose
lactate

FIG. 20

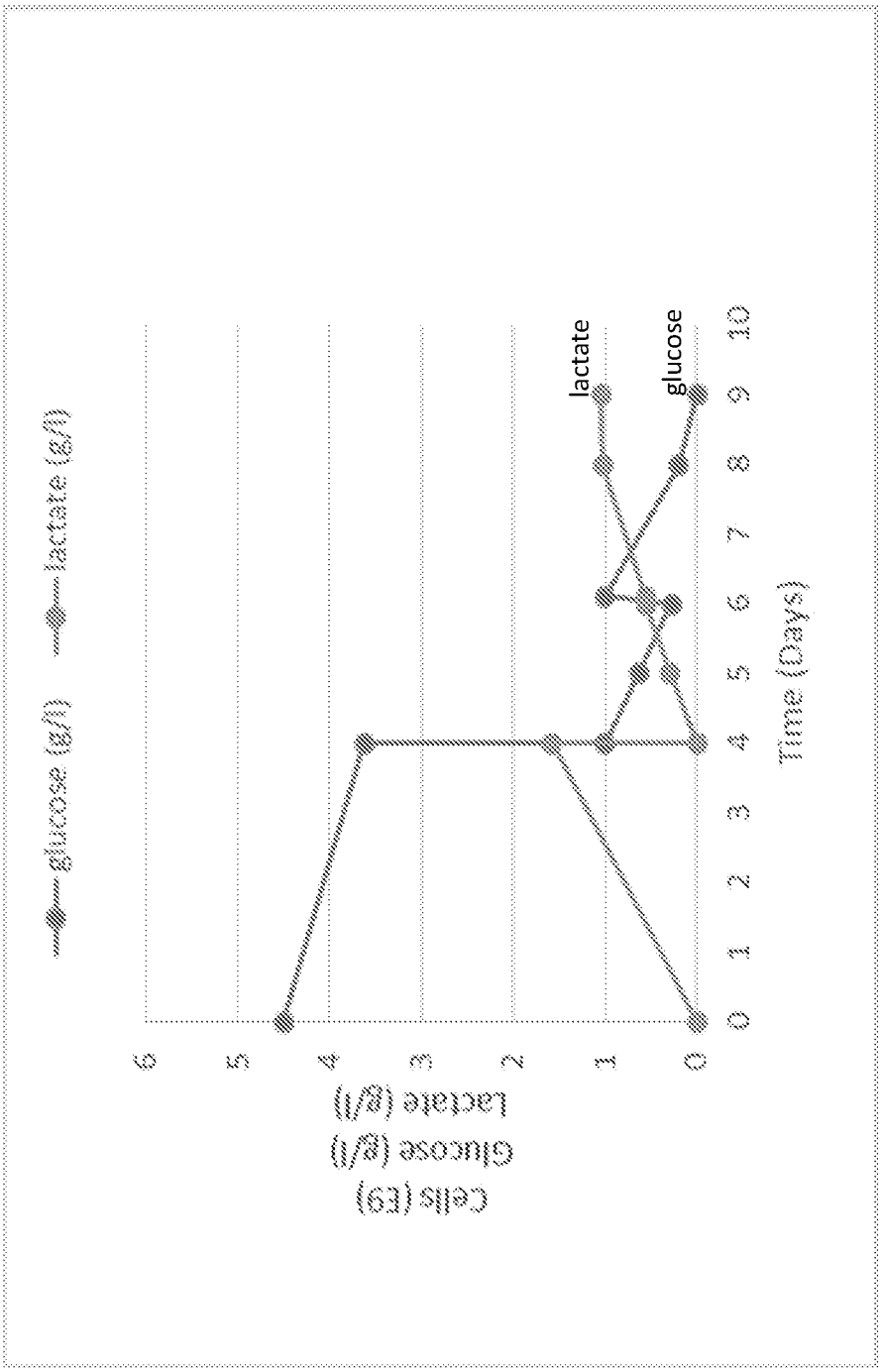

glucose (g/l)
lactate (g/l)
Cells (E9)
Glucose (g/l)
Lactate (g/l)
Time (Days)
lactate
glucose
0
1
2
3
4
5
6
7
8
9
10

FIG. 21

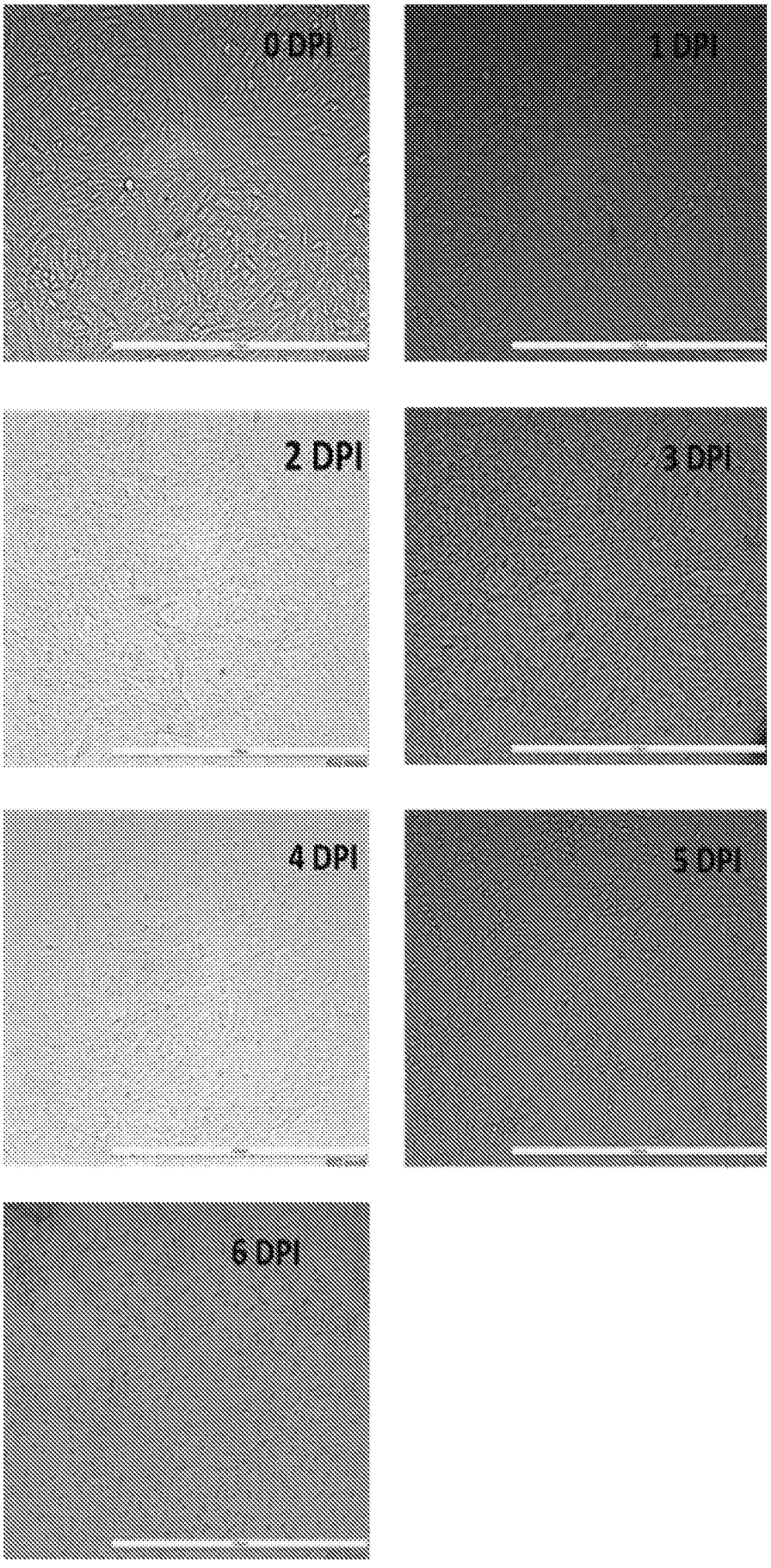


FIG. 22

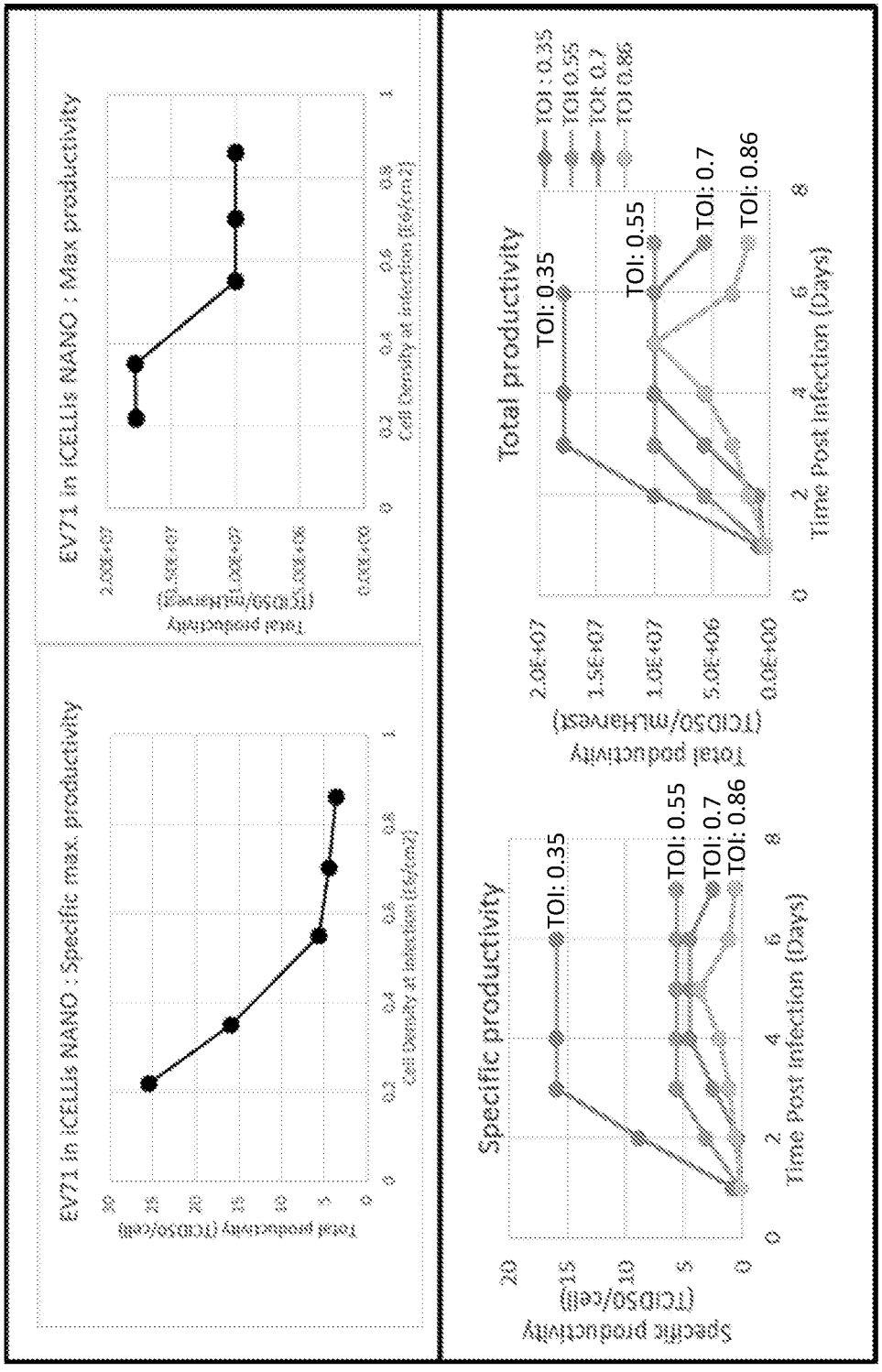


FIG. 23A

FIG. 23B

FIG. 23C

FIG. 23D

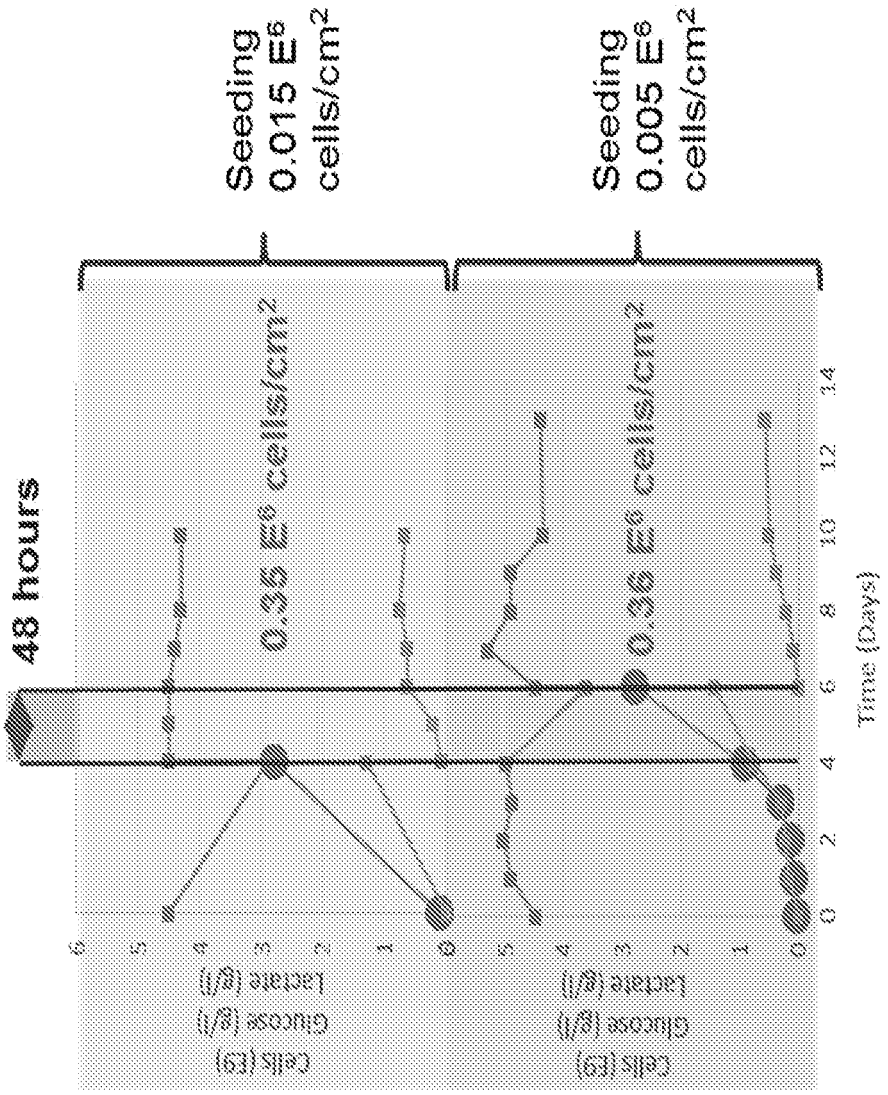

Seeding
0.015 E6
cells/cm2
Seeding
0.005 E6
cells/cm2
48 hours
0.36 E6 cells/cm2
0.36 E6 cells/cm2
Time (Days)
0
2
4
6
8
10
12
14
cells (E9)
Glucose (g/l)
Lactate (g/l)
0
1
2
3
4
5
6

FIG. 24

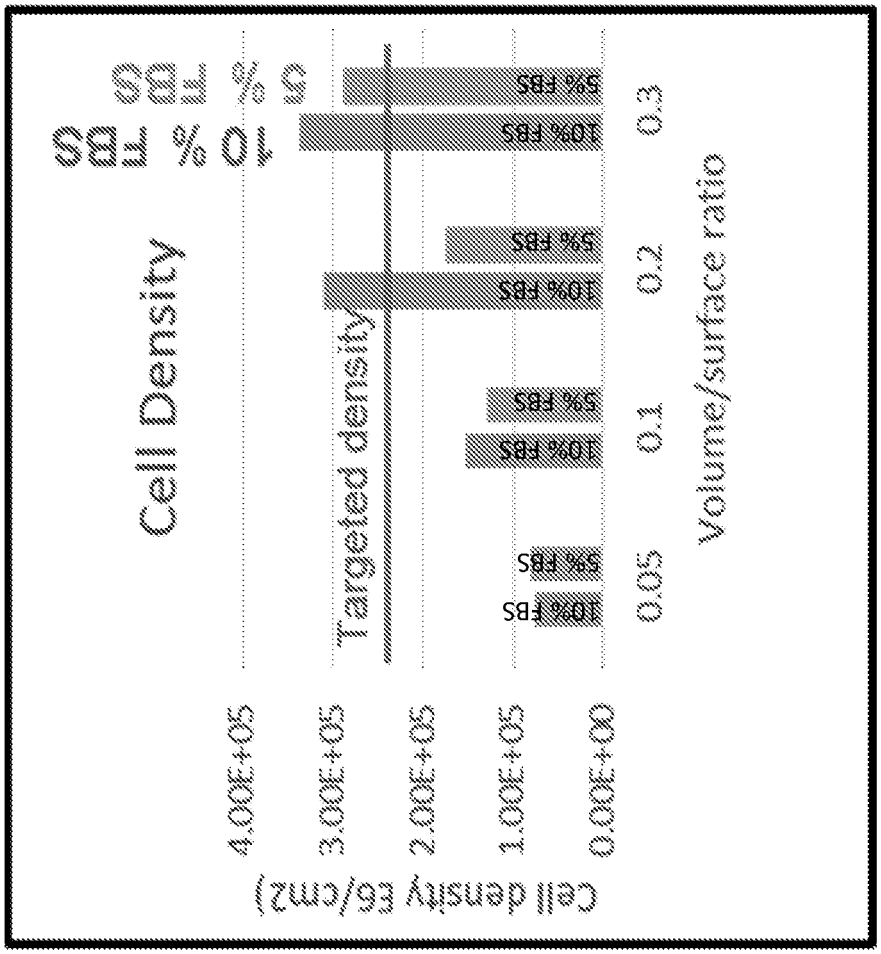

Cell Density
5% FBS
10% FBS
Targeted density
cell density E6/cm2)
4.00E+05
3.00E+05
2.00E+05
1.00E+05
0.00E+00
0.05
0.1
0.2
0.3
Volume/surface ratio
5% FBS
10% FBS
5% FBS
10% FBS
5% FBS
10% FBS
5% FBS
10% FBS

FIG. 25

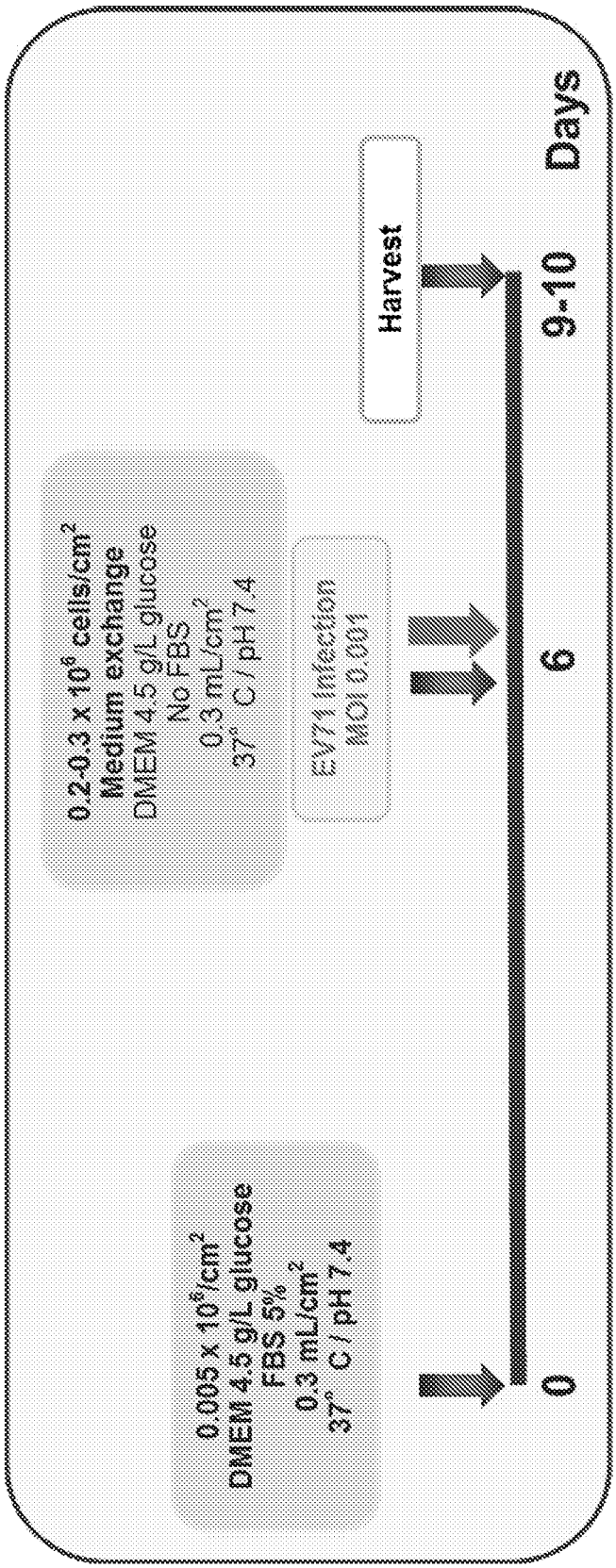

0.2-0.3 x 10^6 cells/cm^2
Medium exchange
DMEM 4.5 g/L glucose
No FBS
0.3 mL/cm^2
37° C / pH 7.4
EV71 infection
MOI 0.001
Harvest
0.005 x 10^6/cm^2
DMEM 4.5 g/L glucose
FBS 5%
0.3 mL/cm^2
37° C / pH 7.4
0
6
9-10
Days

FIG. 26

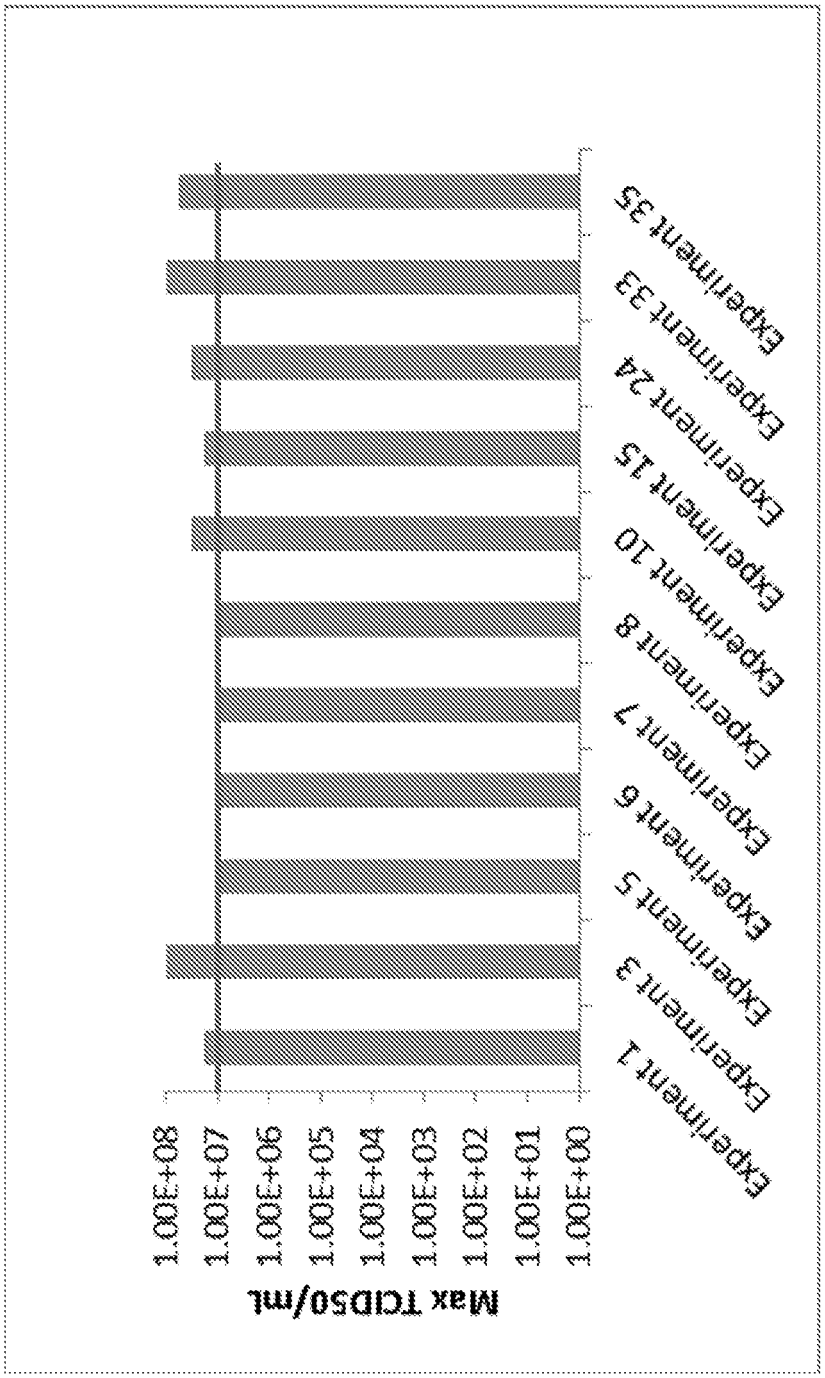

Max TCID50/mL
1.00E+08
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
Experiment 1
Experiment 3
Experiment 5
Experiment 6
Experiment 7
Experiment 8
Experiment 10
Experiment 15
Experiment 24
Experiment 33
Experiment 35

FIG. 27

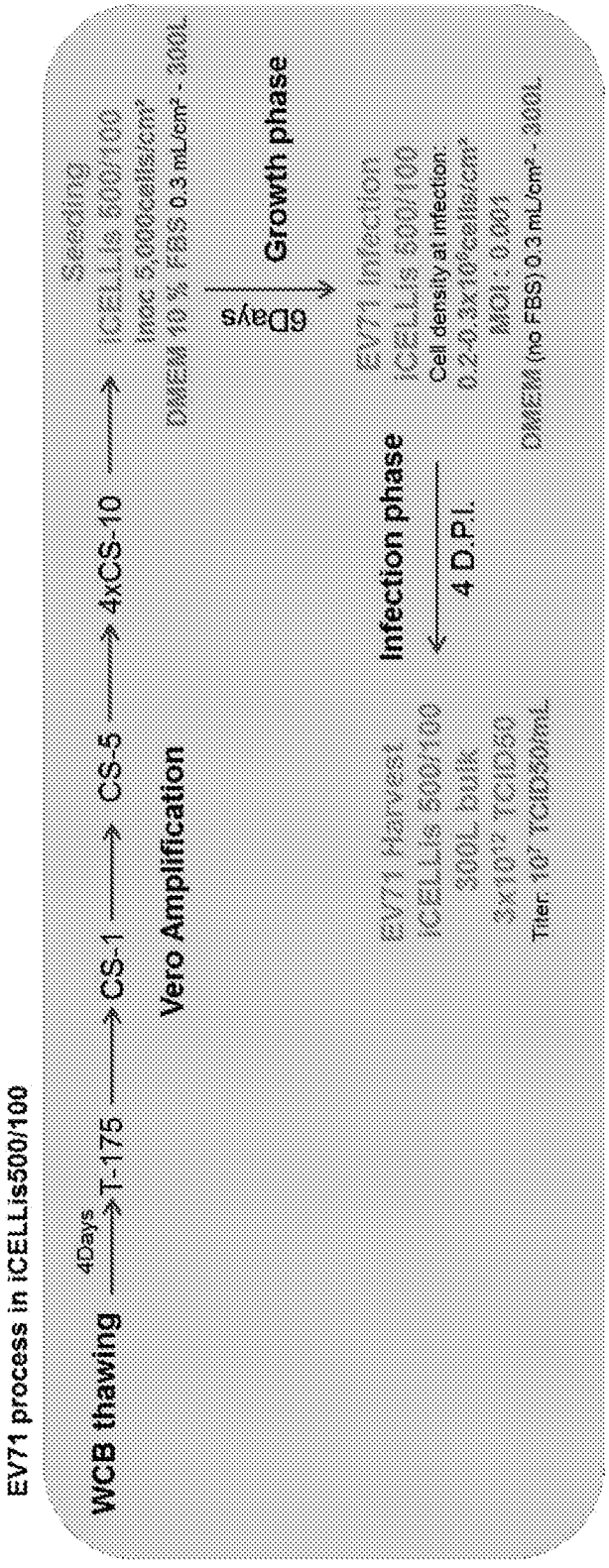

EV71 process in iCELLis500/100
WCB thawing
4Days
T-175
CS-1
CS-5
4xCS-10
iCELLis 500/100
Vero Amplification
Growth phase
6Days
Infection phase
4 D.P.I
EV71 Harvest
iCELLis 500/100
EV71 Infection
iCELLis 500/100
Cell density at infection
MOI : 0.001
4 D.P.I
Titer 10⁷ TCID50/mL

FIG. 28

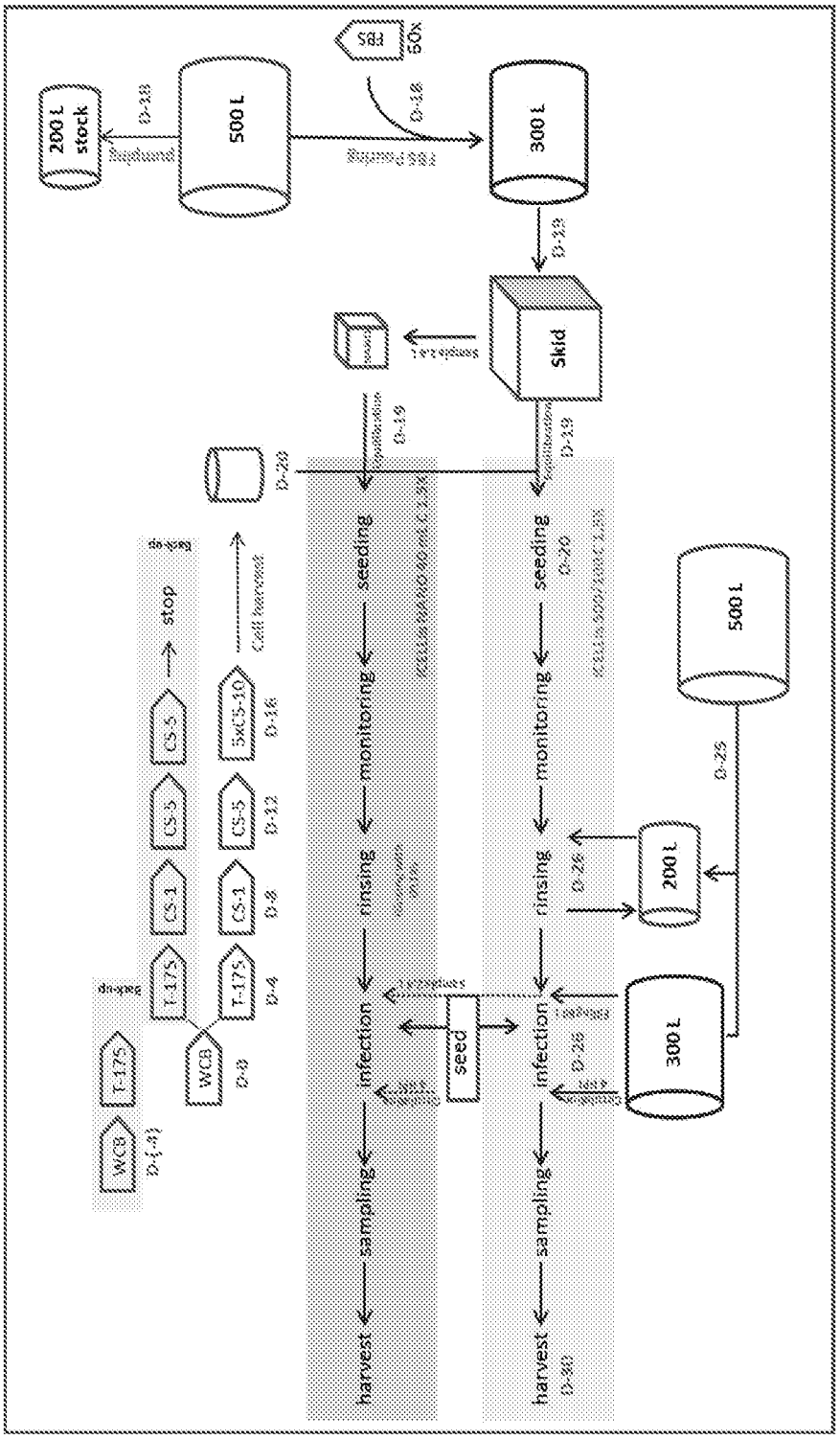

200 L stock
500 L
PBS
300 L
Skid
WCB
T-175
CS-1
CS-5
5xCS-10
stop
Cell harvest
seed
infection
rinsing
monitoring
seeding
sampling
harvest
200 L
500 L
300 L

FIG. 29

FIG. 30A

FIG. 30B

FIG. 30C

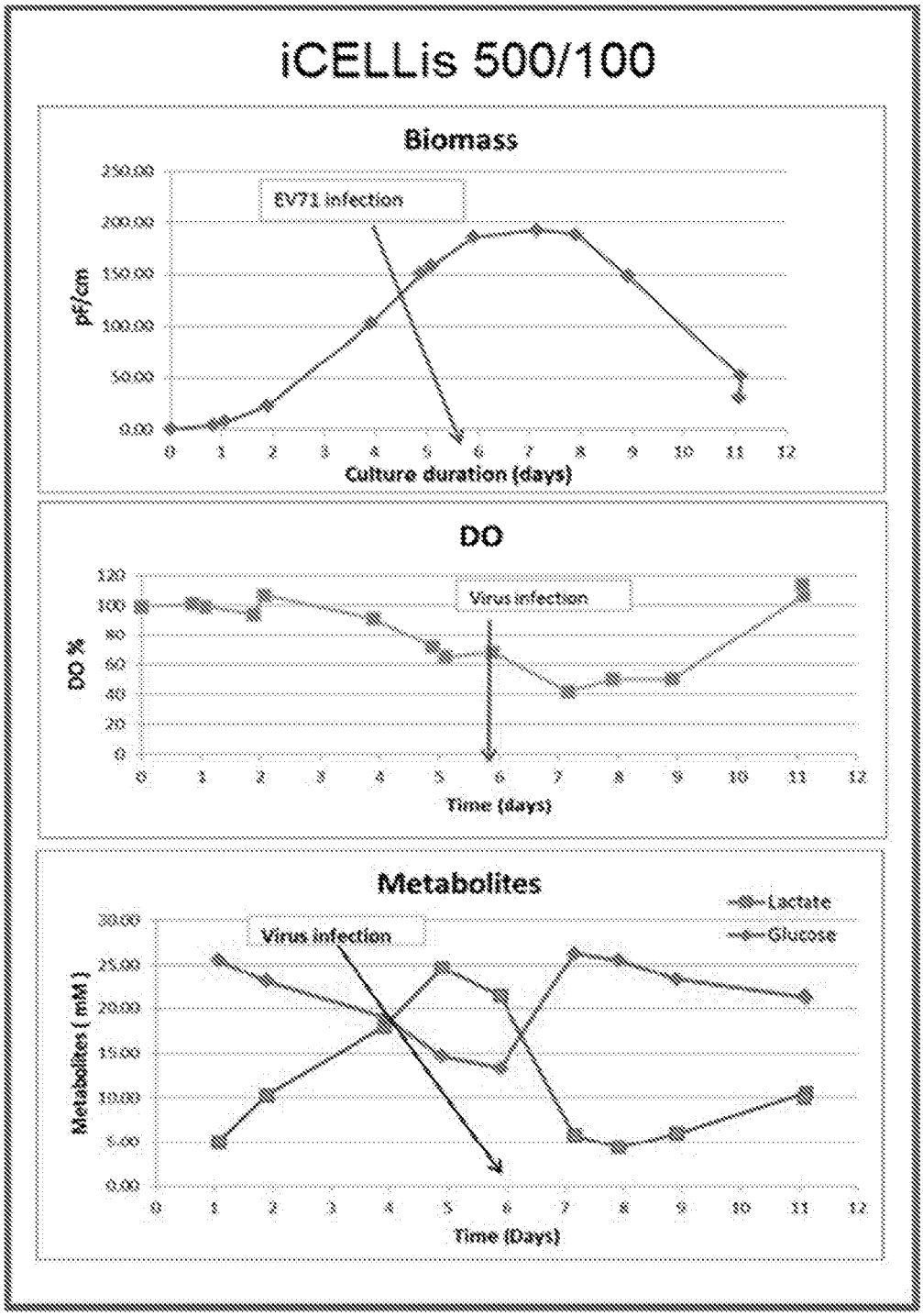


FIG. 30D

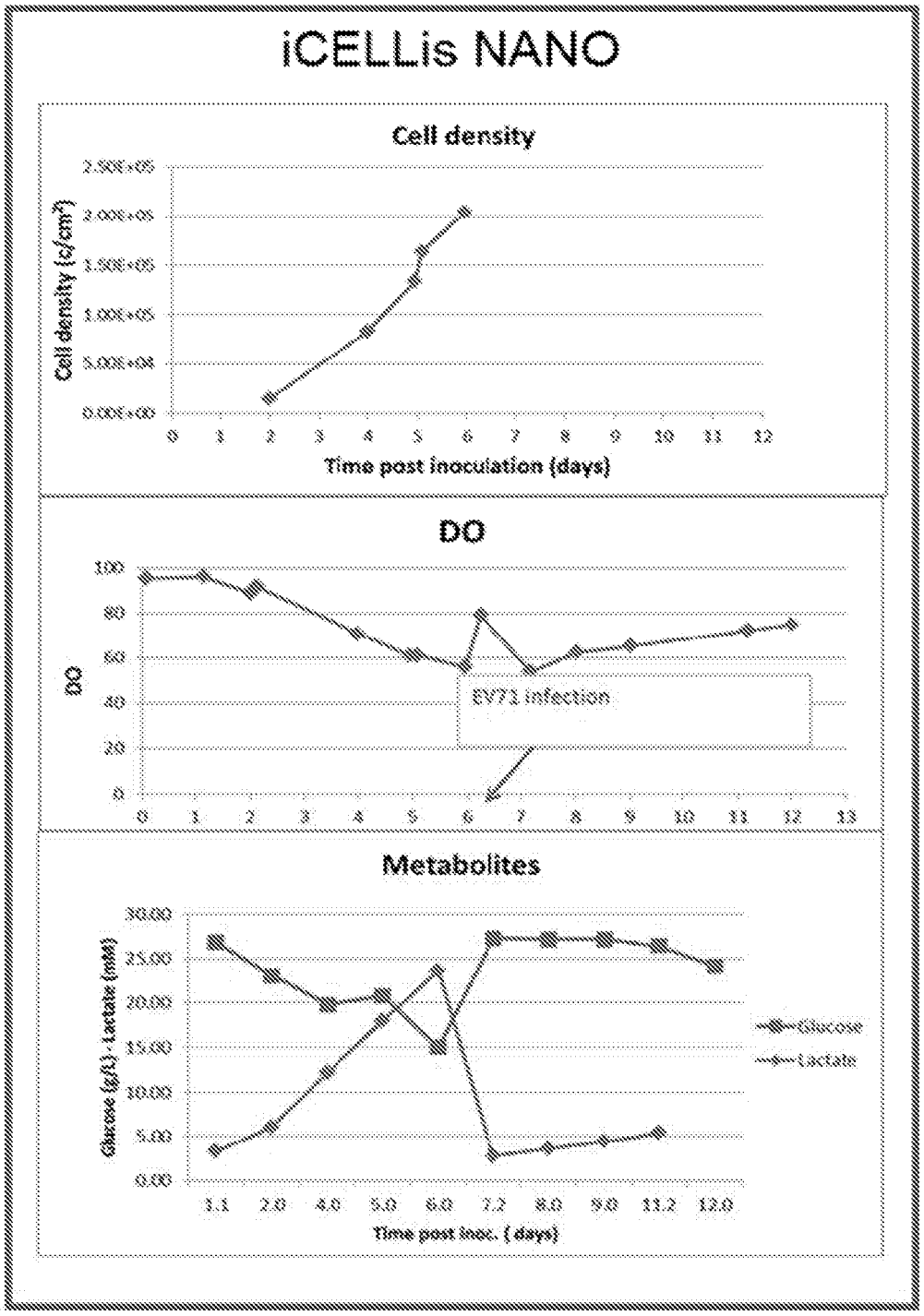


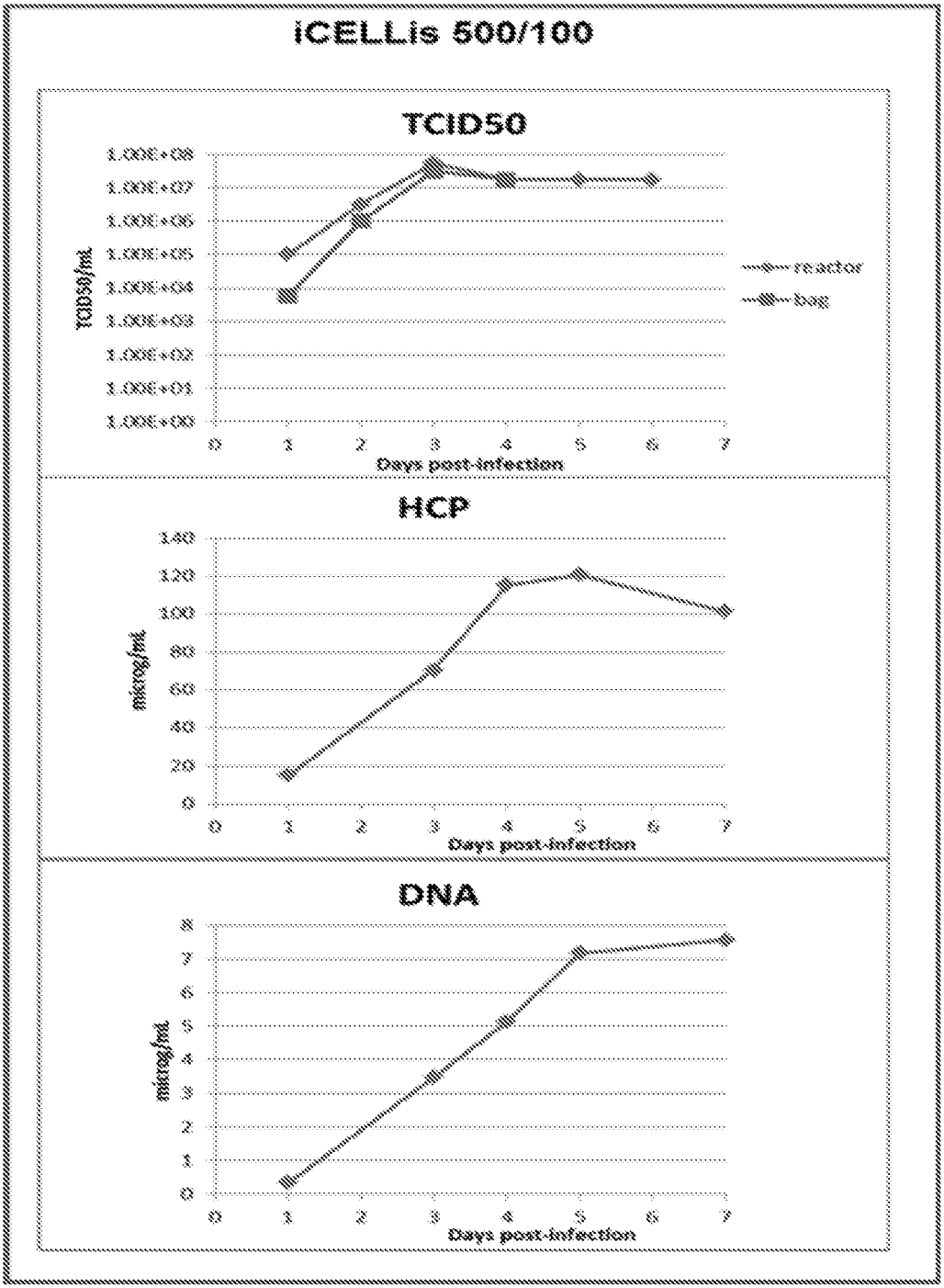
FIG. 30E

FIG. 30F

FIG. 31A

FIG. 31B

FIG. 31C



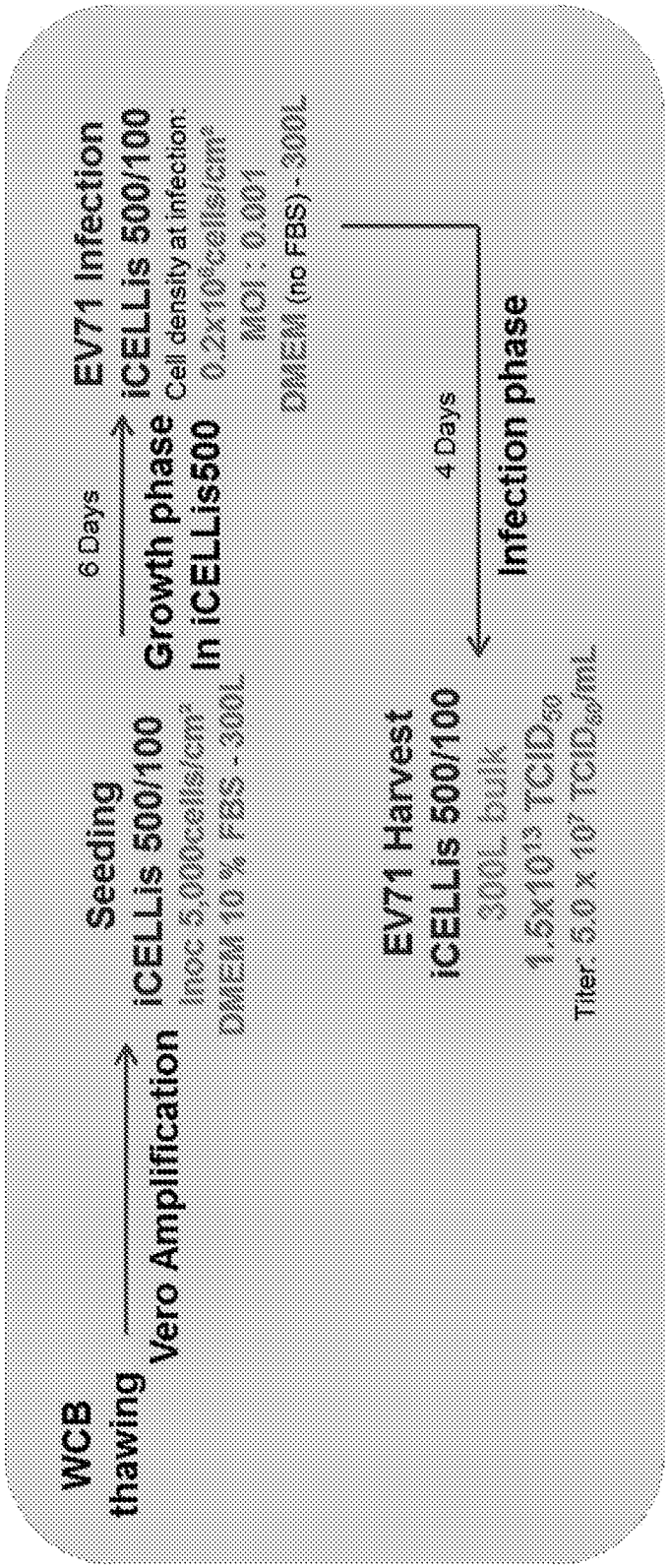

WCB
thawing
Vero Amplification
Seeding
iCELLis 500/100
Inoc. 5,000cells/cm²
DMEM 10 % FBS - 30mL
6 Days
Growth phase
In iCELLis500
EV71 Infection
iCELLis 500/100
Cell density at infection:
0.2x10⁶cells/cm²
MOI : 0.001
DMEM (no FBS) - 30mL
4 Days
Infection phase
EV71 Harvest
iCELLis 500/100
30mL bulk
1.5x10¹³ TCID₅₀
Titer: 5.0 x 10⁸ TCID₅₀/mL

FIG. 32

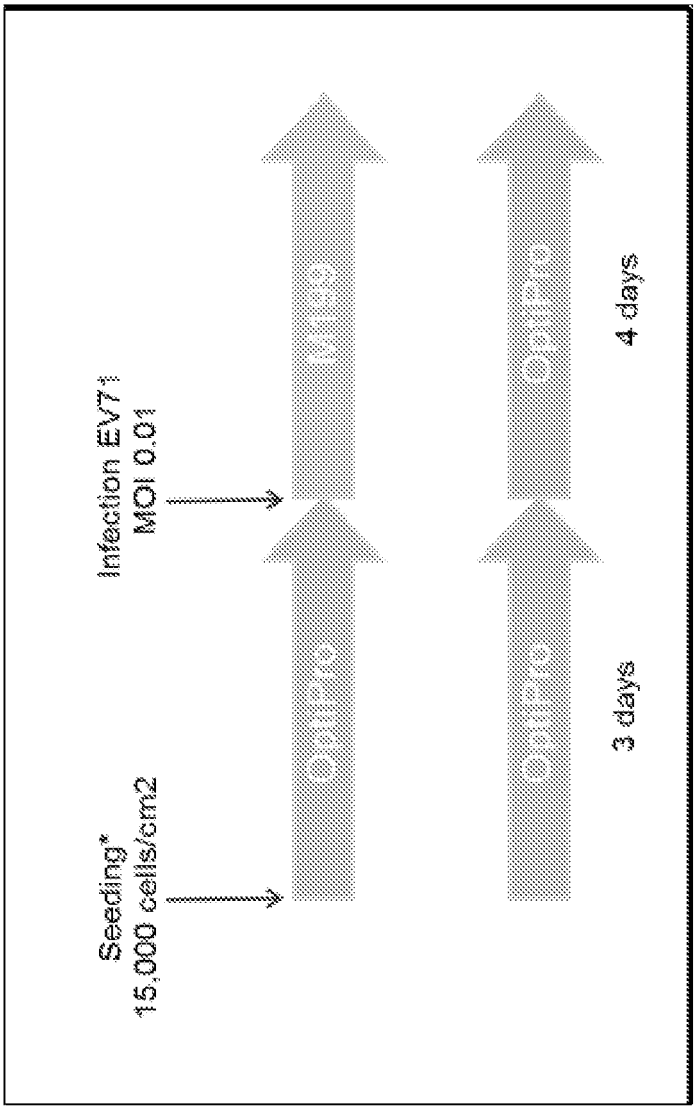

Seeding*
15,000 cells/cm2
Optimize
Infection EV71
MOI 0.01
Optimize
3 days
Optimize
4 days

FIG. 33

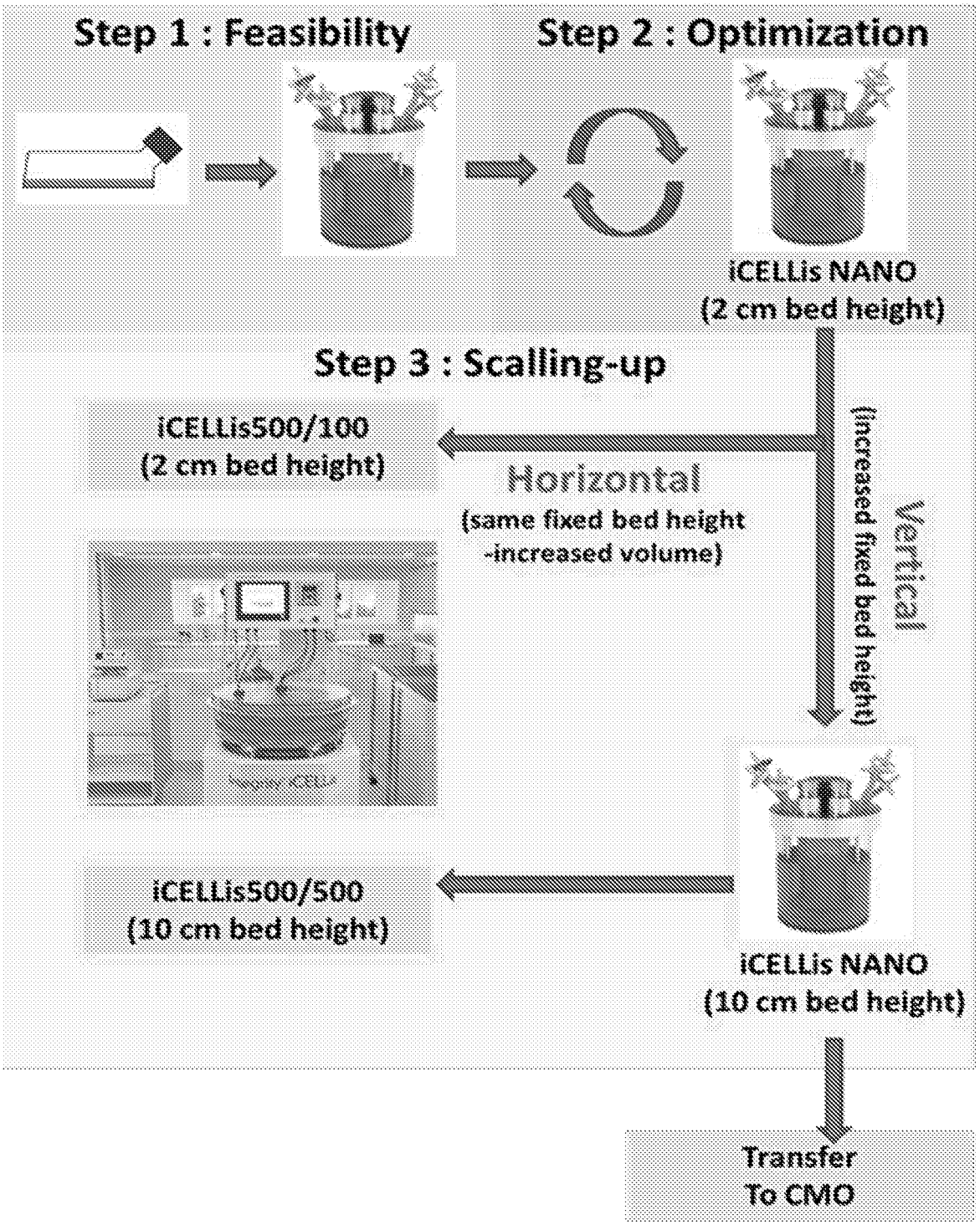

Step 1 : Feasibility
Step 2 : Optimization
iCELLis NANO
(2 cm bed height)
Step 3 : Scalling-up
iCELLis500/100
(2 cm bed height)
Horizontal
(same fixed bed height
-increased volume)
Vertical
(increased fixed bed height)
iCELLis500/500
(10 cm bed height)
iCELLis NANO
(10 cm bed height)
Transfer
To CMO

FIG. 34

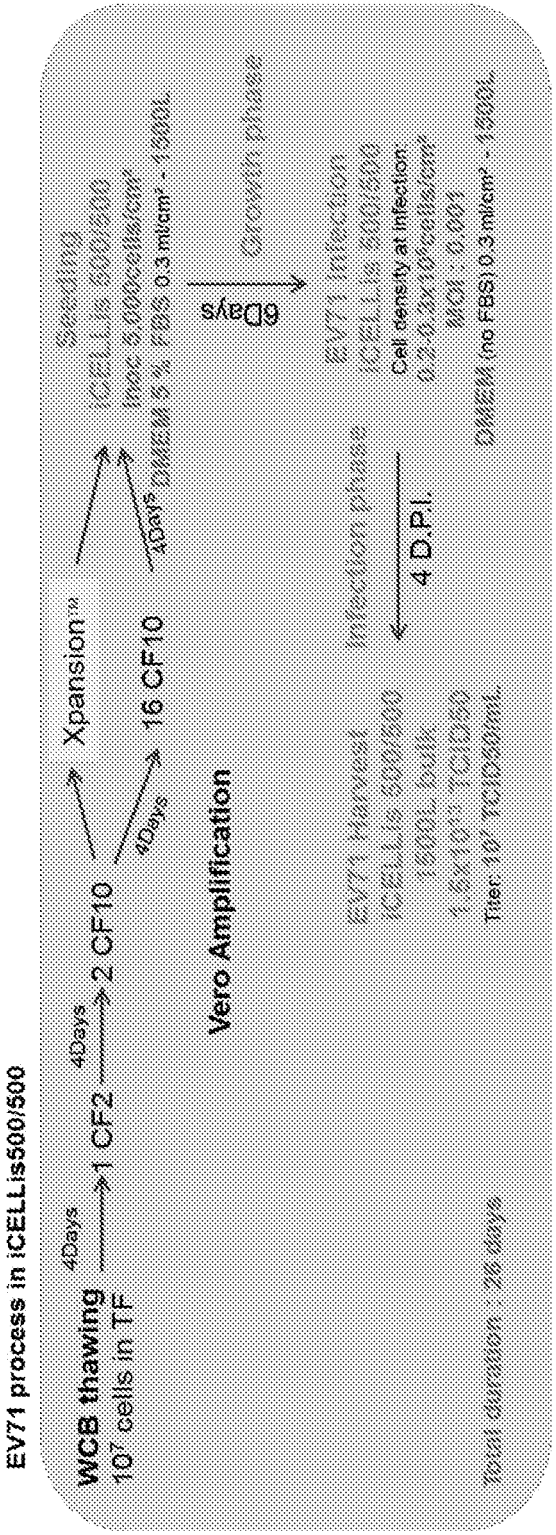

EV71 process in iCELLis500/500
WCB thawing
10⁷ cells in TF
4Days
1 CF2
4Days
2 CF10
Xpansion¹⁰
4Days
16 CF10
Vero Amplification
Seeding
iCELLis 500/500
max 5.000cells/cm²
MEM 5 % FBS 0.3 micm² - 150mL
4Days
6Days
Growth phase
EV71 Infection
iCELLis 500/500
Cel density at infection
0.2-0.3x10⁶cells/cm²
MOI : 0.001
DMEM (no FBS) 0.3 micm² - 150mL
4 D.P.I.
Infection phase
EV71 Harvest
iCELLis 500/500
1600L bulk
1.6x10¹³ TCID50
Titer: 10⁷ TCID50/mL
Total duration : 26 days

FIG. 35