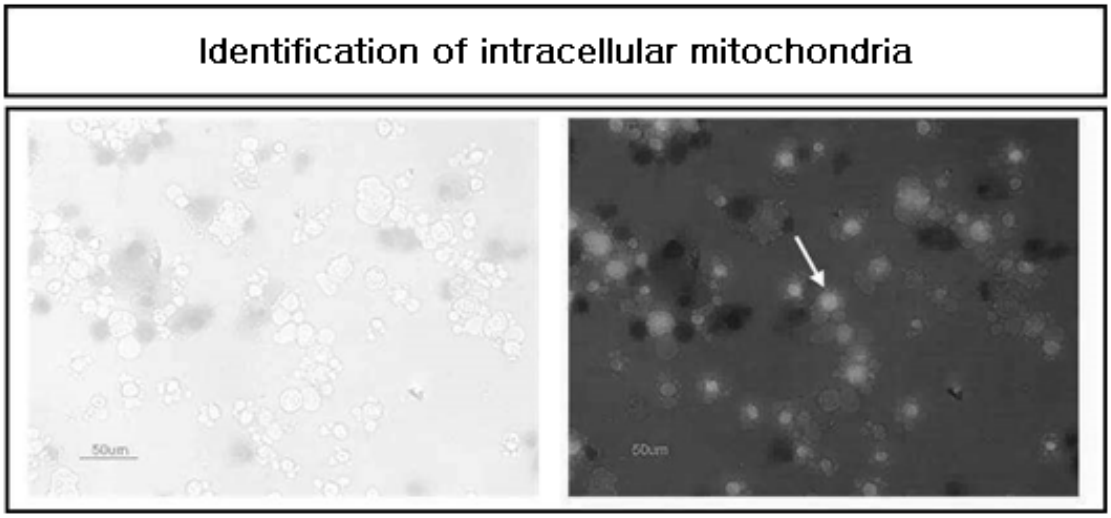
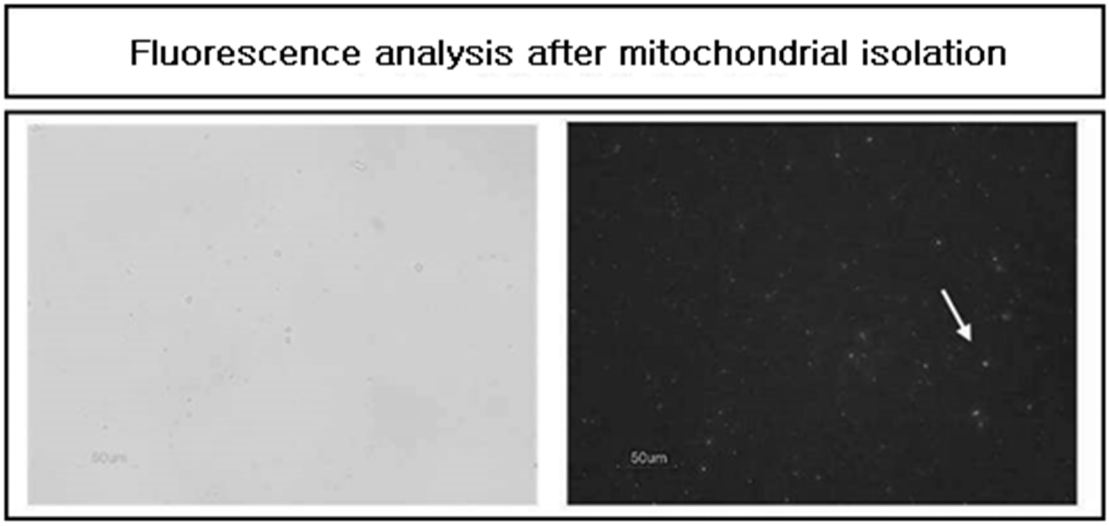
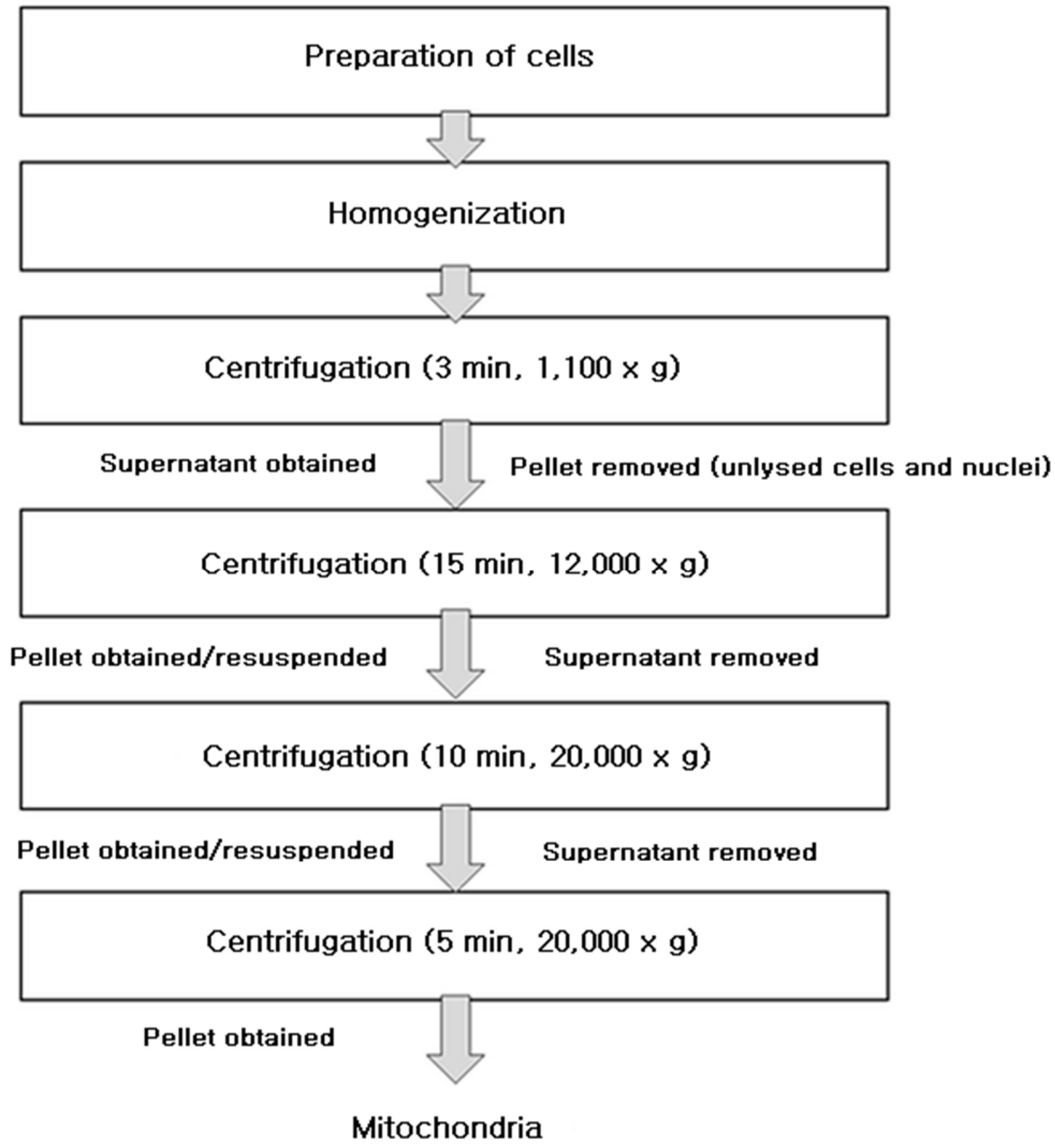
[FIG. 1]


Identification of intracellular mitochondria
50um
50um

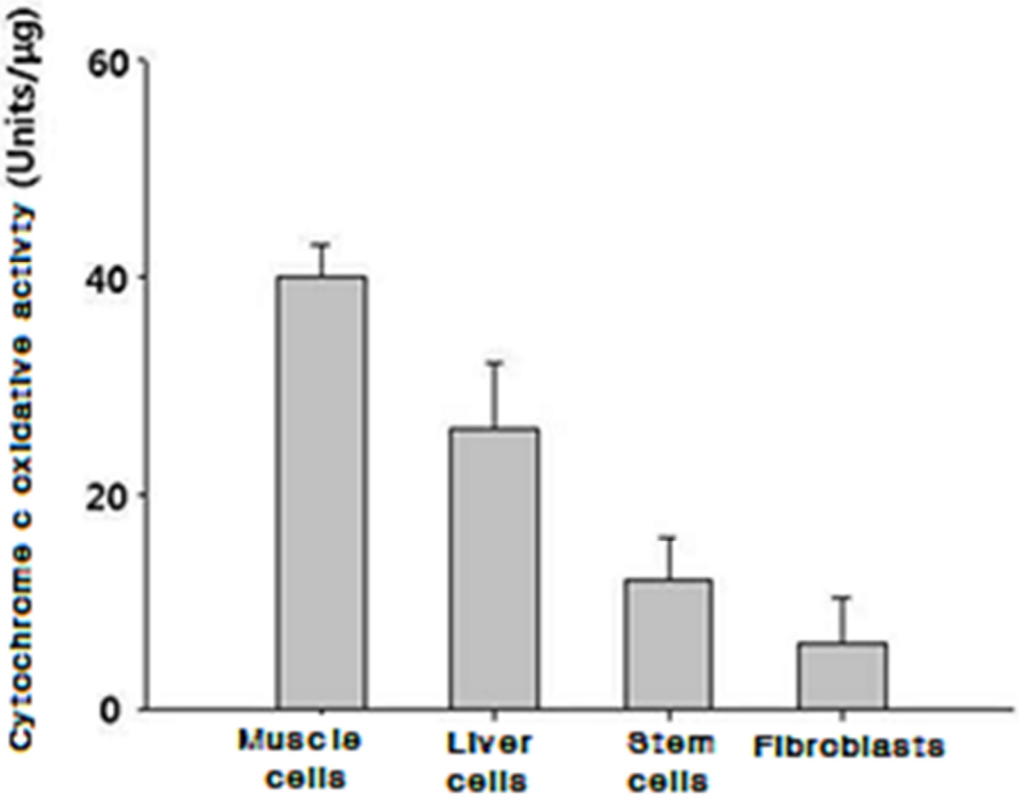
[FIG. 2]


Fluorescence analysis after mitochondrial isolation
50um

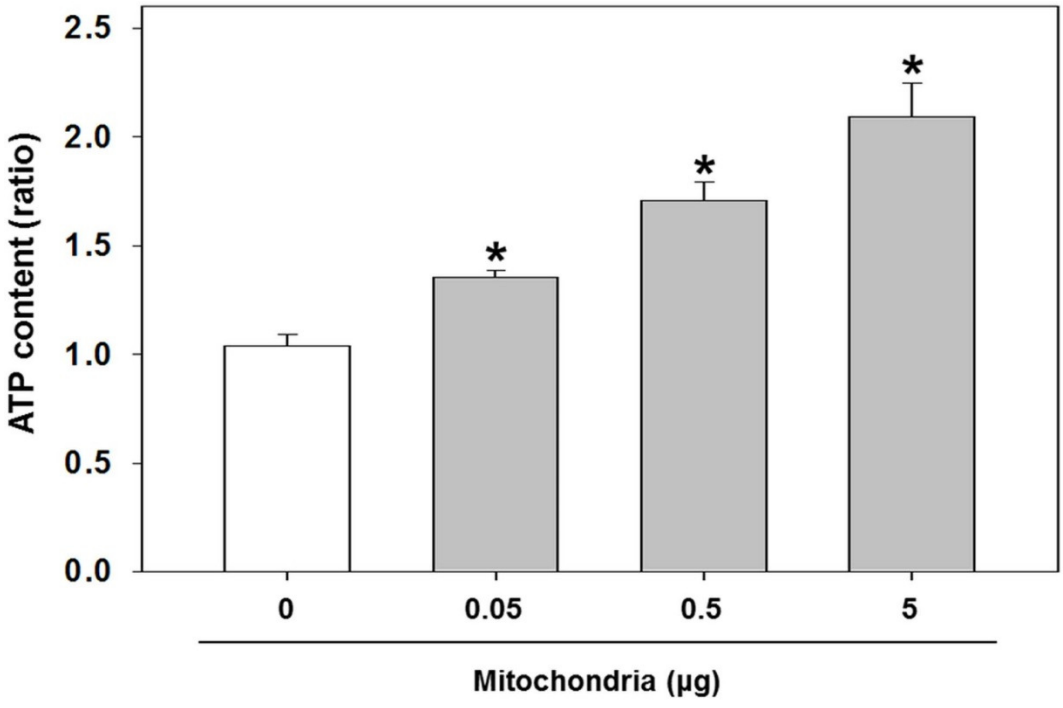
[FIG. 3]


Preparation of cells
Homogenization
Centrifugation (3 min, 1,100 x g)
Supernatant obtained
Pellet removed (unlysed cells and nuclei)
Centrifugation (15 min, 12,000 x g)
Pellet obtained/resuspended
Supernatant removed
Centrifugation (10 min, 20,000 x g)
Pellet obtained/resuspended
Supernatant removed
Centrifugation (5 min, 20,000 x g)
Pellet obtained
Mitochondria

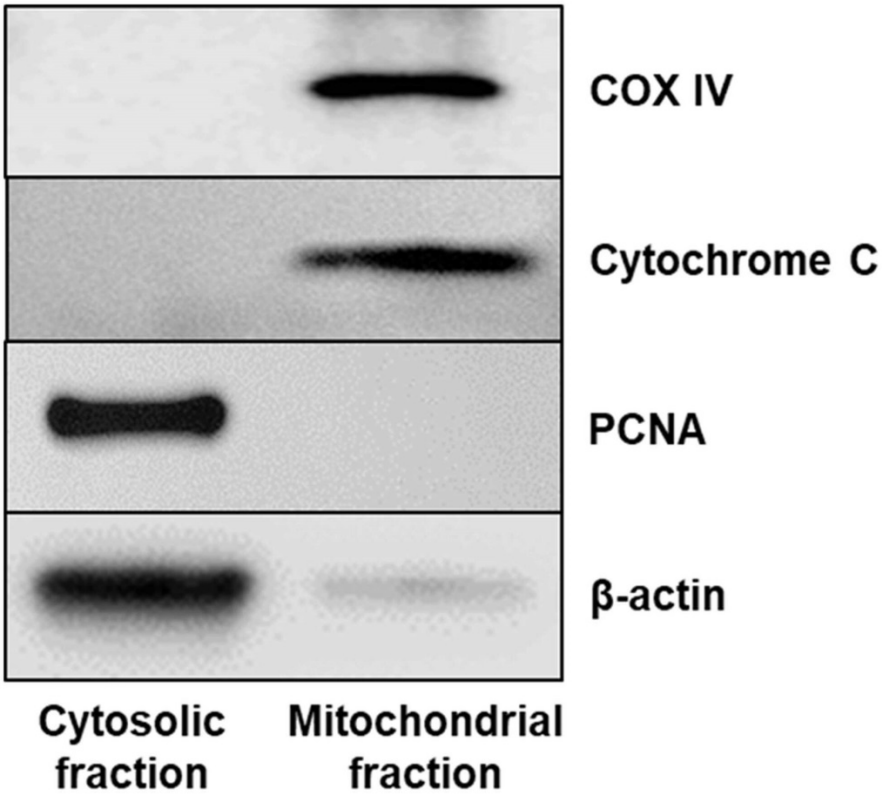
[FIG. 4]


Cytochrome c oxidative activity (Units/μg)
60
40
20
0
Muscle cells
Liver cells
Stem cells
Fibroblasts

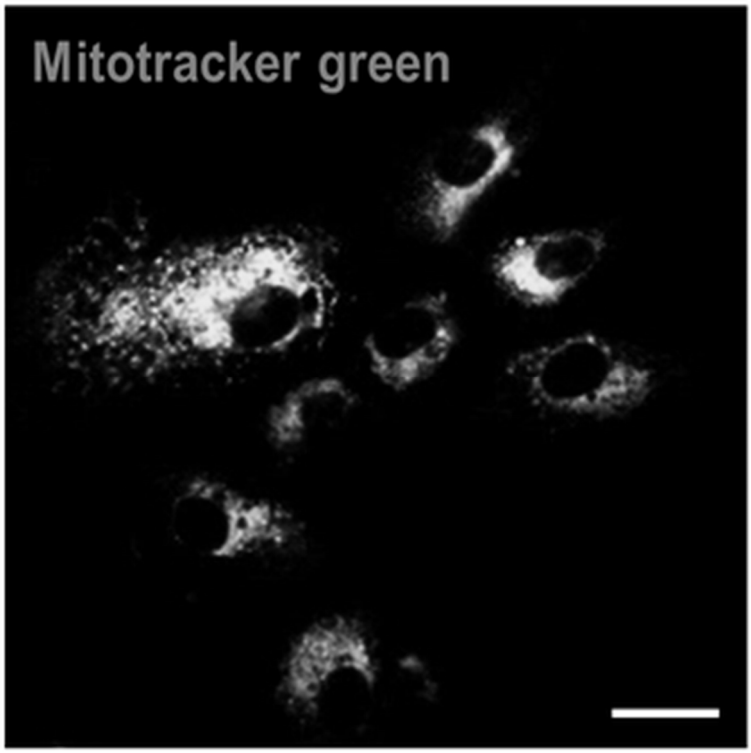
[FIG. 5]


ATP content (ratio)
2.5
2.0
1.5
1.0
0.5
0.0
*
*
*
0
0.05
0.5
5
Mitochondria (μg)

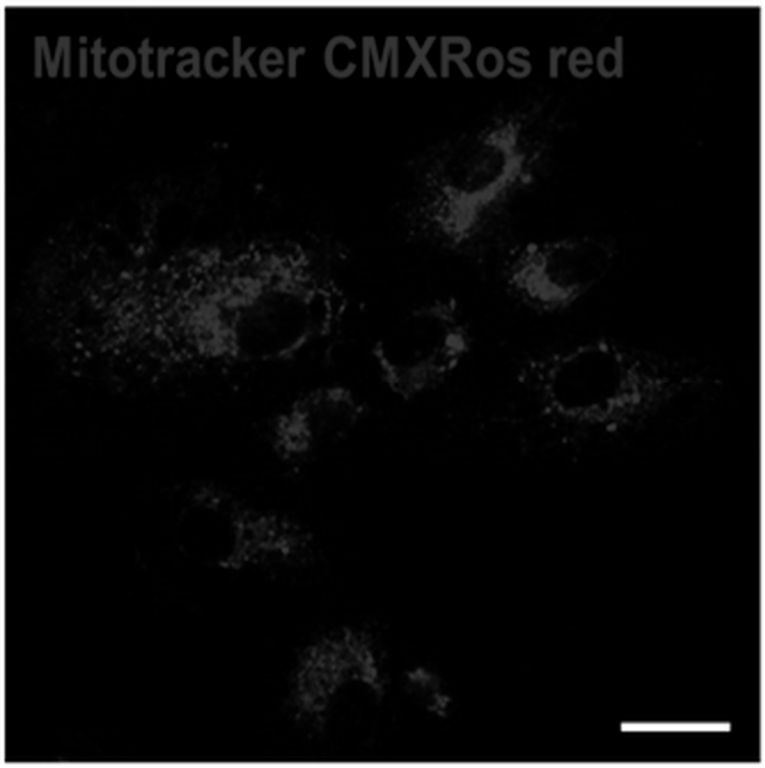
[FIG. 6]


COX IV
Cytochrome C
PCNA
β-actin
Cytosolic fraction
Mitochondrial fraction

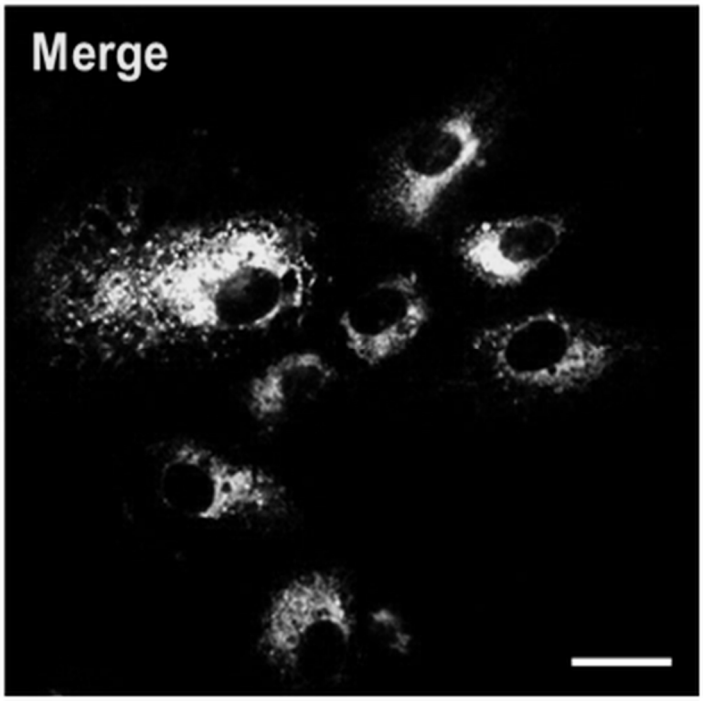
[FIG. 7A]



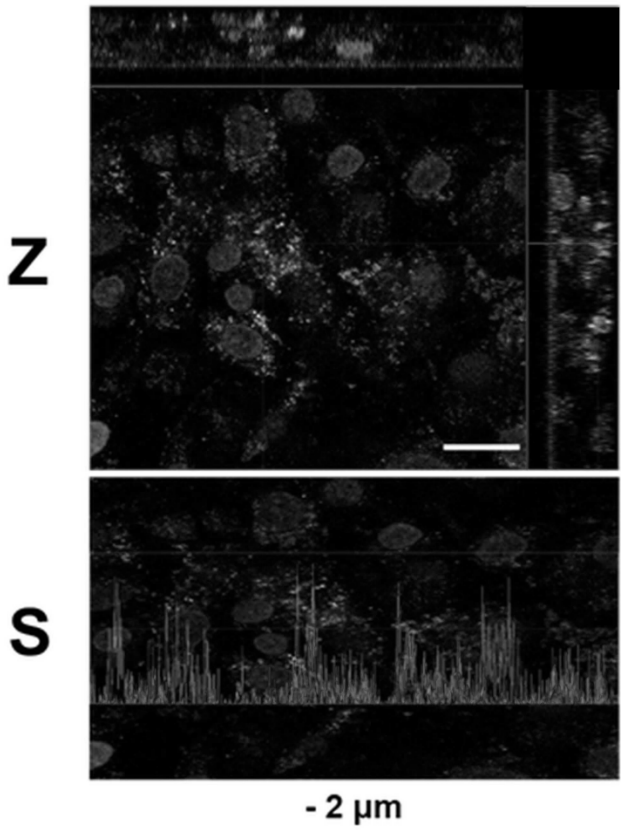
[FIG. 7B]



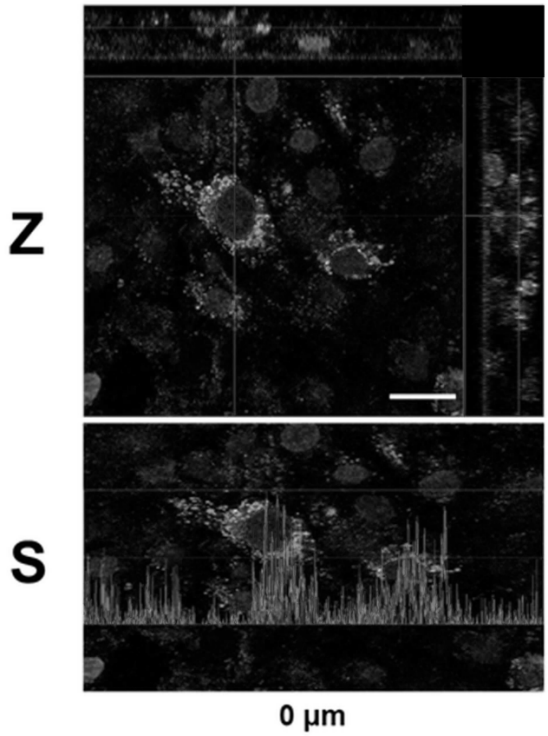
[FIG. 7C]



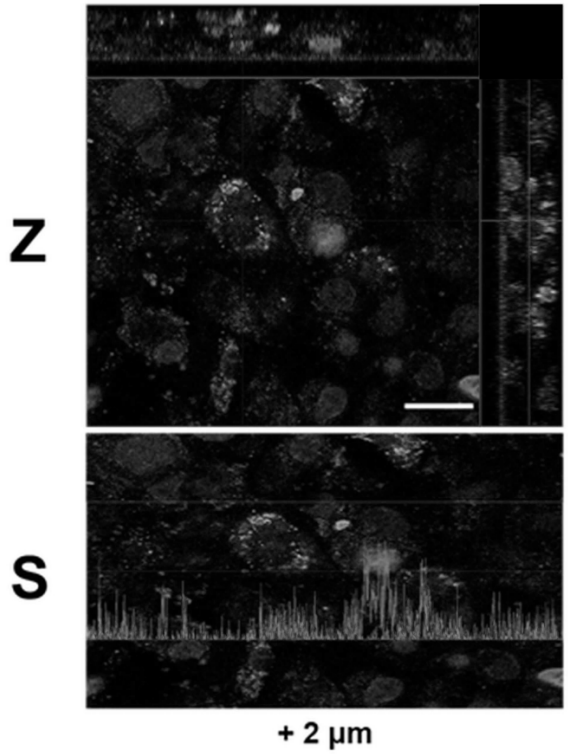
[FIG. 7D]



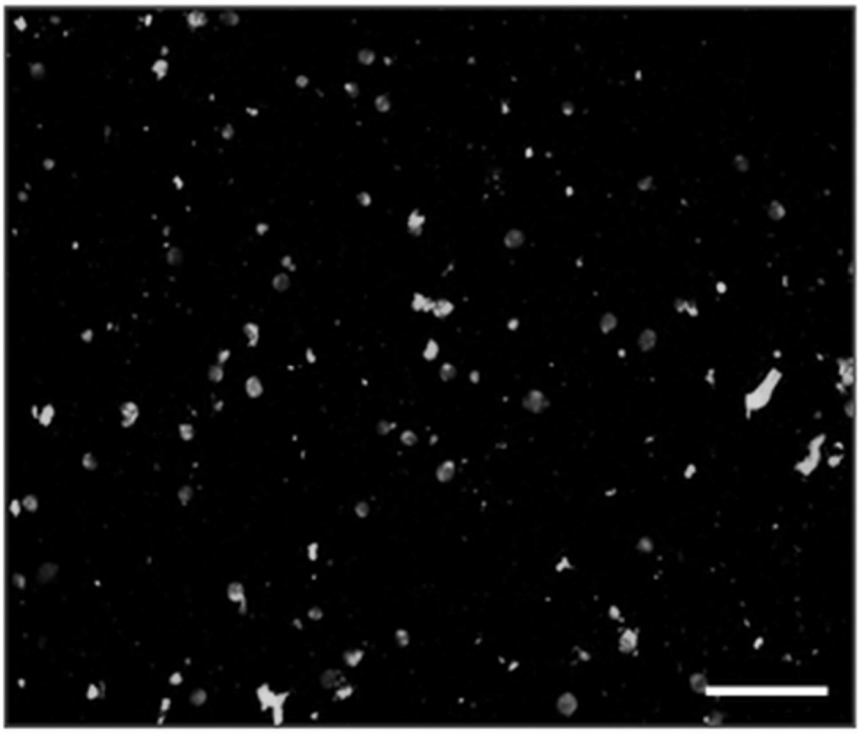
[FIG. 7E]



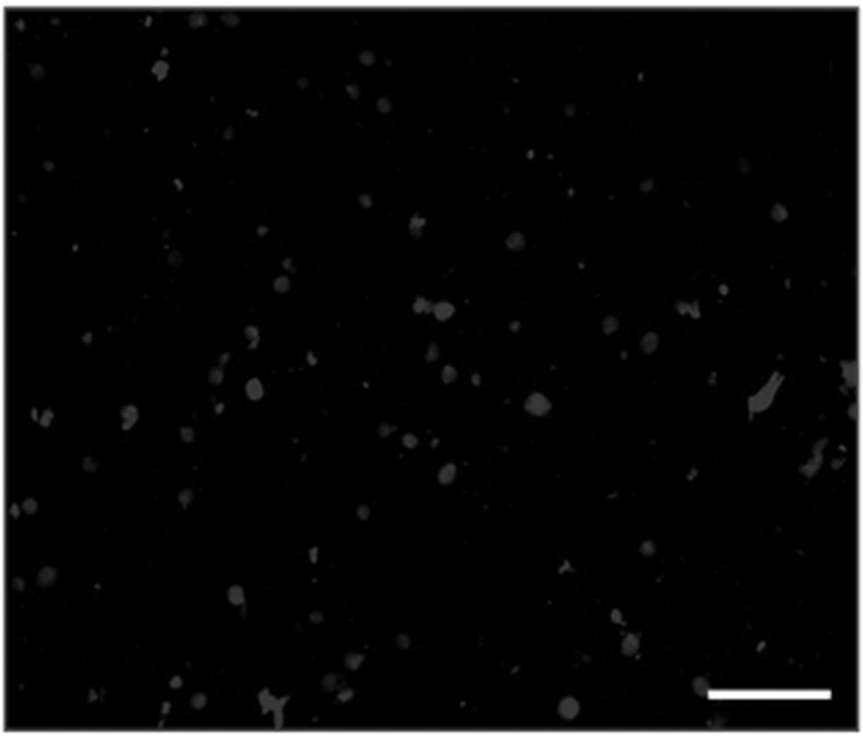
[FIG. 7F]



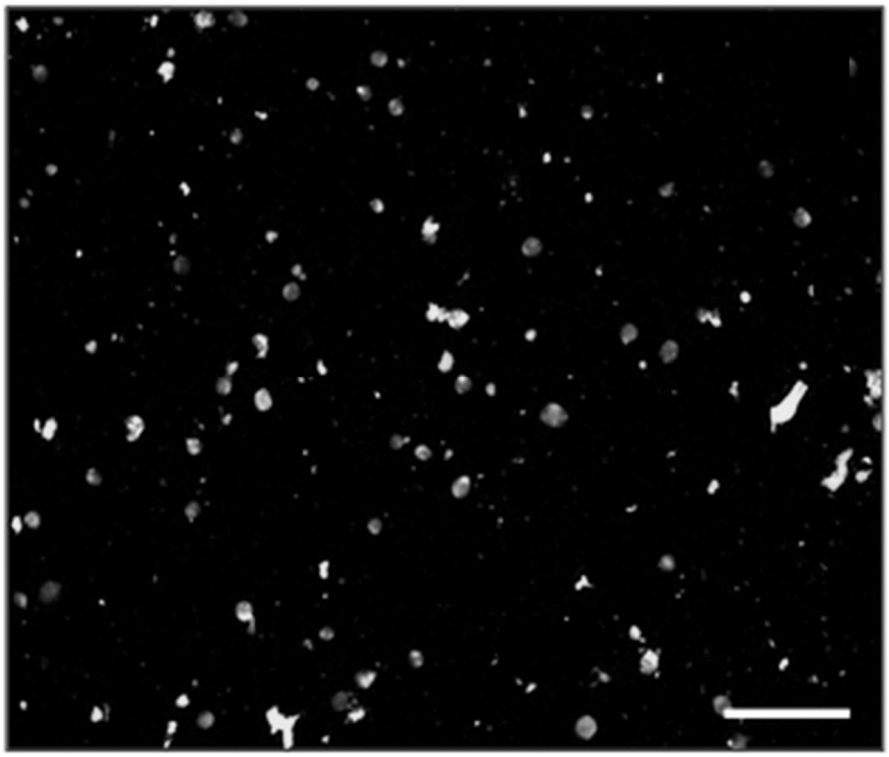
[FIG. 7G]



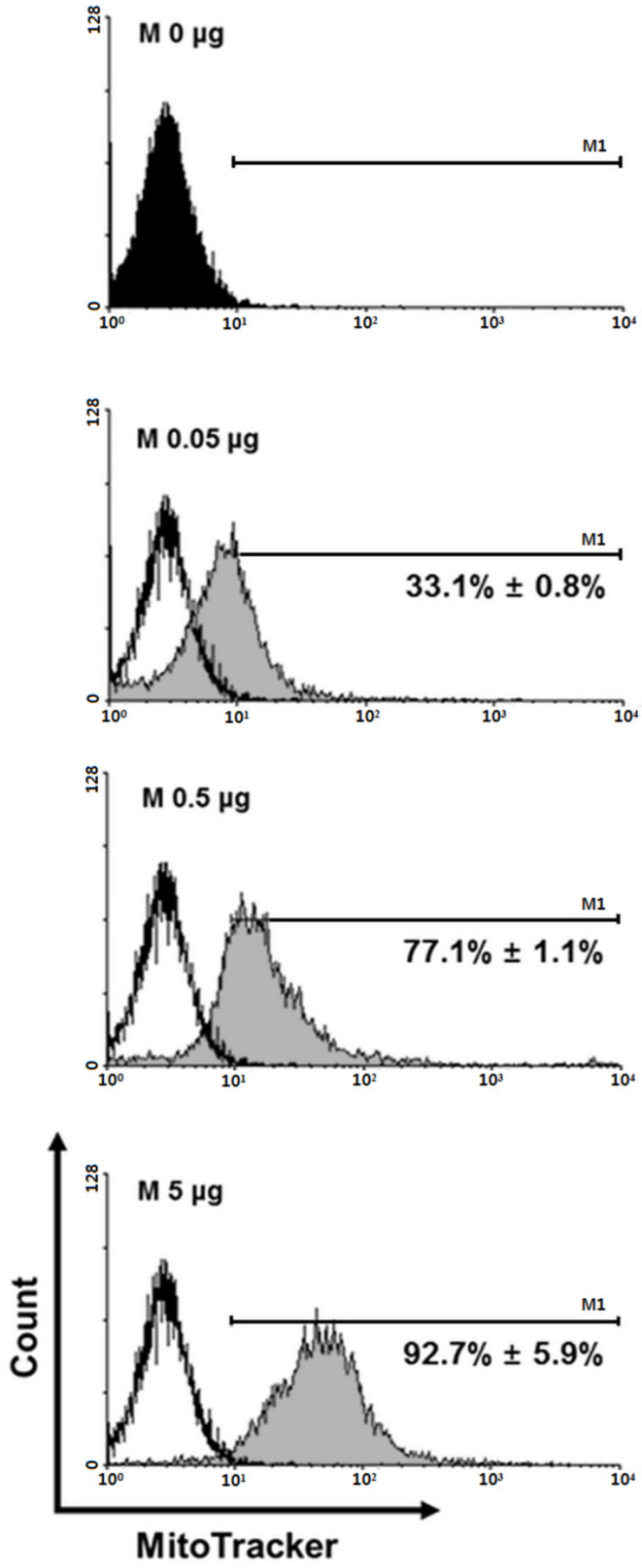
[FIG. 7H]



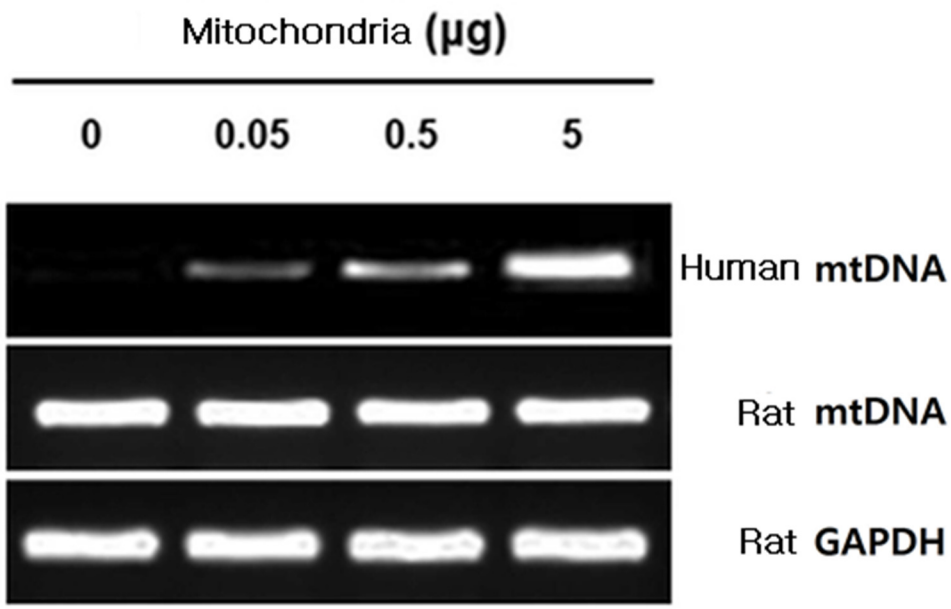
[FIG. 7I]



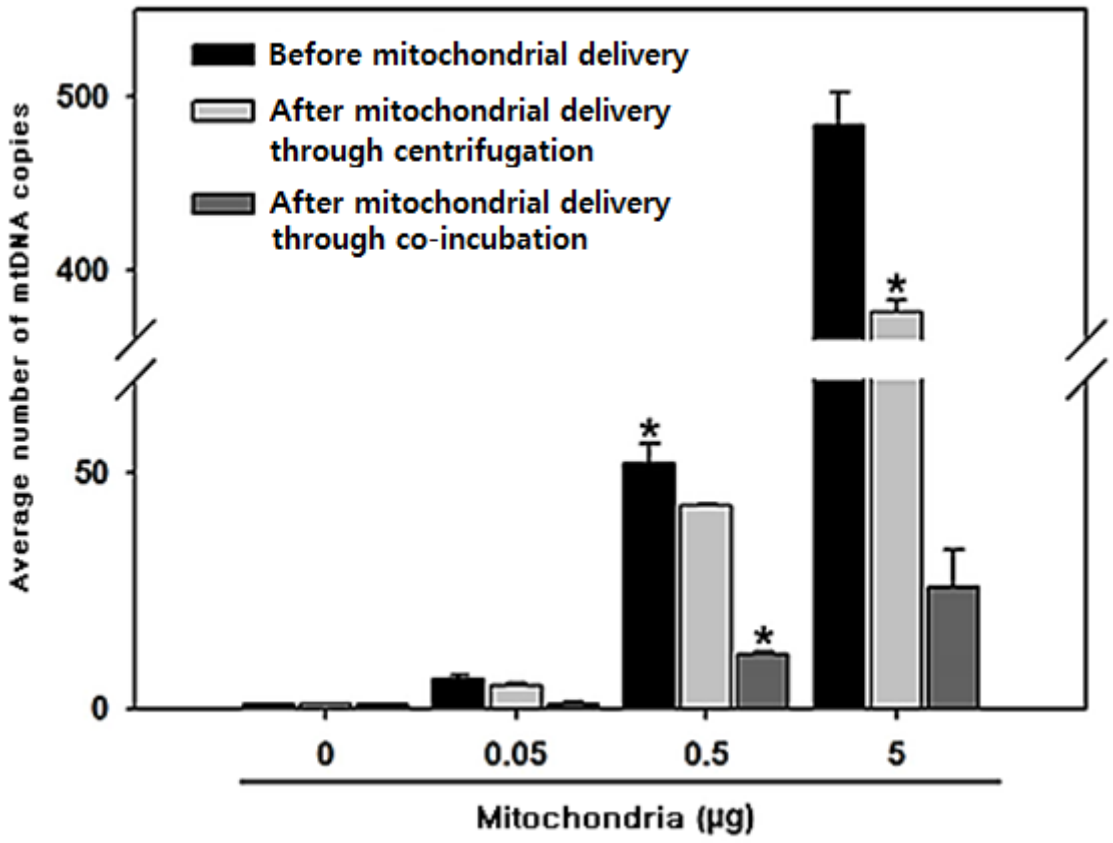
[FIG. 8]


M 0 μg
M1
M 0.05 μg
M1
33.1% ± 0.8%
M 0.5 μg
M1
77.1% ± 1.1%
M 5 μg
M1
92.7% ± 5.9%
Count
MitoTracker

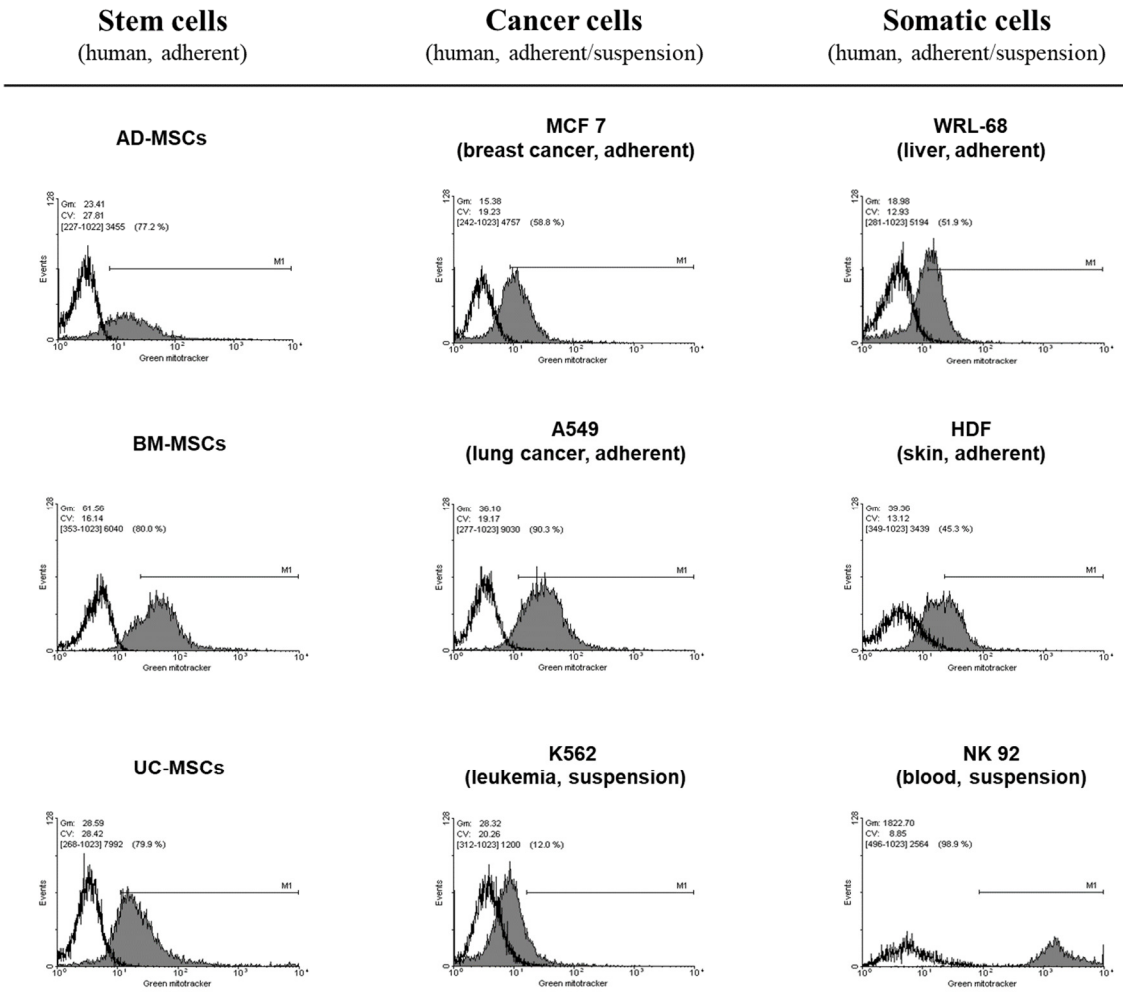
[FIG. 9]


Mitochondria (μg)
0
0.05
0.5
5
Human mtDNA
Rat mtDNA
Rat GAPDH

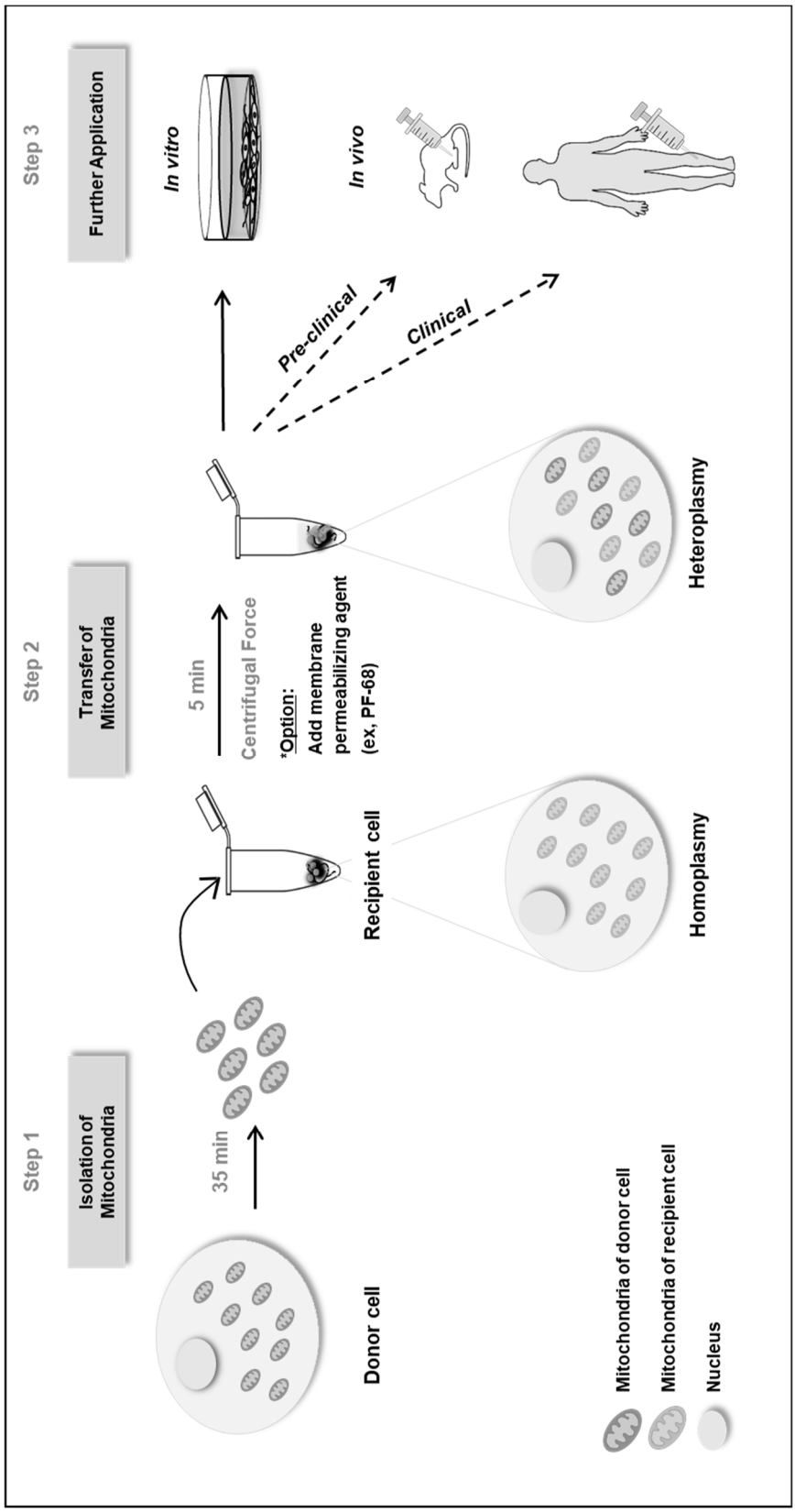
[FIG. 10]


Before mitochondrial delivery
After mitochondrial delivery through centrifugation
After mitochondrial delivery through co-incubation
Average number of mtDNA copies
500
400
50
0
*
*
*
0
0.05
0.5
5
Mitochondria (μg)

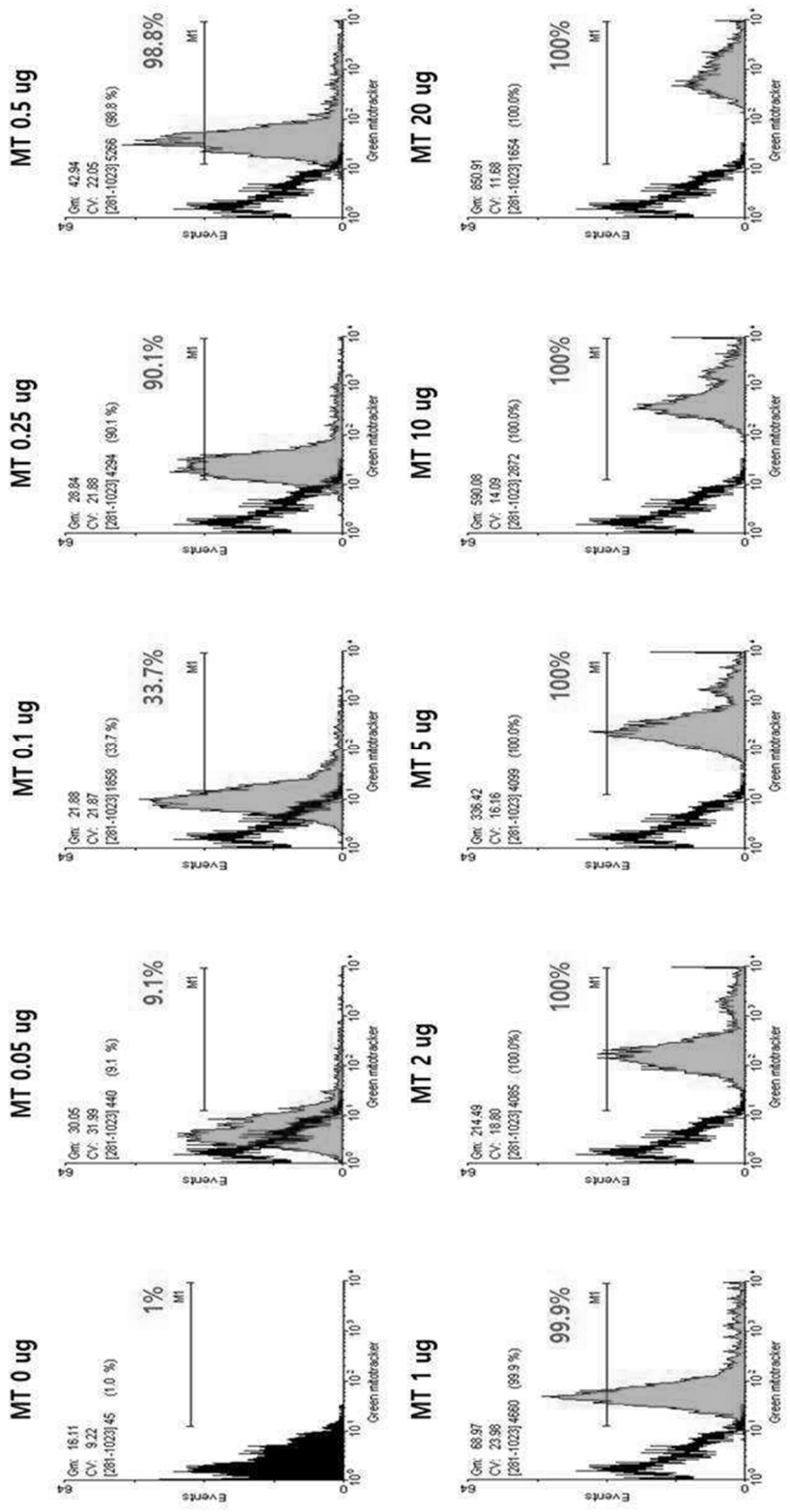
[FIG. 11]


Stem cells
(human, adherent)
Cancer cells
(human, adherent/suspension)
Somatic cells
(human, adherent/suspension)
AD-MSCs
MCF 7
(breast cancer, adherent)
WRL-68
(liver, adherent)
BM-MSCs
A549
(lung cancer, adherent)
HDF
(skin, adherent)
UC-MSCs
K562
(leukemia, suspension)
NK 92
(blood, suspension)
Green mitotracker
Events
M1

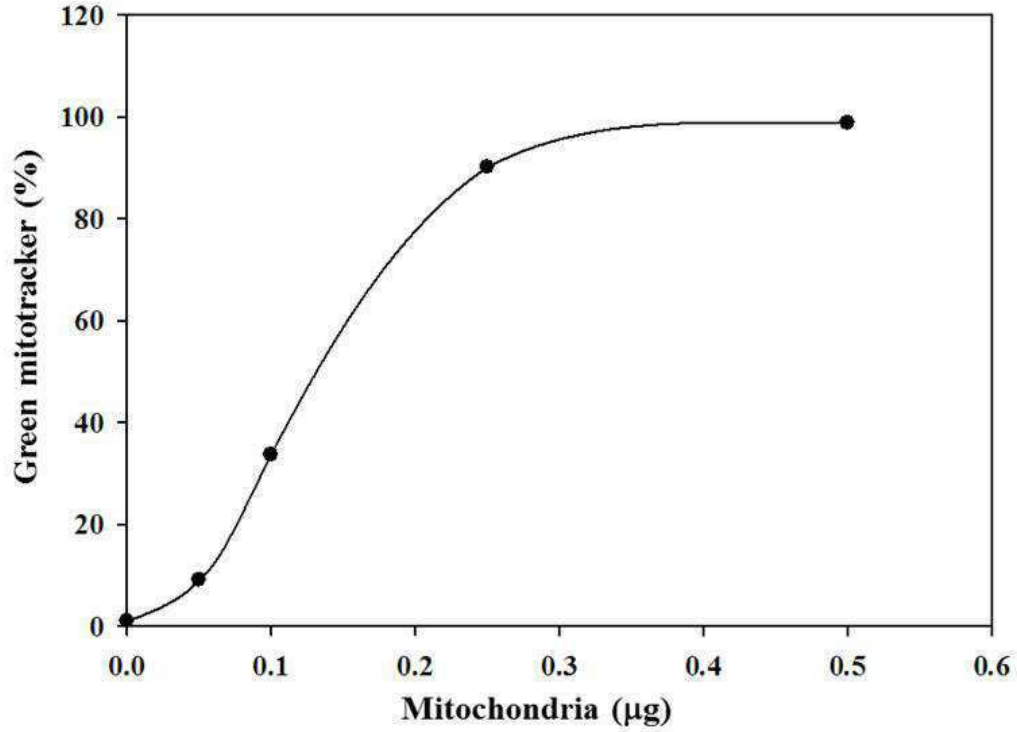
[FIG. 12]


Step 1
Isolation of Mitochondria
35 min
Donor cell
Step 2
Transfer of Mitochondria
5 min
Centrifugal Force
*Option:
Add membrane permeabilizing agent (ex, PF-68)
Recipient cell
Homoplasmy
Heteroplasmy
Step 3
Further Application
In vitro
Pre-clinical
Clinical
In vivo
Mitochondria of donor cell
Mitochondria of recipient cell
Nucleus

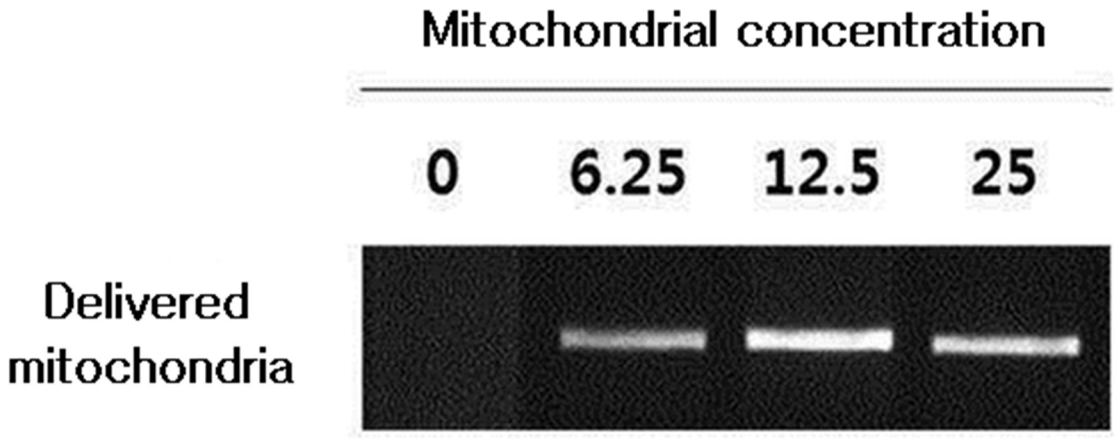
[FIG. 13]


MT 0 ug
MT 0.05 ug
MT 0.1 ug
MT 0.25 ug
MT 0.5 ug
MT 1 ug
MT 2 ug
MT 5 ug
MT 10 ug
MT 20 ug
1%
9.1%
33.7%
90.1%
98.8%
99.9%
100%
100%
100%
100%
M1
Events
Green mitotracker

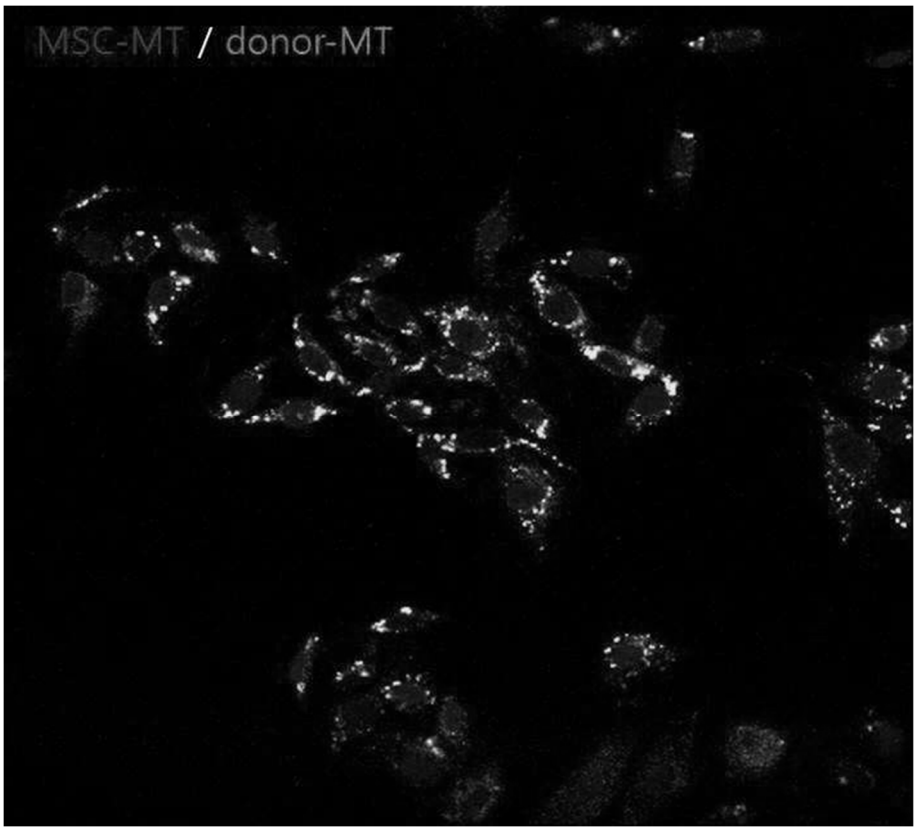
[FIG. 14]


120
100
80
60
40
20
0
Green mitotracker (%)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
Mitochondria (μg)

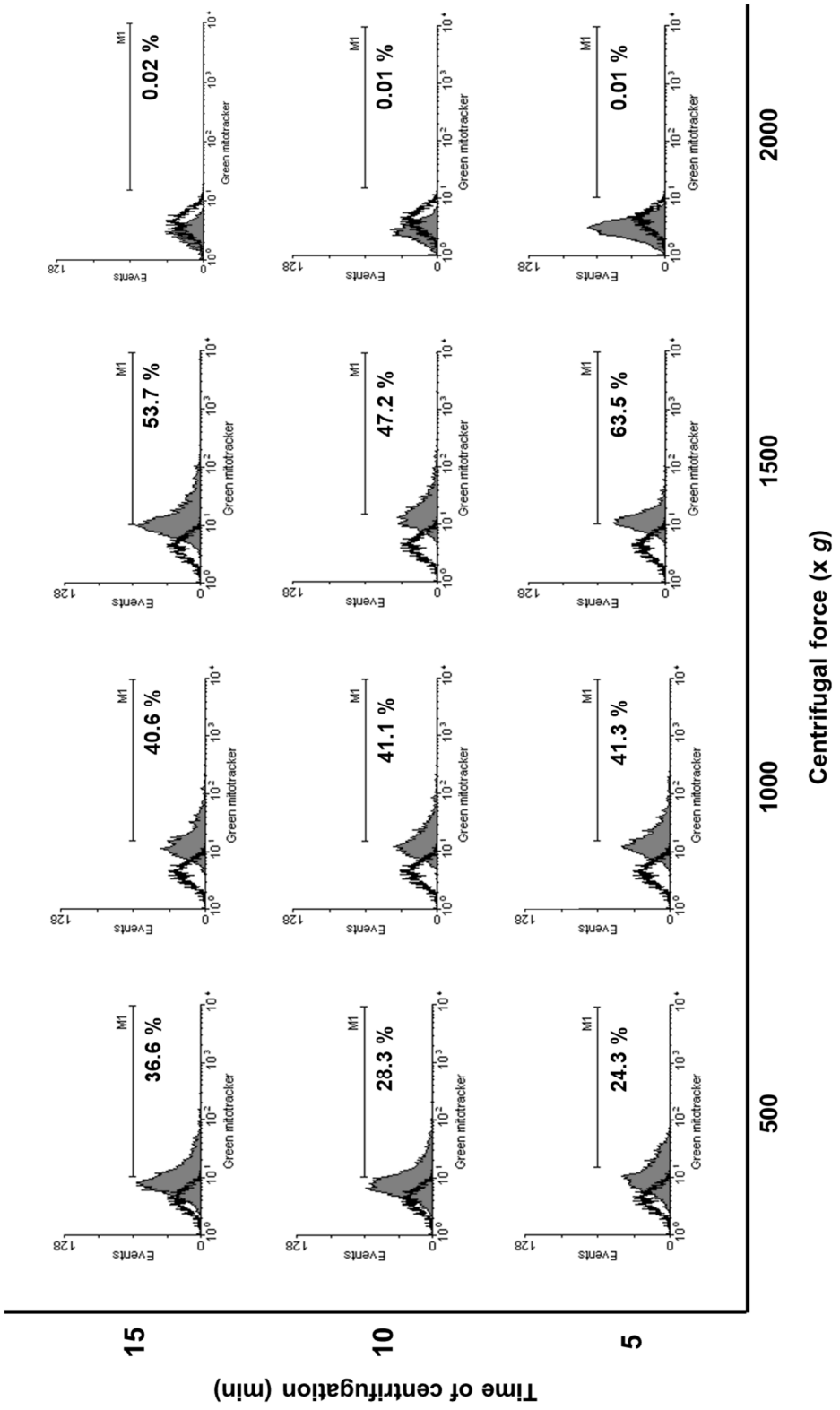
[FIG. 15]


Mitochondrial concentration
0
6.25
12.5
25
Delivered mitochondria

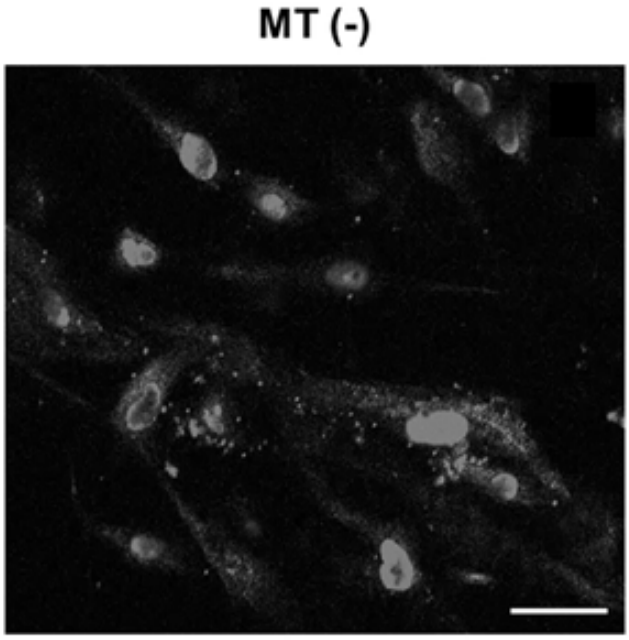
[FIG. 16]



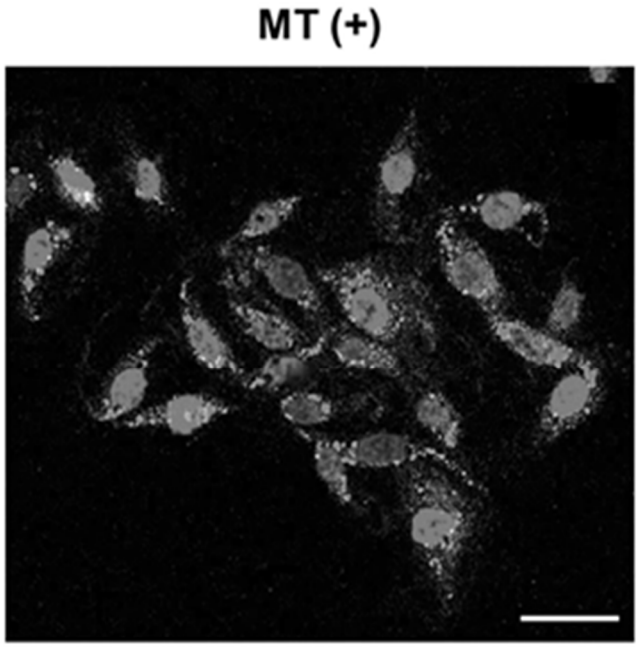
[FIG. 17]


Time of centrifugation (min)
15
10
5
Centrifugal force (x g)
500
1000
1500
2000
36.6 %
40.6 %
53.7 %
0.02 %
28.3 %
41.1 %
47.2 %
0.01 %
24.3 %
41.3 %
63.5 %
0.01 %
M1
Green mitotracker
Events

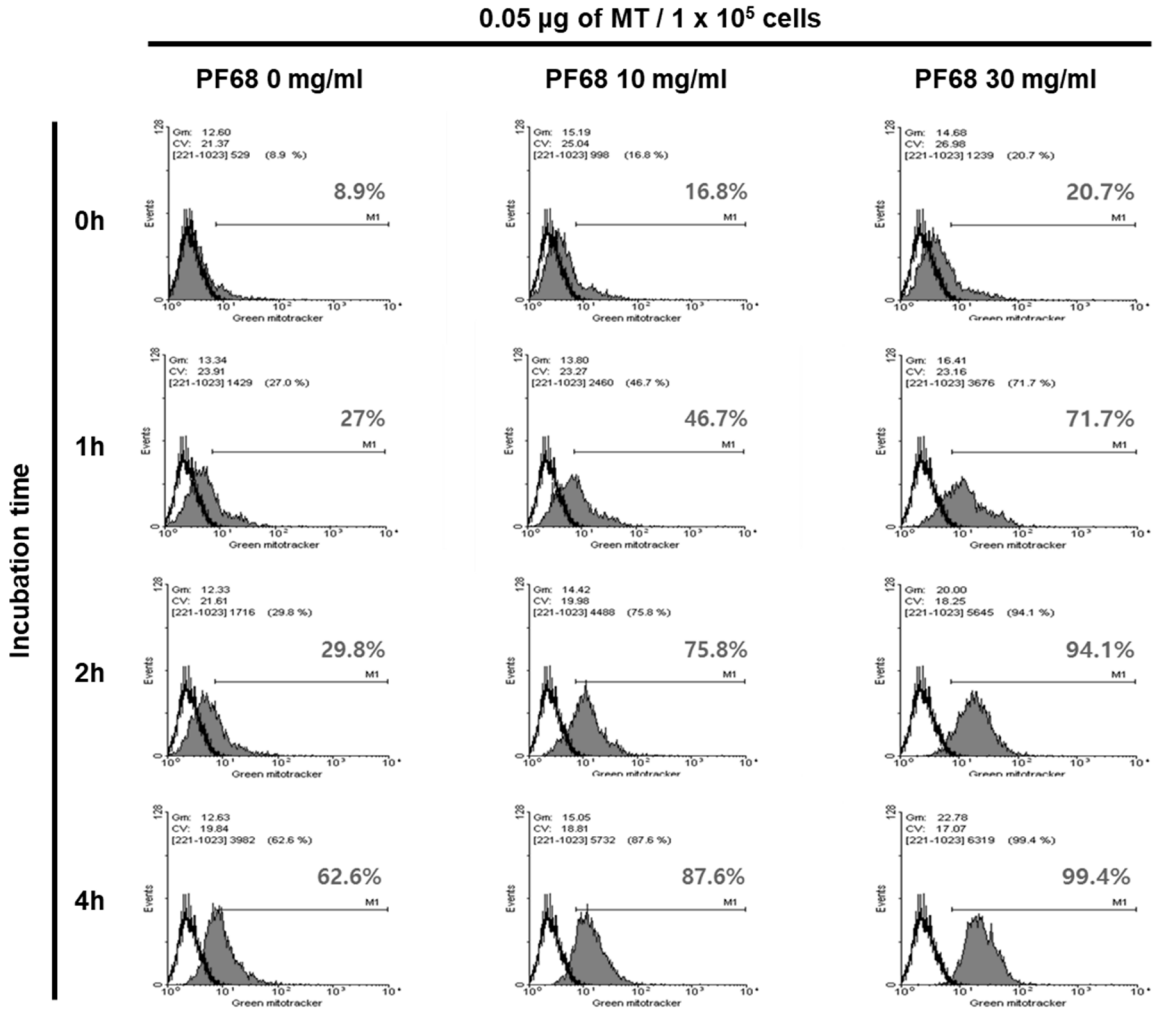
[FIG. 18A]



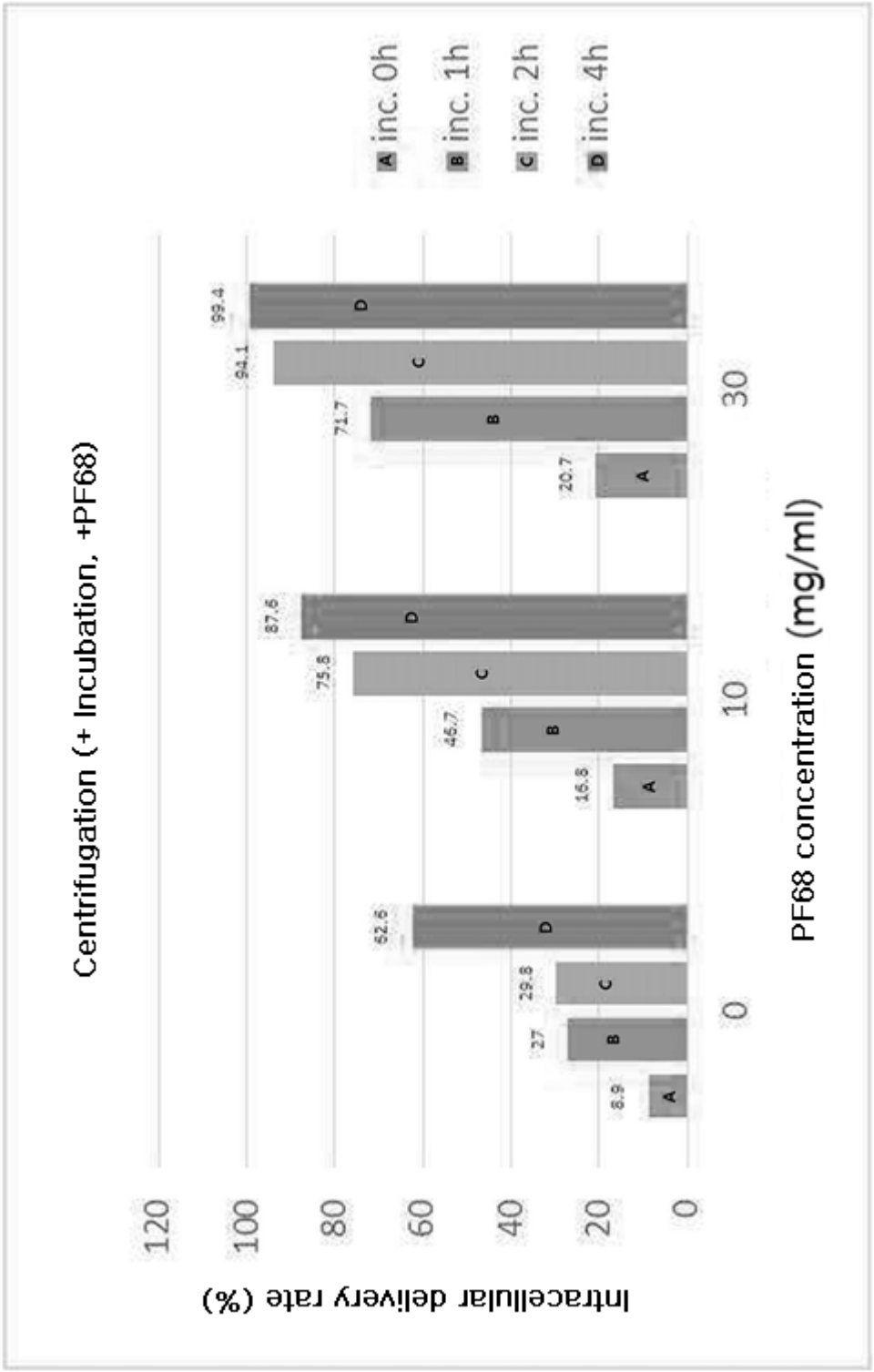
[FIG. 18B]



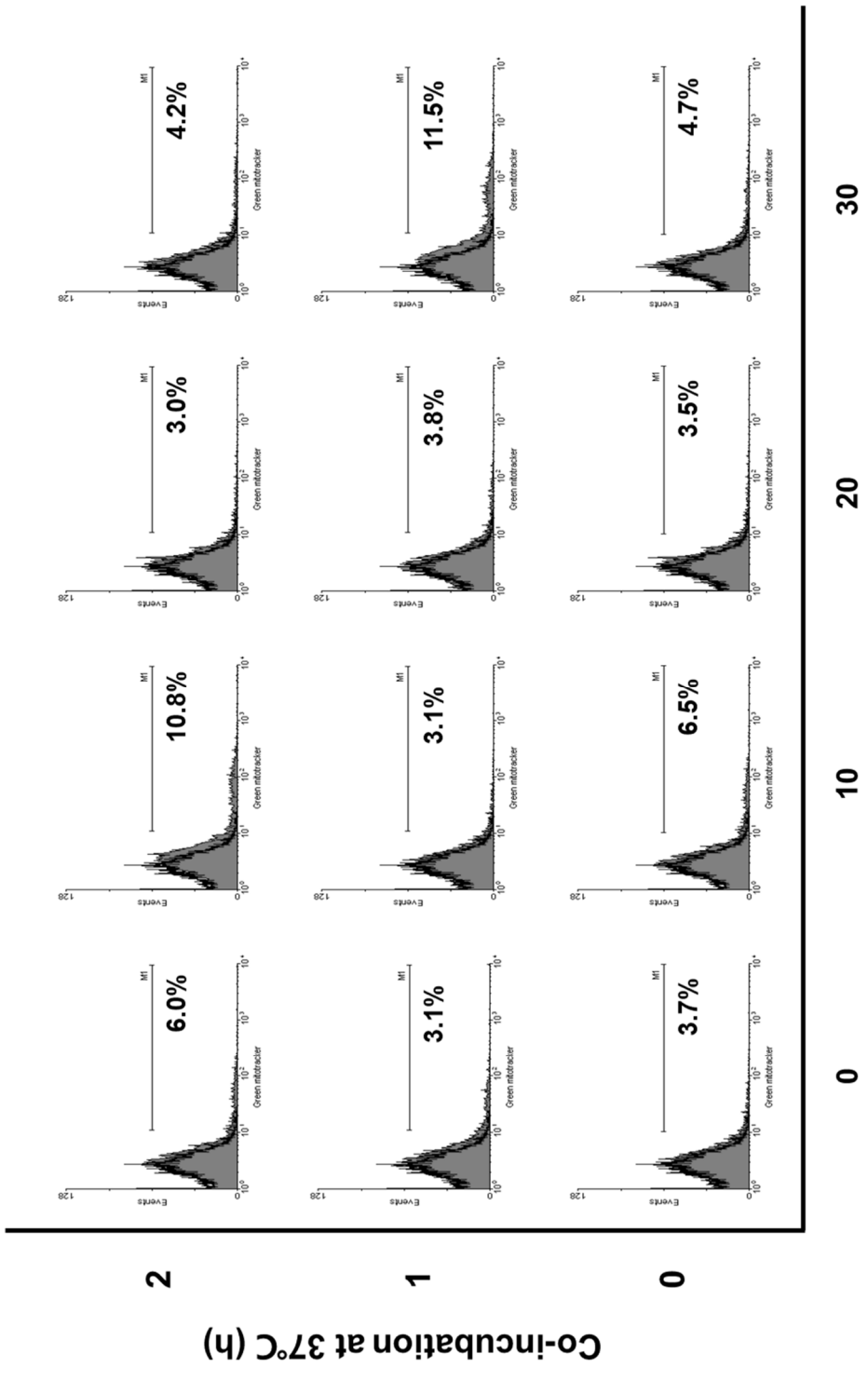
[FIG. 19]


0.05 μg of MT / 1 x 10^5 cells
PF68 0 mg/ml
PF68 10 mg/ml
PF68 30 mg/ml
Incubation time
0h
1h
2h
4h
8.9%
16.8%
20.7%
27%
46.7%
71.7%
29.8%
75.8%
94.1%
62.6%
87.6%
99.4%
Events
Green mitotracker
M1

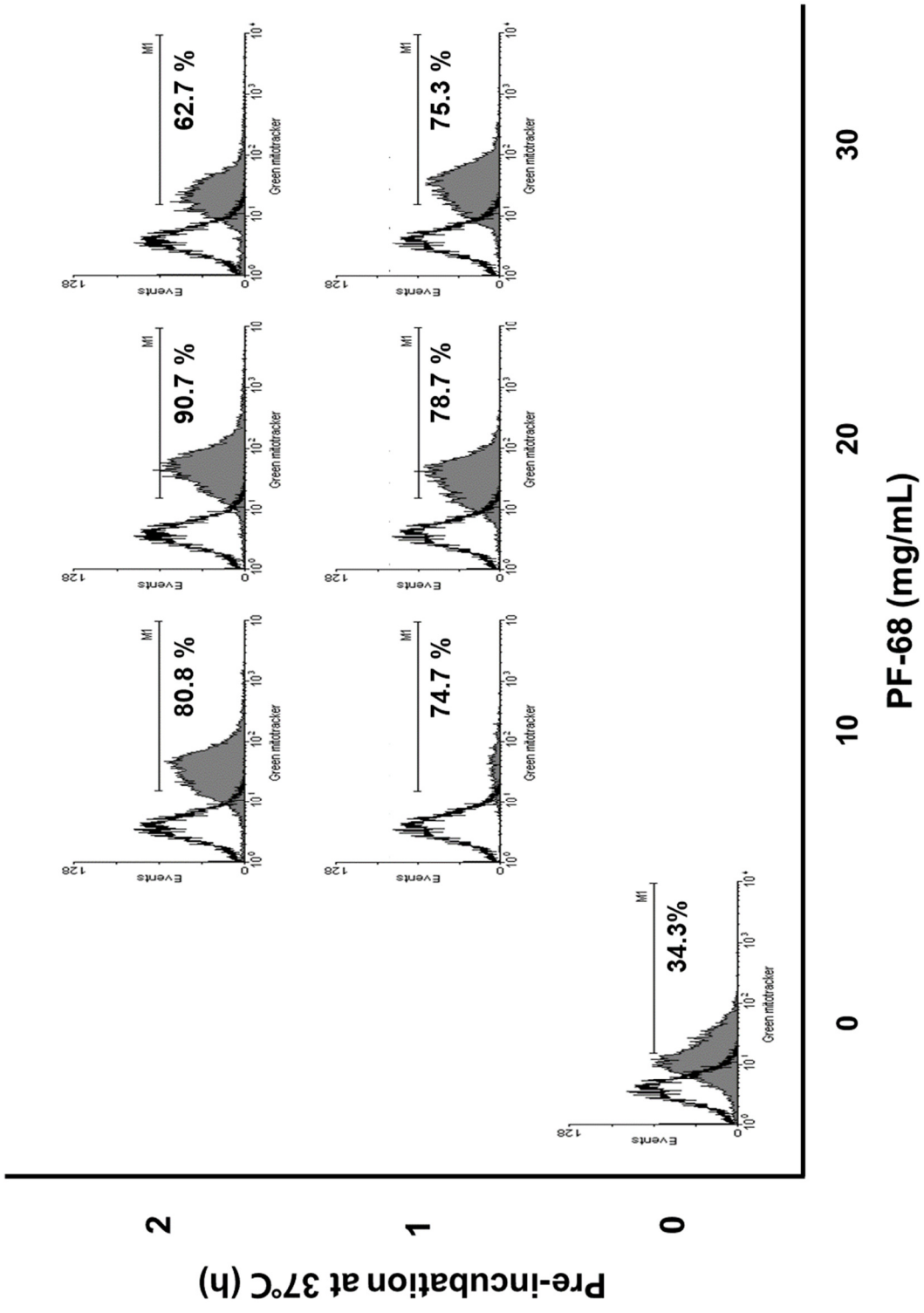
[FIG. 20]


Centrifugation (+ Incubation, +PF68)
Intracellular delivery rate (%)
120
100
80
60
40
20
0
0
10
30
PF68 concentration (mg/ml)
A inc. 0h
B inc. 1h
C inc. 2h
D inc. 4h
8.9
27
29.8
62.6
16.8
45.7
75.8
87.6
20.7
71.7
94.1
99.4
A
B
C
D

[FIG. 21]


Co-incubation at 37°C (h)
2
1
0
PF-68 (mg/mL)
0
10
20
30
6.0%
10.8%
3.0%
4.2%
3.1%
3.1%
3.8%
11.5%
3.7%
6.5%
3.5%
4.7%
M1
Green mitotracker
Events

[FIG. 22]


Pre-incubation at 37°C (h)
2
1
0
PF-68 (mg/mL)
0
10
20
30
34.3%
74.7 %
78.7 %
75.3 %
80.8 %
90.7 %
62.7 %
M1
Events
Green mitotracker