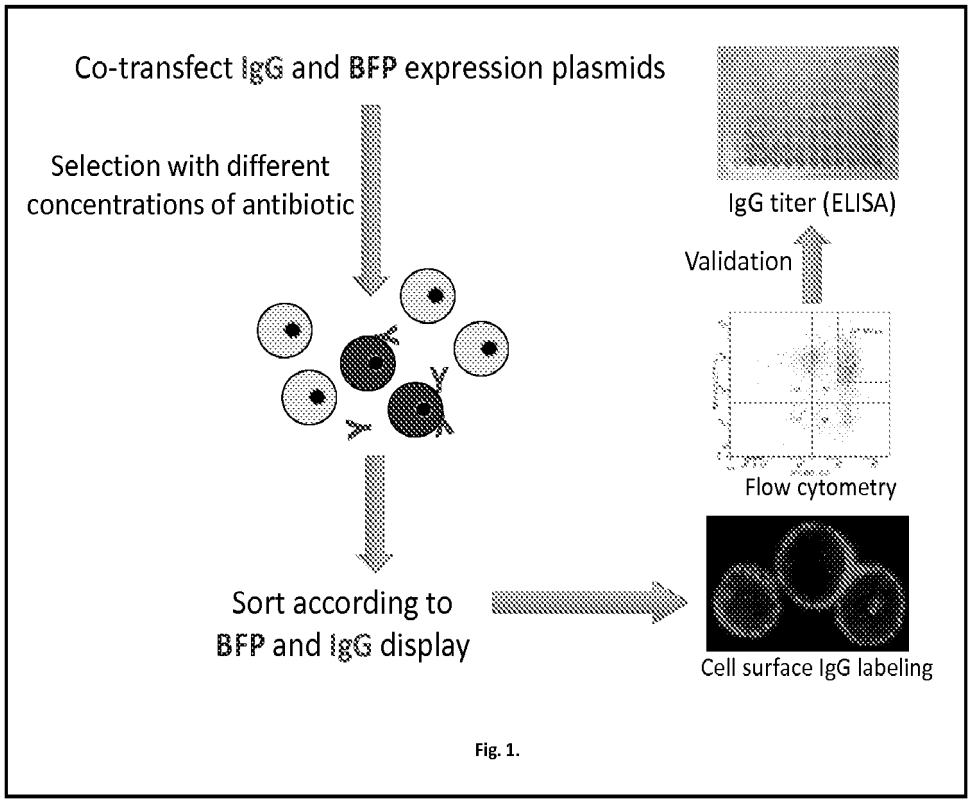

Co-transfect IgG and BFP expression plasmids
Selection with different concentrations of antibiotic
IgG titer (ELISA)
Validation
Flow cytometry
Sort according to BFP and IgG display
Cell surface IgG labeling

Fig. 1.

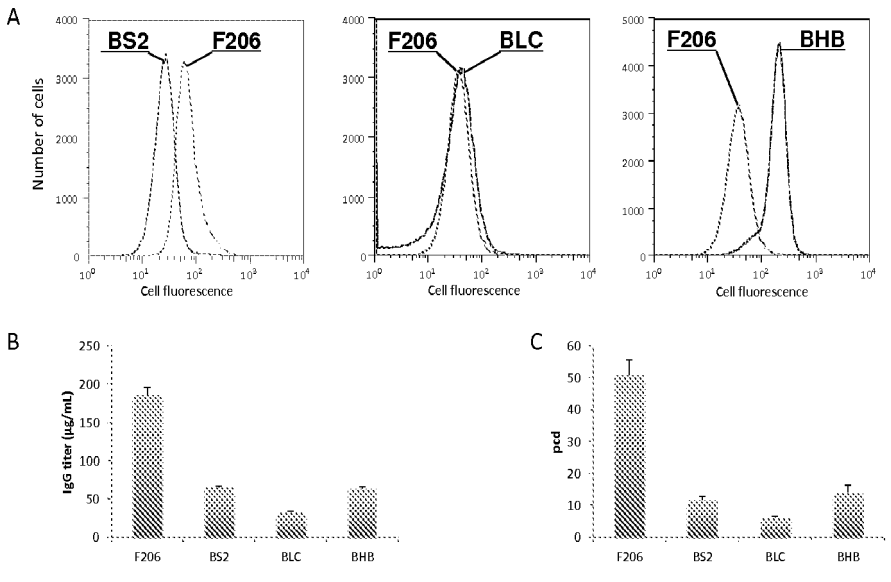

A
BS2
F206
F206
BLC
F206
BHB
Number of cells
Cell fluorescence
B
IgG titer (μg/mL)
F206
BS2
BLC
BHB
C
pcd
F206
BS2
BLC
BHB

Fig. 2

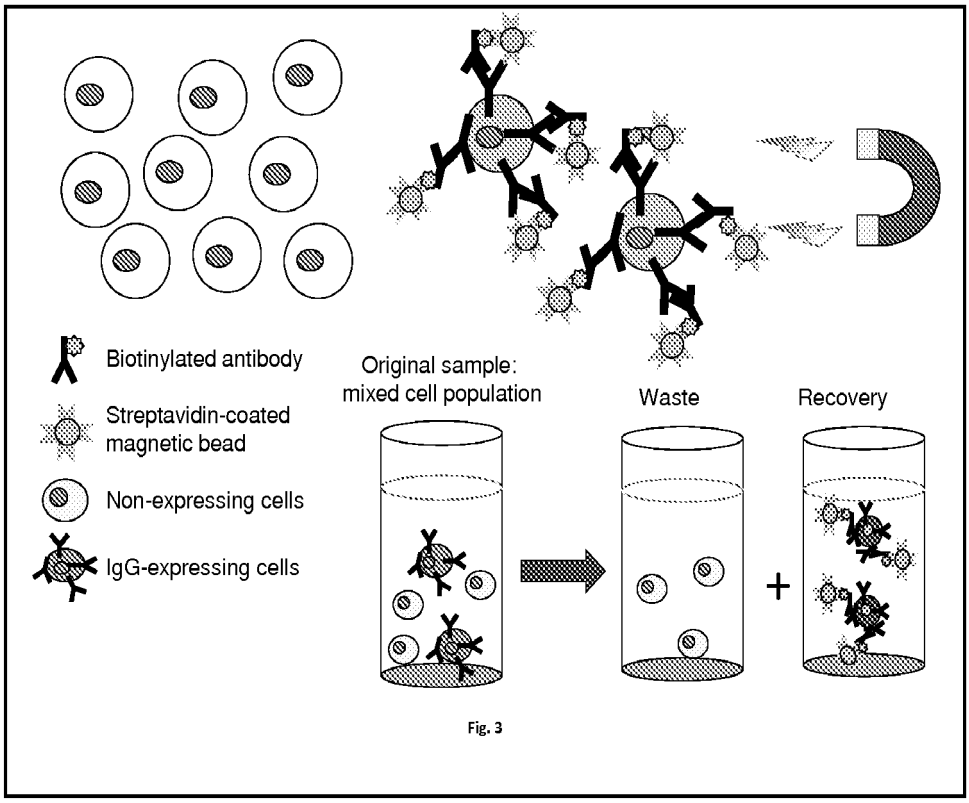

Biotinylated antibody
Streptavidin-coated magnetic bead
Non-expressing cells
IgG-expressing cells
Original sample: mixed cell population
Waste
Recovery

Fig. 3

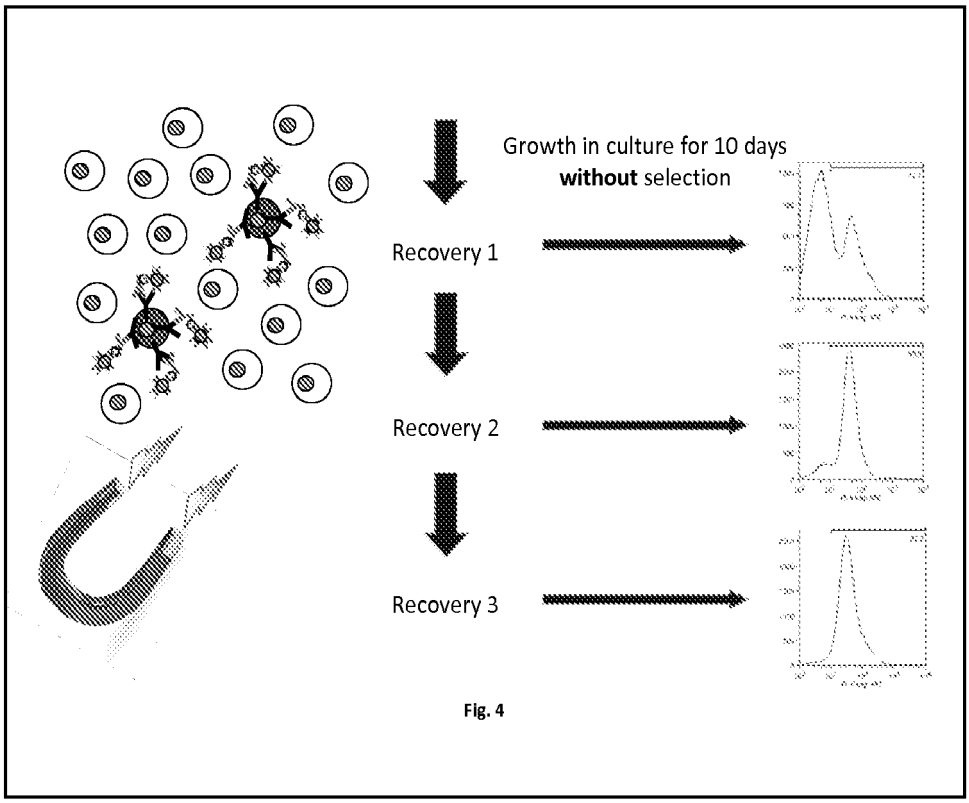

Growth in culture for 10 days **without** selection
Recovery 1
Recovery 2
Recovery 3

Fig. 4

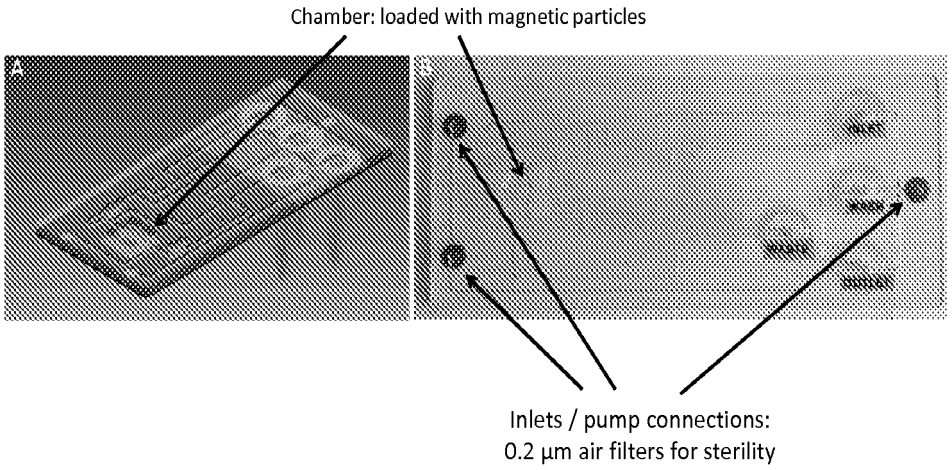



Fig. 5

## Superparamagnetic

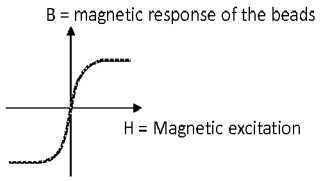
Advantages:
- No remanent magnetization, and thus
  - no aggregation of beads
  - easy resuspension and cell release
- Ideal for cell-sorting applications

Inconveniences:
- Not handled properly by MagPhase
- Magnetic field too weak, no remanence

## Ferromagnetic

Advantages:
- Strongly responsive to magnetic fields
- Works well with MagPhase settings

Inconveniences:
- Permanent magnets: form bead lattices
- Strong magnetization / aggregation of cells



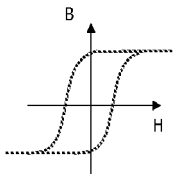




Fig. 6

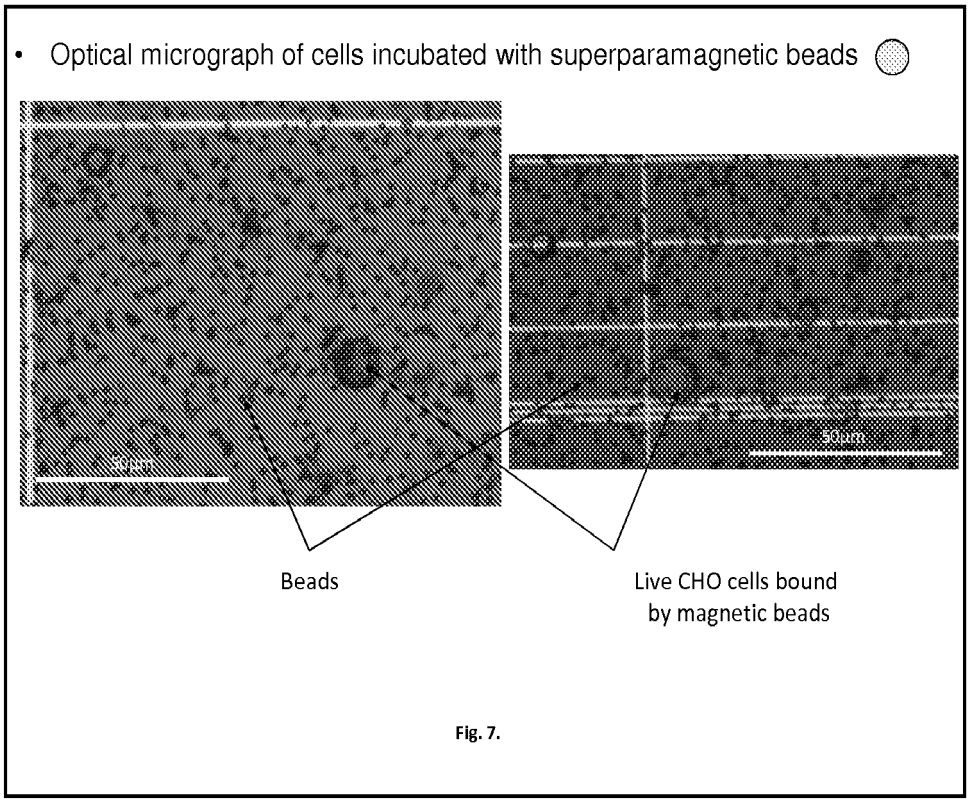

• Optical micrograph of cells incubated with superparamagnetic beads
50µm
50µm
Beads
Live CHO cells bound
by magnetic beads

Fig. 7.

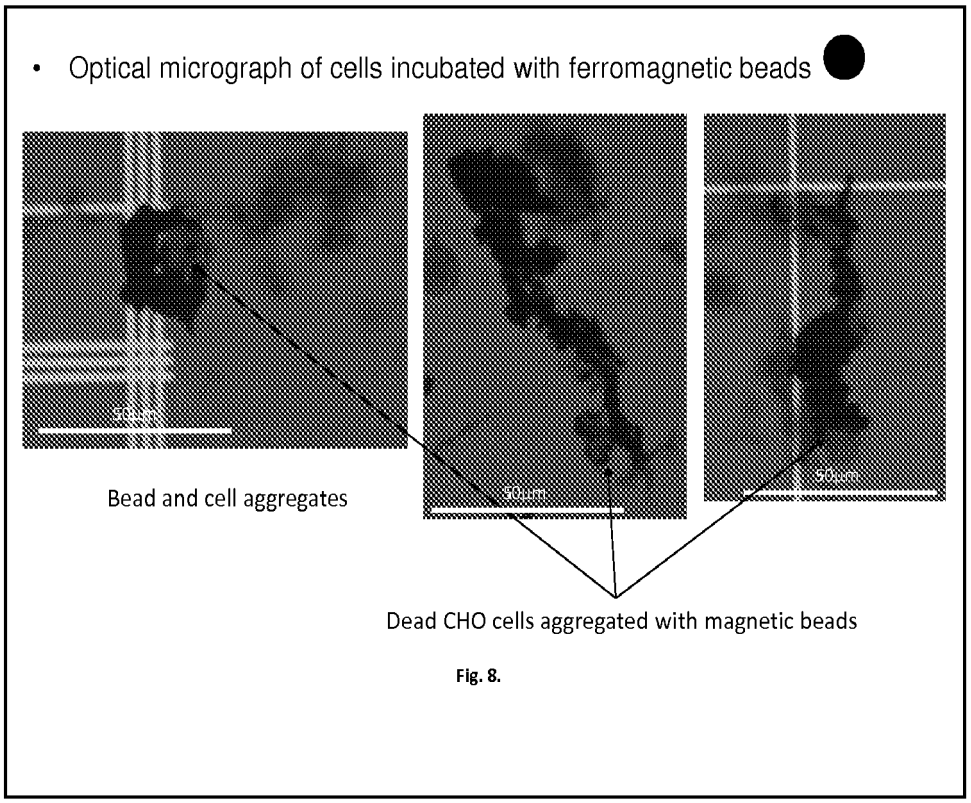

• Optical micrograph of cells incubated with ferromagnetic beads
50µm
50µm
50µm
Bead and cell aggregates
Dead CHO cells aggregated with magnetic beads

Fig. 8.

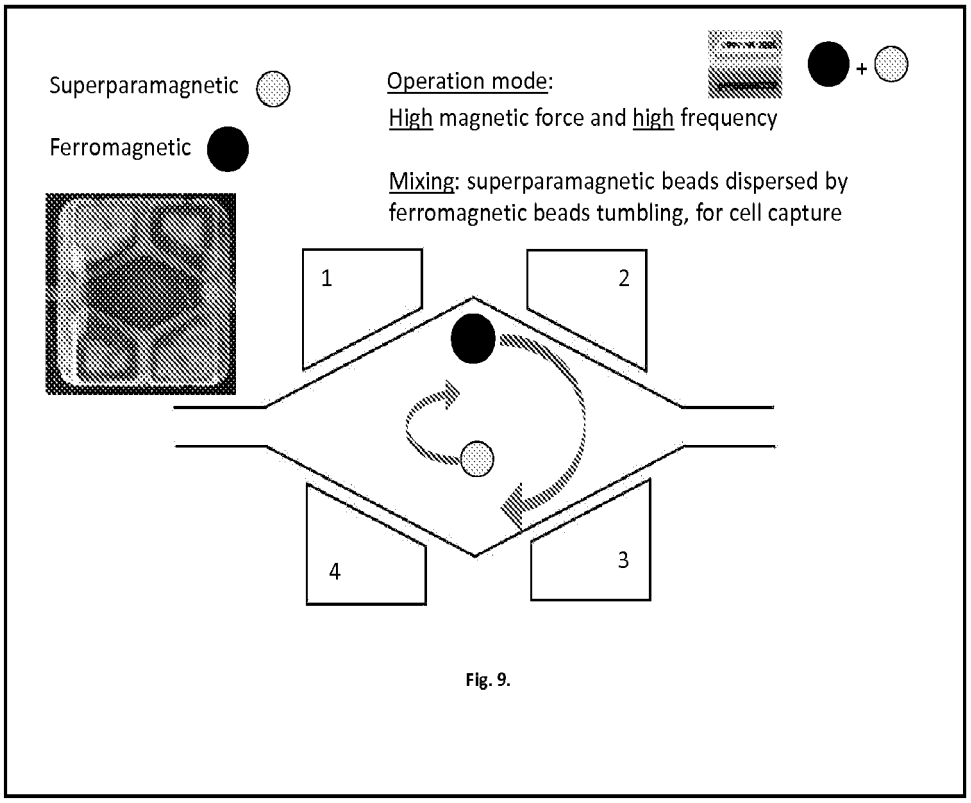

Superparamagnetic
Ferromagnetic
Operation mode:
High magnetic force and high frequency
Mixing: superparamagnetic beads dispersed by ferromagnetic beads tumbling, for cell capture
1
2
3
4

Fig. 9.

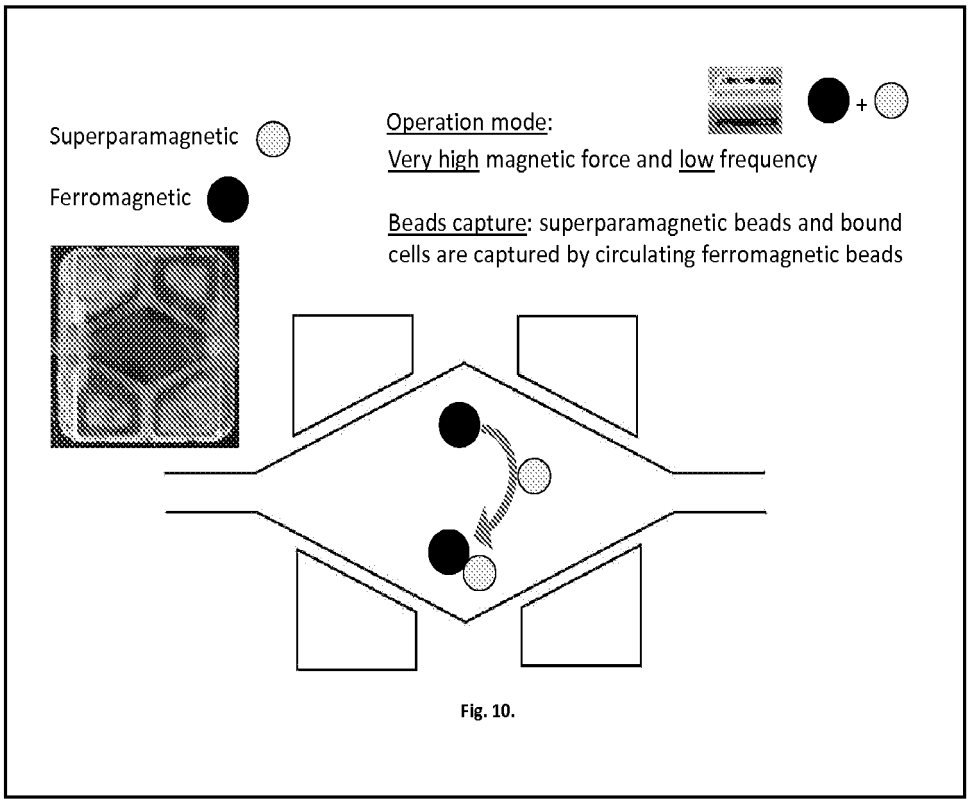

Superparamagnetic
Ferromagnetic
Operation mode:
Very high magnetic force and low frequency
Beads capture: superparamagnetic beads and bound cells are captured by circulating ferromagnetic beads

Fig. 10.

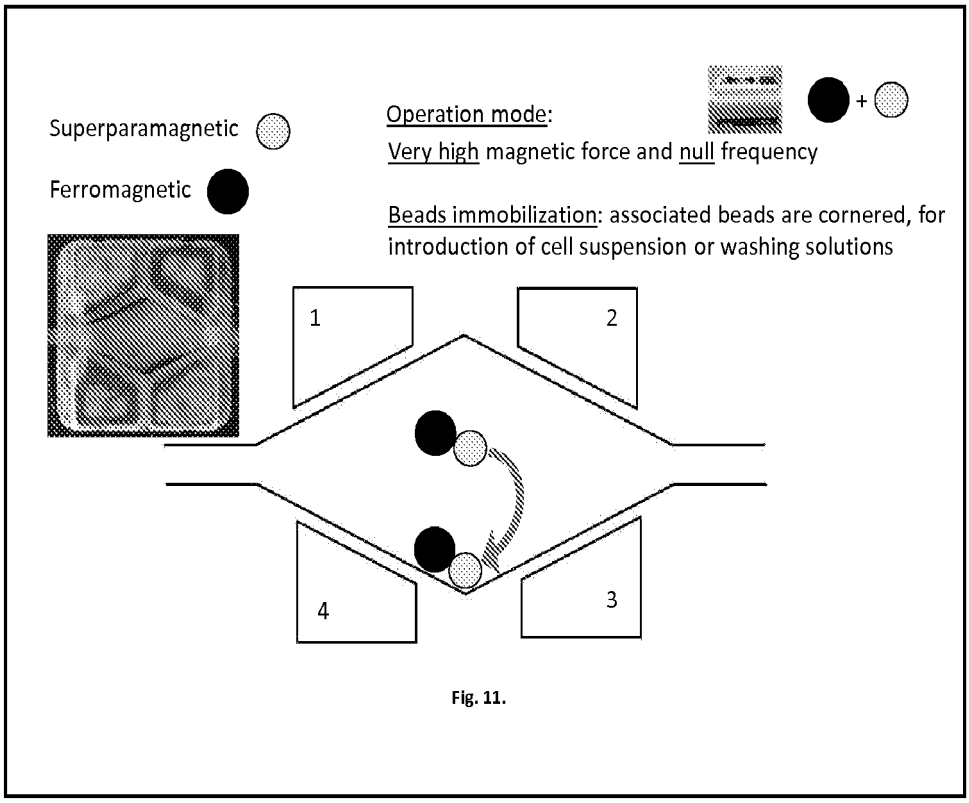

Superparamagnetic
Ferromagnetic
Operation mode:
Very high magnetic force and null frequency
Beads immobilization: associated beads are cornered, for introduction of cell suspension or washing solutions
1
2
3
4

Fig. 11.

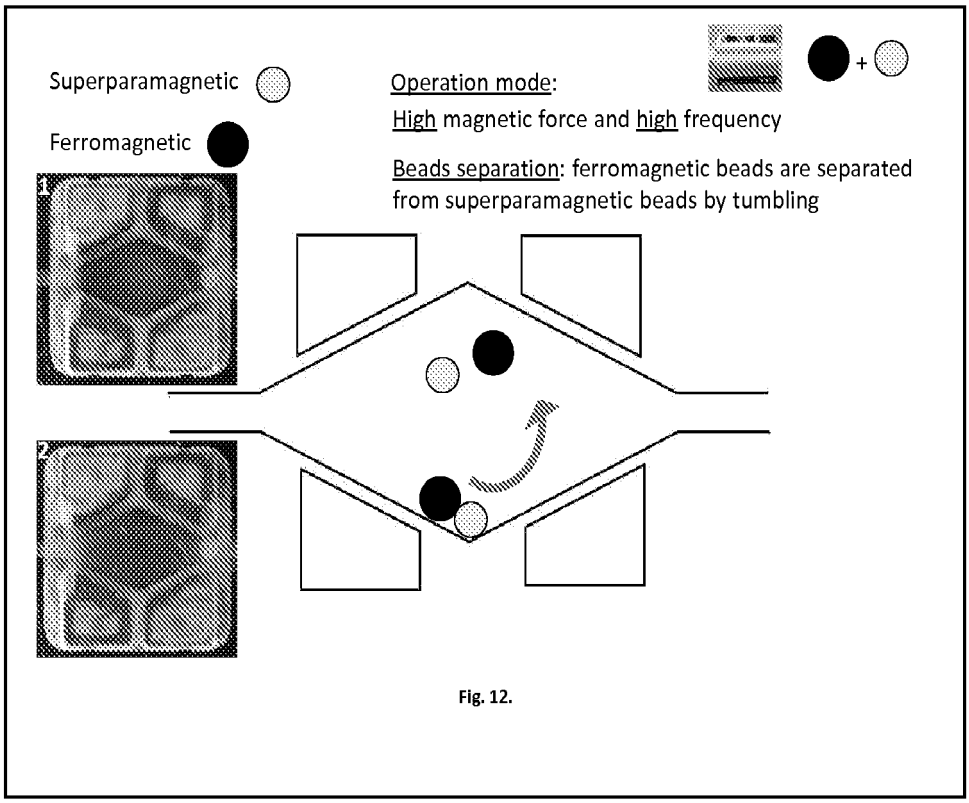

Superparamagnetic
Ferromagnetic
Operation mode:
High magnetic force and high frequency
Beads separation: ferromagnetic beads are separated from superparamagnetic beads by tumbling
1
2

Fig. 12.

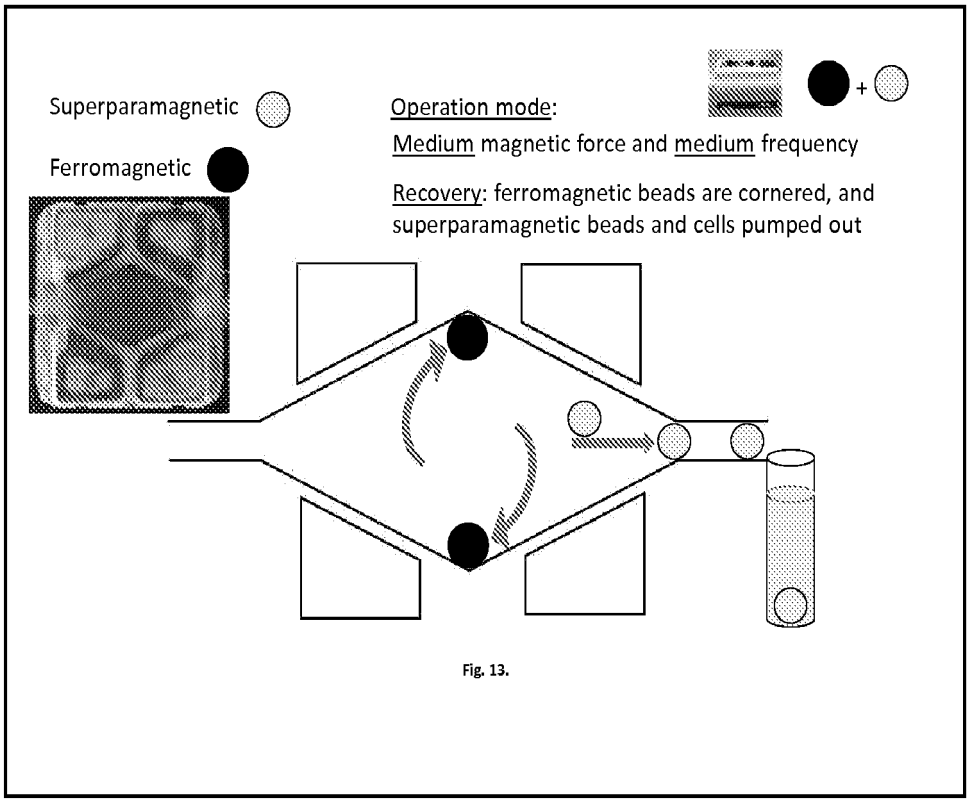

Superparamagnetic
Ferromagnetic
Operation mode:
Medium magnetic force and medium frequency
Recovery: ferromagnetic beads are cornered, and superparamagnetic beads and cells pumped out

Fig. 13.

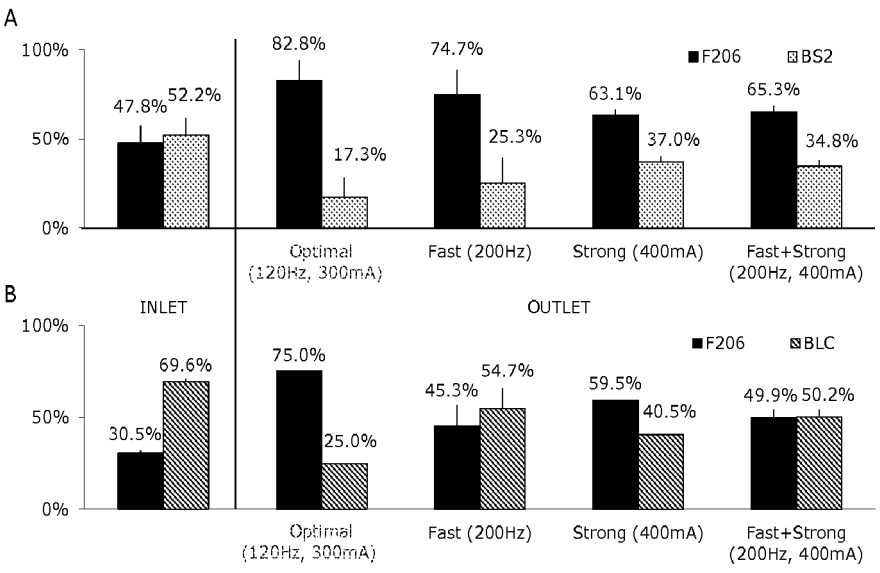

A
100%
50%
0%
47.8%
52.2%
82.8%
17.3%
74.7%
25.3%
63.1%
37.0%
65.3%
34.8%
F206
BS2
Optimal (120Hz, 300mA)
Fast (200Hz)
Strong (400mA)
Fast+Strong (200Hz, 400mA)
B
INLET
OUTLET
30.5%
69.6%
75.0%
25.0%
45.3%
54.7%
59.5%
40.5%
49.9%
50.2%
F206
BLC

Fig. 14.

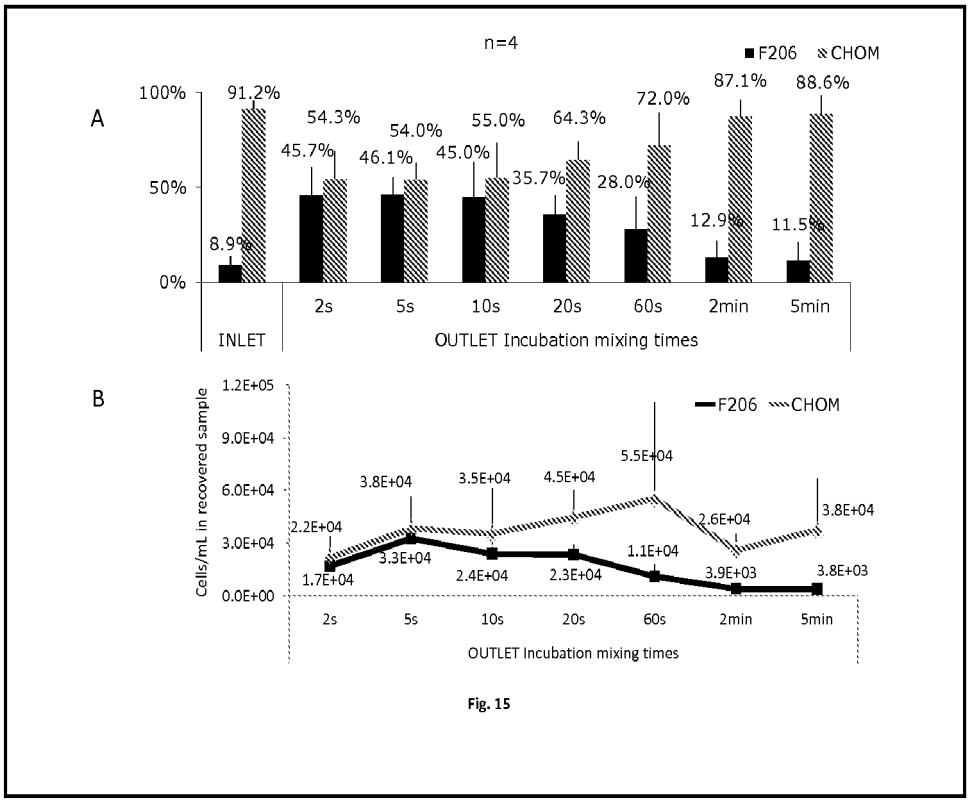

n=4
F206
CHOM
A
100%
50%
0%
91.2%
54.3%
54.0%
55.0%
64.3%
72.0%
87.1%
88.6%
45.7%
46.1%
45.0%
35.7%
28.0%
12.9%
11.5%
8.9%
INLET
2s
5s
10s
20s
60s
2min
5min
OUTLET Incubation mixing times
B
Cells/mL in recovered sample
1.2E+05
9.0E+04
6.0E+04
3.0E+04
0.0E+00
F206
CHOM
5.5E+04
3.8E+04
3.5E+04
4.5E+04
3.8E+04
2.6E+04
2.2E+04
3.3E+04
1.1E+04
1.7E+04
2.4E+04
2.3E+04
3.9E+03
3.8E+03
2s
5s
10s
20s
60s
2min
5min
OUTLET Incubation mixing times

Fig. 15

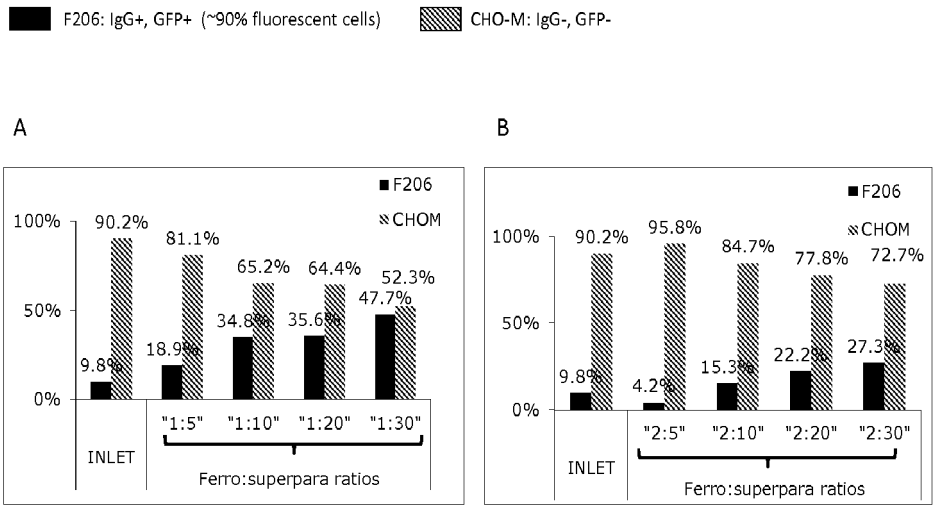

F206: IgG+, GFP+ (~90% fluorescent cells)
CHO-M: IgG-, GFP-
A
B
F206
CHOM
100%
50%
0%
90.2%
81.1%
65.2%
64.4%
52.3%
47.7%
34.8%
35.6%
18.9%
9.8%
INLET
"1:5"
"1:10"
"1:20"
"1:30"
Ferro:superpara ratios
F206
CHOM
100%
50%
0%
90.2%
95.8%
84.7%
77.8%
72.7%
27.3%
22.2%
15.3%
9.8%
4.2%
INLET
"2:5"
"2:10"
"2:20"
"2:30"
Ferro:superpara ratios

Fig. 16

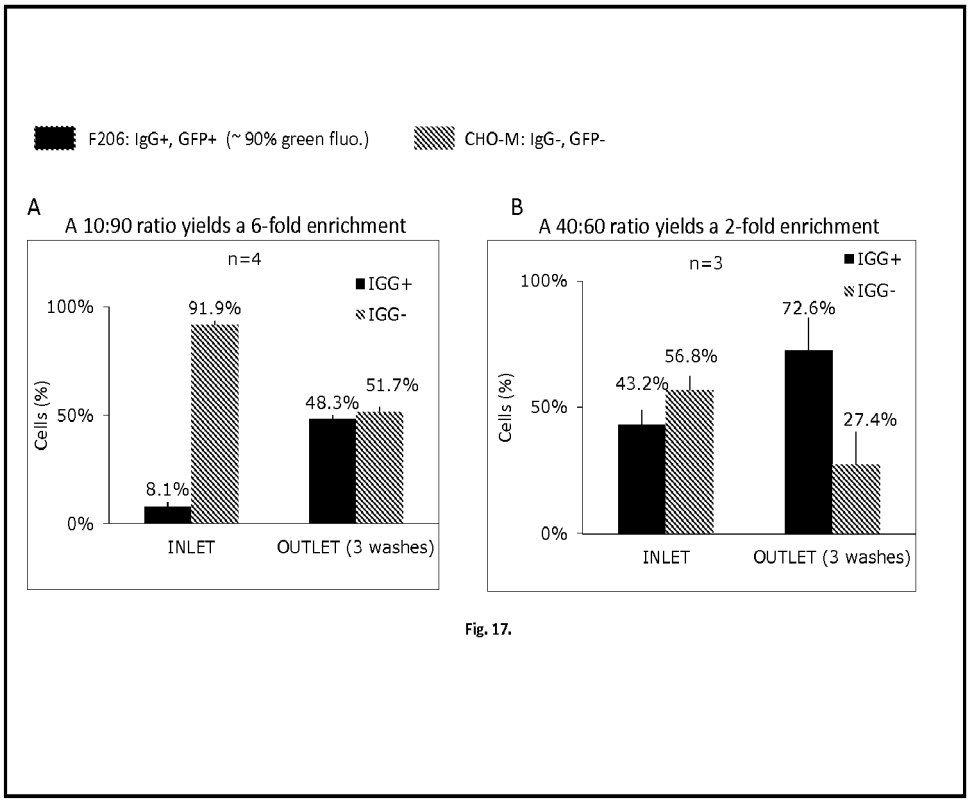

F206: IgG+, GFP+ (~ 90% green fluo.)
CHO-M: IgG-, GFP-
A
A 10:90 ratio yields a 6-fold enrichment
n=4
IGG+
IGG-
100%
50%
0%
Cells (%)
91.9%
8.1%
48.3%
51.7%
INLET
OUTLET (3 washes)
B
A 40:60 ratio yields a 2-fold enrichment
n=3
IGG+
IGG-
100%
50%
0%
Cells (%)
43.2%
56.8%
72.6%
27.4%
INLET
OUTLET (3 washes)

Fig. 17.

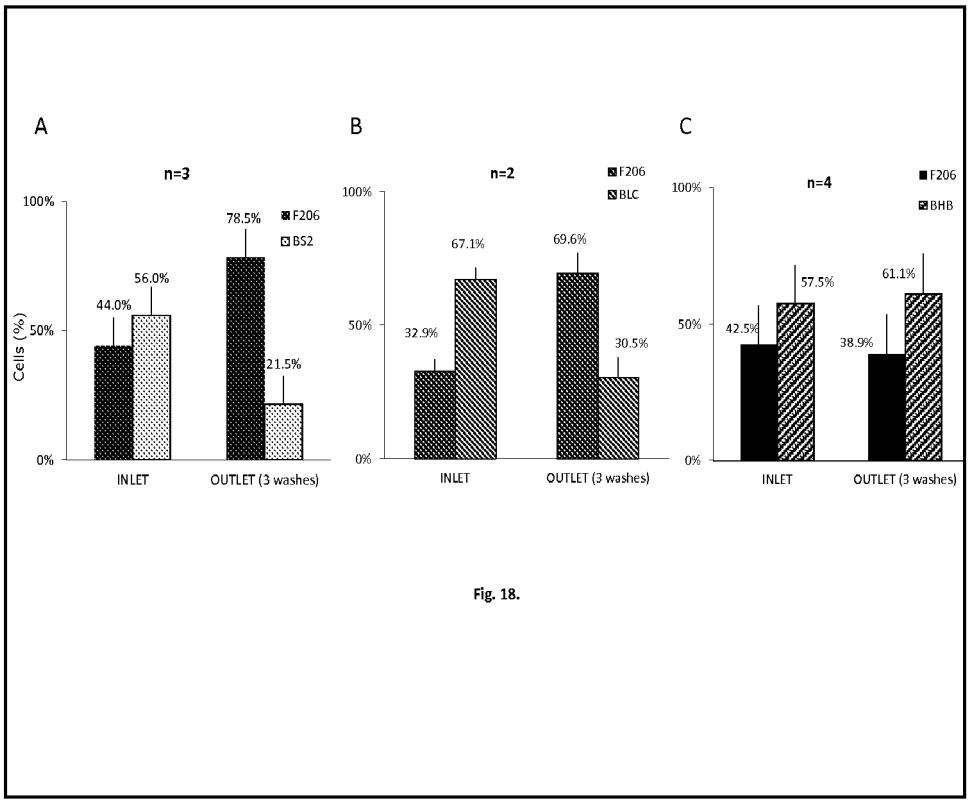

A
n=3
F206
BS2
100%
50%
0%
Cells (%)
44.0%
56.0%
78.5%
21.5%
INLET
OUTLET (3 washes)
B
n=2
F206
BLC
100%
50%
0%
32.9%
67.1%
69.6%
30.5%
INLET
OUTLET (3 washes)
C
n=4
F206
BHB
100%
50%
0%
42.5%
57.5%
38.9%
61.1%
INLET
OUTLET (3 washes)

Fig. 18.

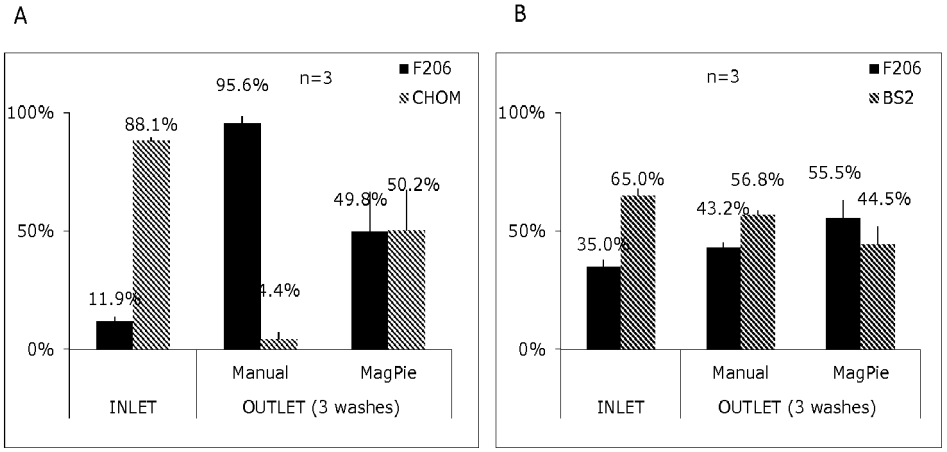

A
B
n=3
F206
CHOM
BS2
100%
50%
0%
88.1%
11.9%
95.6%
4.4%
49.8%
50.2%
65.0%
35.0%
56.8%
43.2%
55.5%
44.5%
Manual
MagPie
INLET
OUTLET (3 washes)

Fig. 19

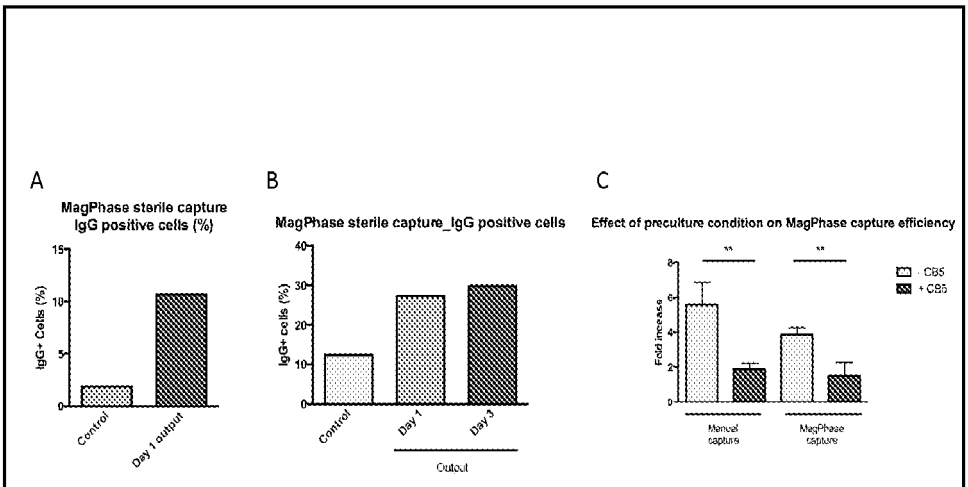

A
MagPhase sterile capture
IgG positive cells (%)
IgG+ Cells (%)
Control
Day 1 output
B
MagPhase sterile capture_IgG positive cells
IgG+ cells (%)
Control
Day 1
Day 3
Output
C
Effect of preculture condition on MagPhase capture efficiency
- CB5
+ CB5
Fold increase
Manual capture
MagPhase capture

Fig. 20

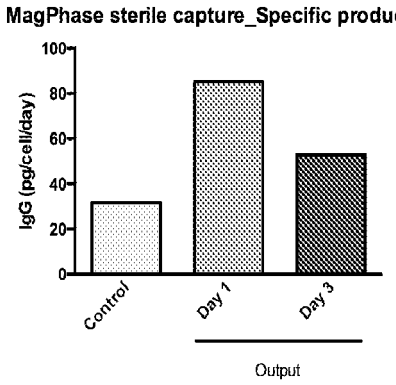

MagPhase sterile capture_Specific productivity
IgG (pg/cell/day)
100
80
60
40
20
0
Control
Day 1
Day 3
Output

Fig. 21

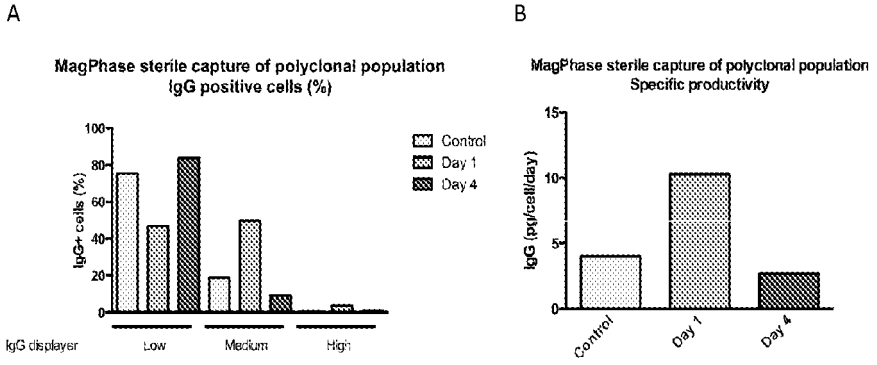

A
MagPhase sterile capture of polyclonal population
IgG positive cells (%)
IgG+ cells (%)
100
80
60
40
20
0
Control
Day 1
Day 4
IgG displayer
Low
Medium
High
B
MagPhase sterile capture of polyclonal population
Specific productivity
IgG (pg/cell/day)
15
10
5
0
Control
Day 1
Day 4

Fig. 22

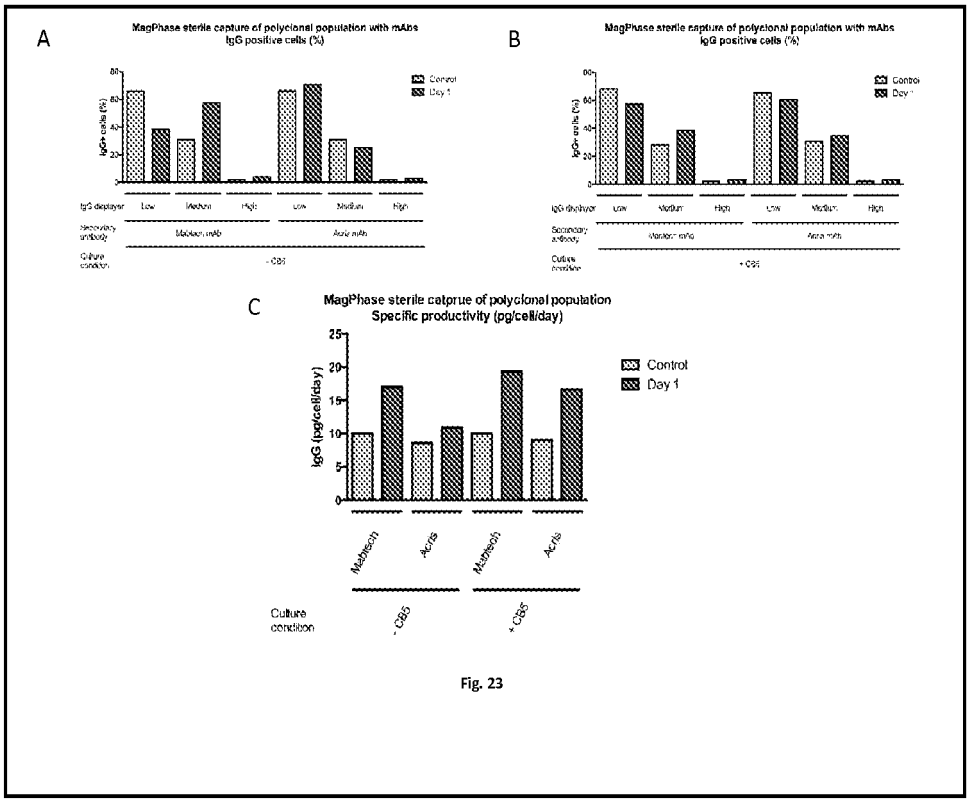

A
MagPhase sterile capture of polyclonal population with mAbs
IgG positive cells (%)
Control
Day 1
IgG+ cells (%)
IgG displayed
Low
Medium
High
Low
Medium
High
Secondary antibody
Mabtech mAb
Acris mAb
Culture condition
- CBS
B
MagPhase sterile capture of polyclonal population with mAbs
IgG positive cells (%)
Control
Day 1
IgG+ cells (%)
IgG displayed
Low
Medium
High
Low
Medium
High
Secondary antibody
Mabtech mAb
Acris mAb
Culture condition
+ CBS
C
MagPhase sterile catprue of polyclonal population
Specific productivity (pg/cell/day)
Control
Day 1
IgG (pg/cell/day)
Mabtech
Acris
Mabtech
Acris
Culture condition
- CBS
+ CBS

Fig. 23

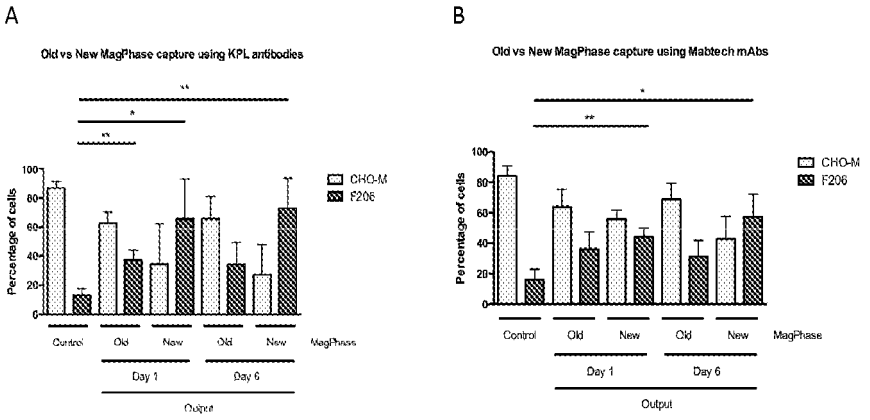

A
Old vs New MagPhase capture using KPL antibodies
CHO-M
F206
Percentage of cells
Control
Old
New
Old
New
MagPhase
Day 1
Day 6
Output
B
Old vs New MagPhase capture using Mabtech mAbs
CHO-M
F206
Percentage of cells
Control
Old
New
Old
New
MagPhase
Day 1
Day 6
Output

Fig. 24

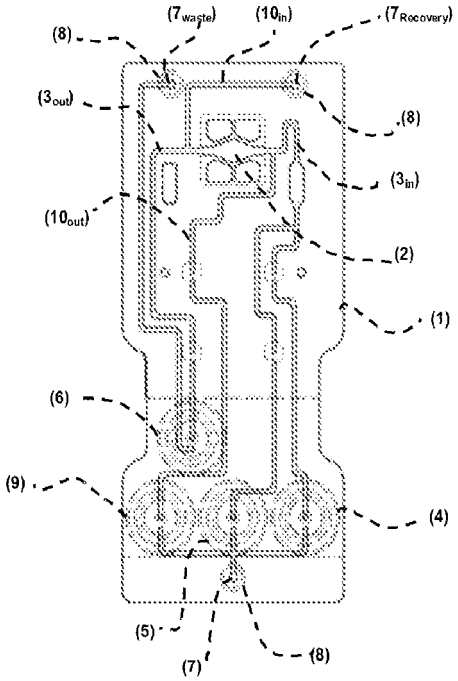

(7waste)
(10in)
(7Recovery)
(8)
(3out)
(8)
(3in)
(10out)
(2)
(1)
(6)
(9)
(4)
(5)
(7)
(8)

Fig. 25

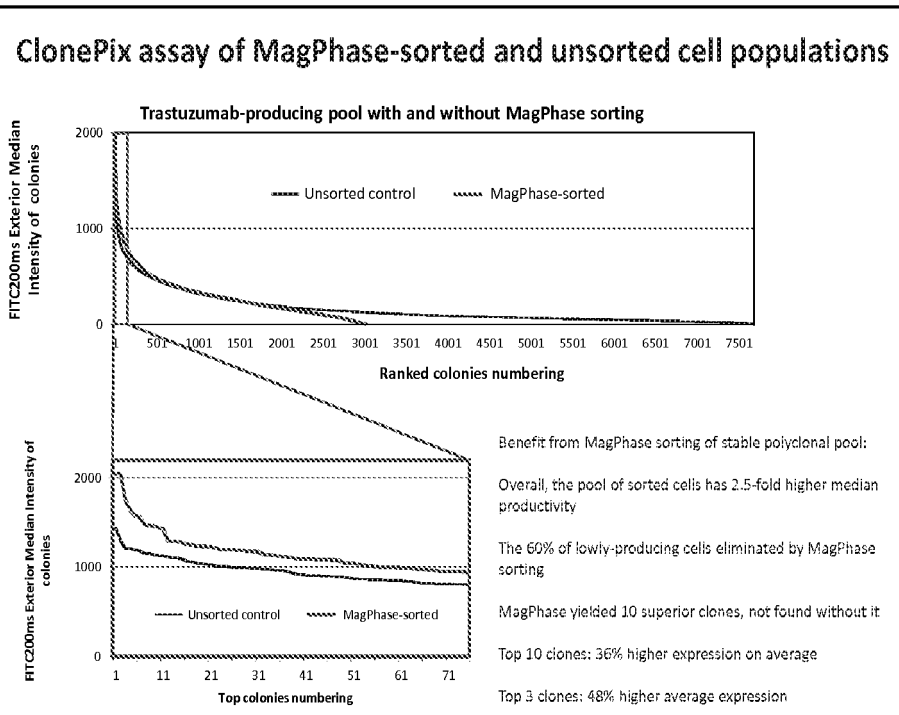

ClonePix assay of MagPhase-sorted and unsorted cell populations
Trastuzumab-producing pool with and without MagPhase sorting
FITC200ms Exterior Median Intensity of colonies
2000
1000
0
Unsorted control
MagPhase-sorted
1
501
1001
1501
2001
2501
3001
3501
4001
4501
5001
5501
6001
6501
7001
7501
Ranked colonies numbering
FITC200ms Exterior Median Intensity of colonies
2000
1000
0
Unsorted control
MagPhase-sorted
1
11
21
31
41
51
61
71
Top colonies numbering
Benefit from MagPhase sorting of stable polyclonal pool:
Overall, the pool of sorted cells has 2.5-fold higher median productivity
The 60% of lowly-producing cells eliminated by MagPhase sorting
MagPhase yielded 10 superior clones, not found without it
Top 10 clones: 36% higher expression on average
Top 3 clones: 48% higher average expression

Fig. 26

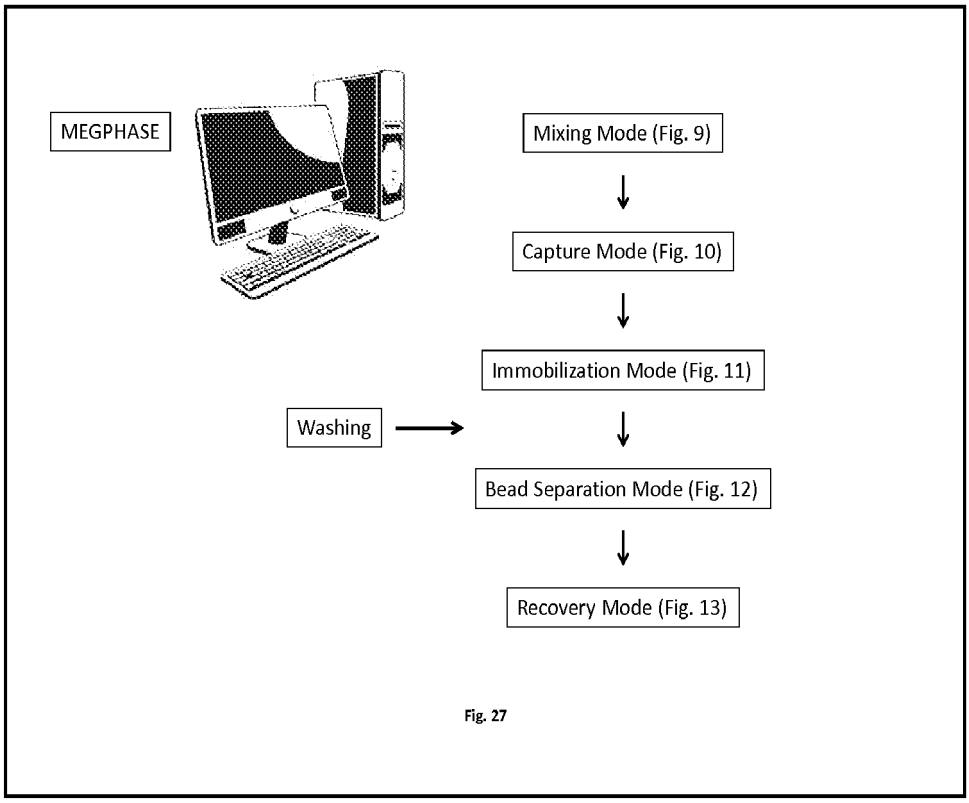

MEGPHASE
Mixing Mode (Fig. 9)
Capture Mode (Fig. 10)
Immobilization Mode (Fig. 11)
Washing
Bead Separation Mode (Fig. 12)
Recovery Mode (Fig. 13)

Fig. 27