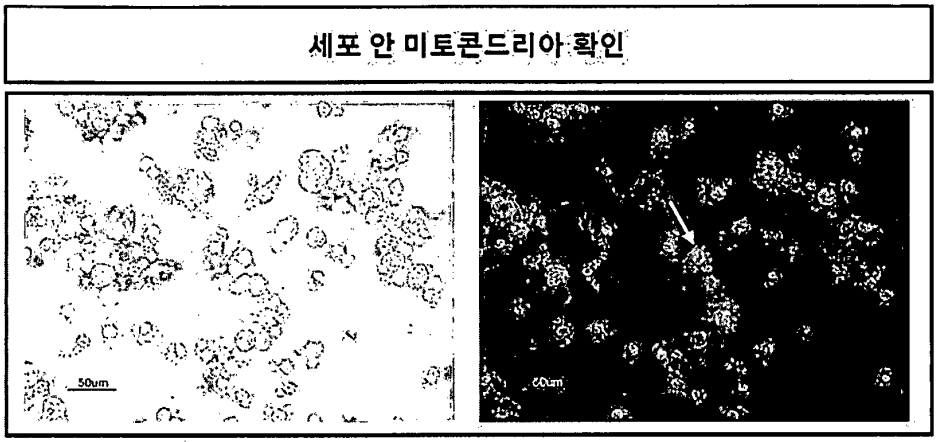
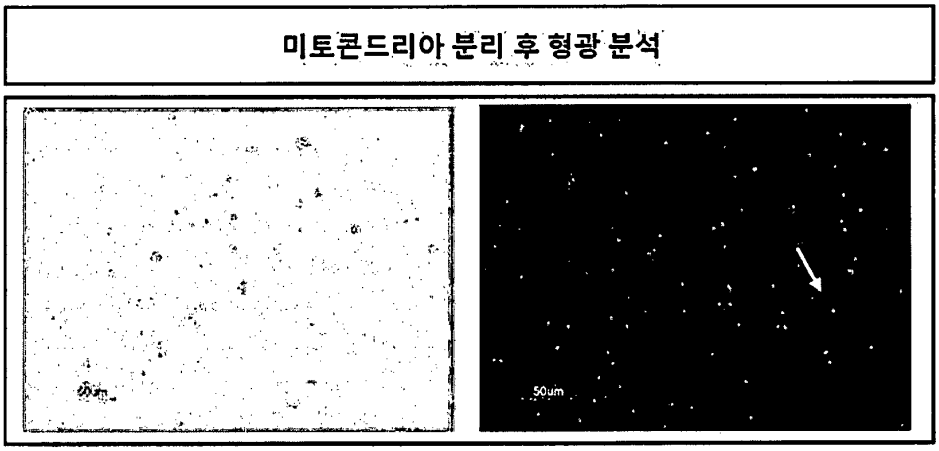
【도면】

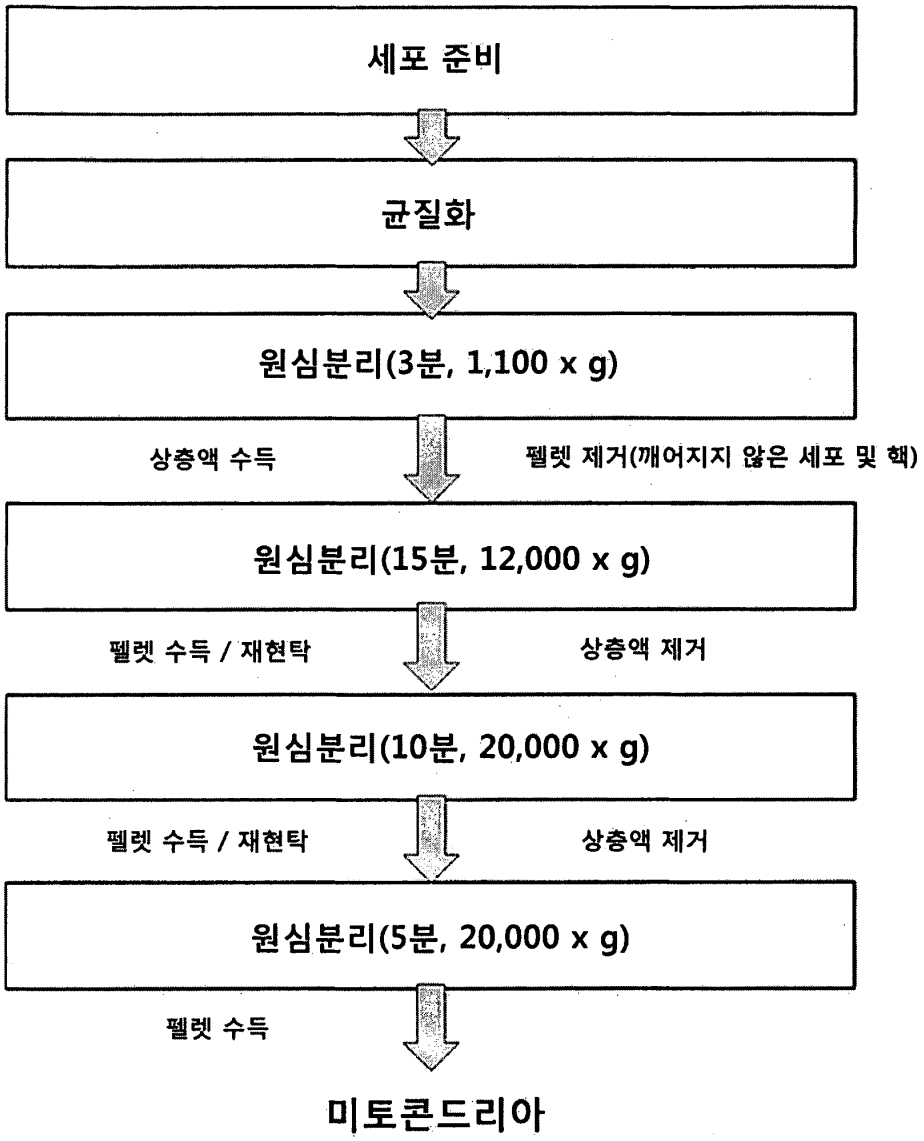
【도 1】



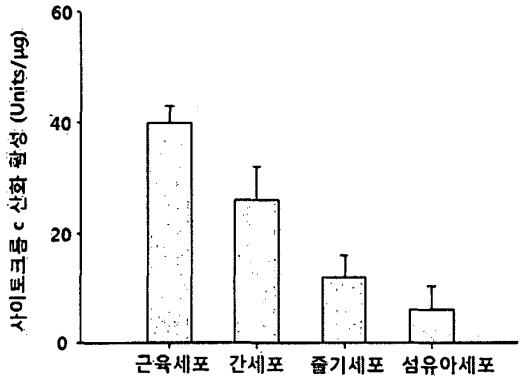
【도 2】



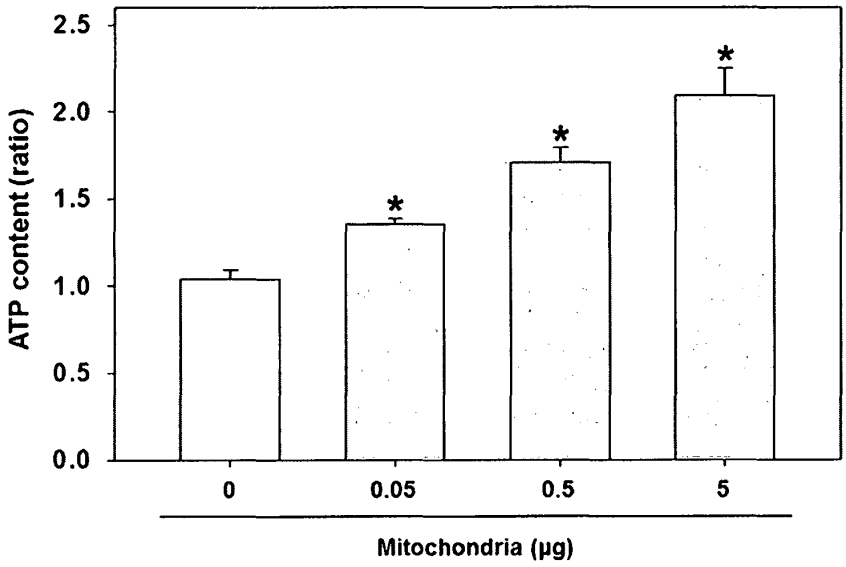
【도 3】


세포 준비
균질화
원심분리(3분, 1,100 x g)
상층액 수득
펠렛 제거(깨어지지 않은 세포 및 핵)
원심분리(15분, 12,000 x g)
펠렛 수득 / 재현탁
상층액 제거
원심분리(10분, 20,000 x g)
펠렛 수득 / 재현탁
상층액 제거
원심분리(5분, 20,000 x g)
펠렛 수득
미토콘드리아

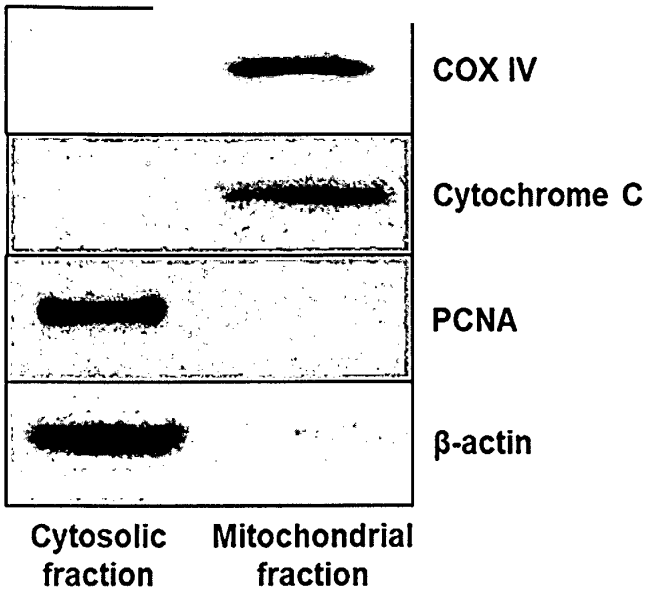
【도 4】


60
40
20
0
사이토크롬 c 산화 활성 (Units/μg)
근육세포
간세포
줄기세포
섬유아세포

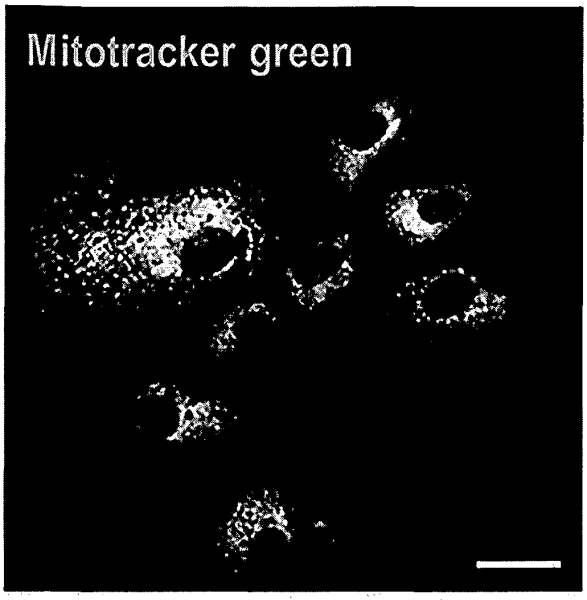
【도 5】


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

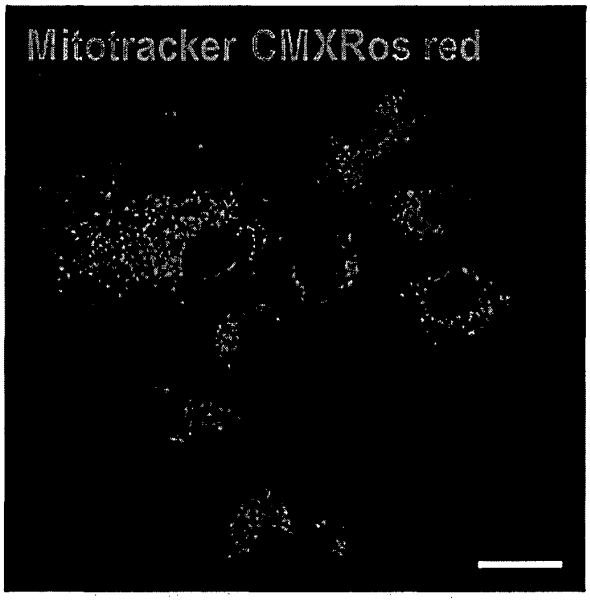
【도 6】


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

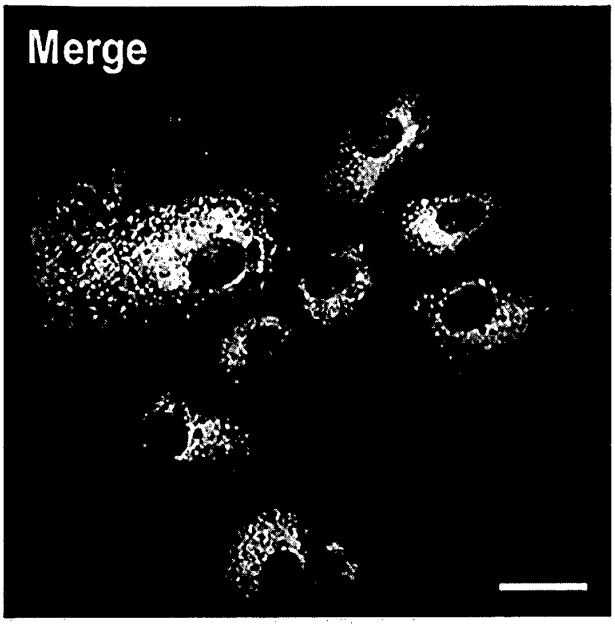
【도 7a】



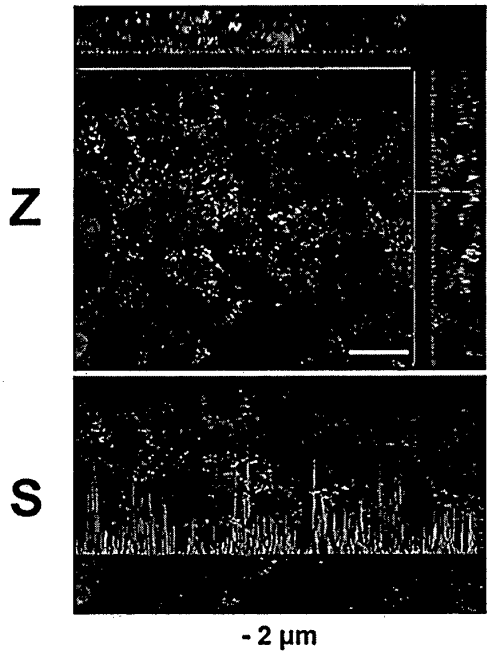
【도 7b】



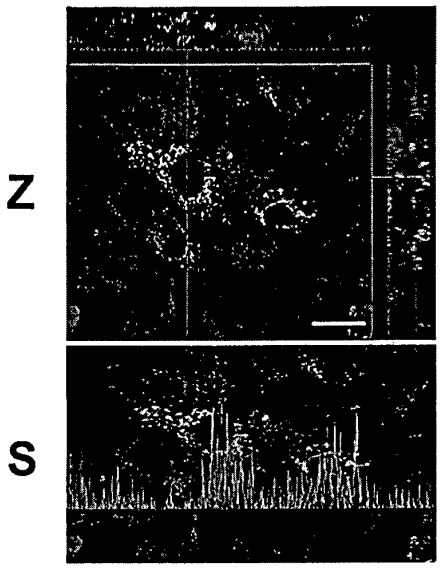
【도 7c】



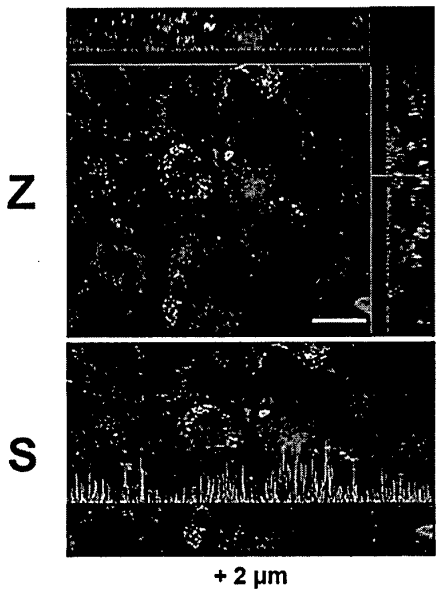
【도 7d】



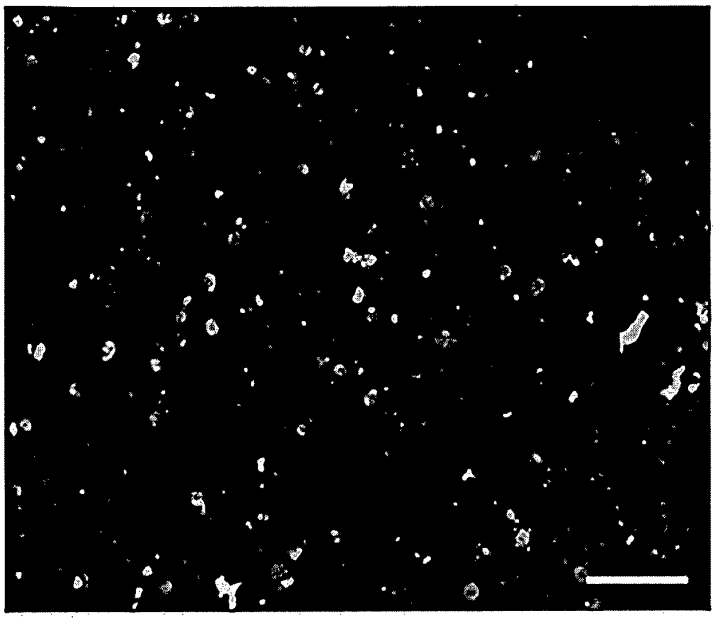
【도 7e】



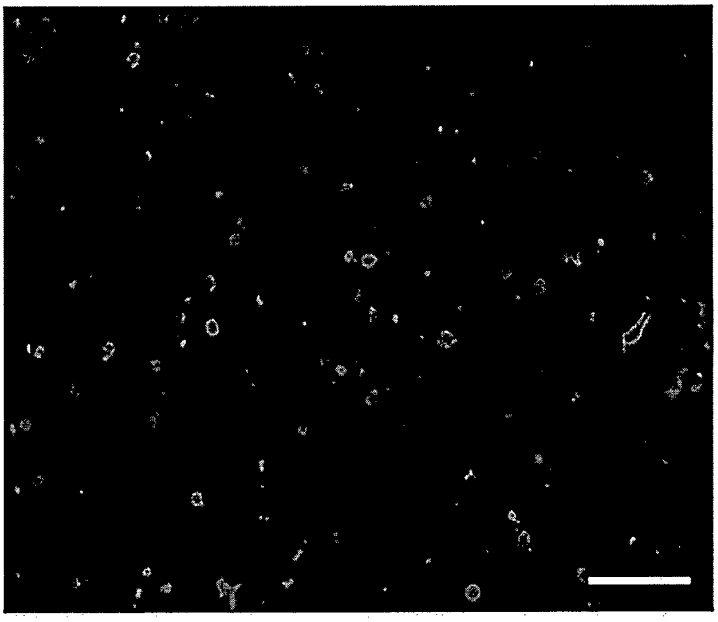
【도 7f】



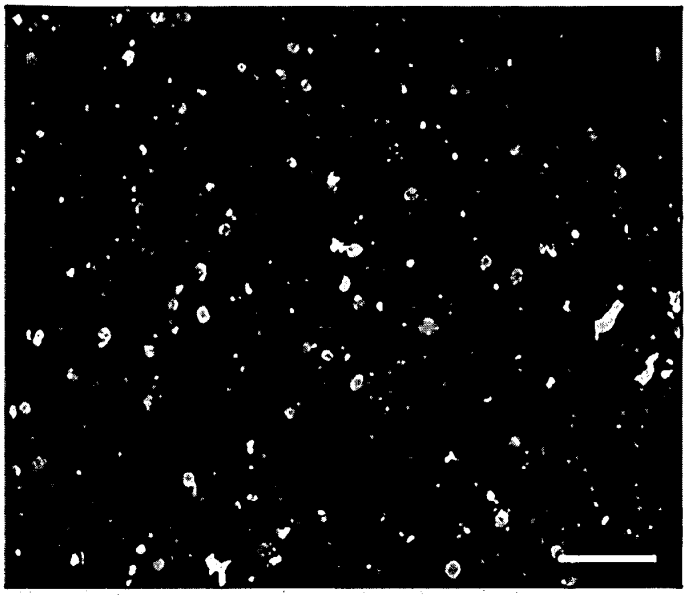
【도 7g】



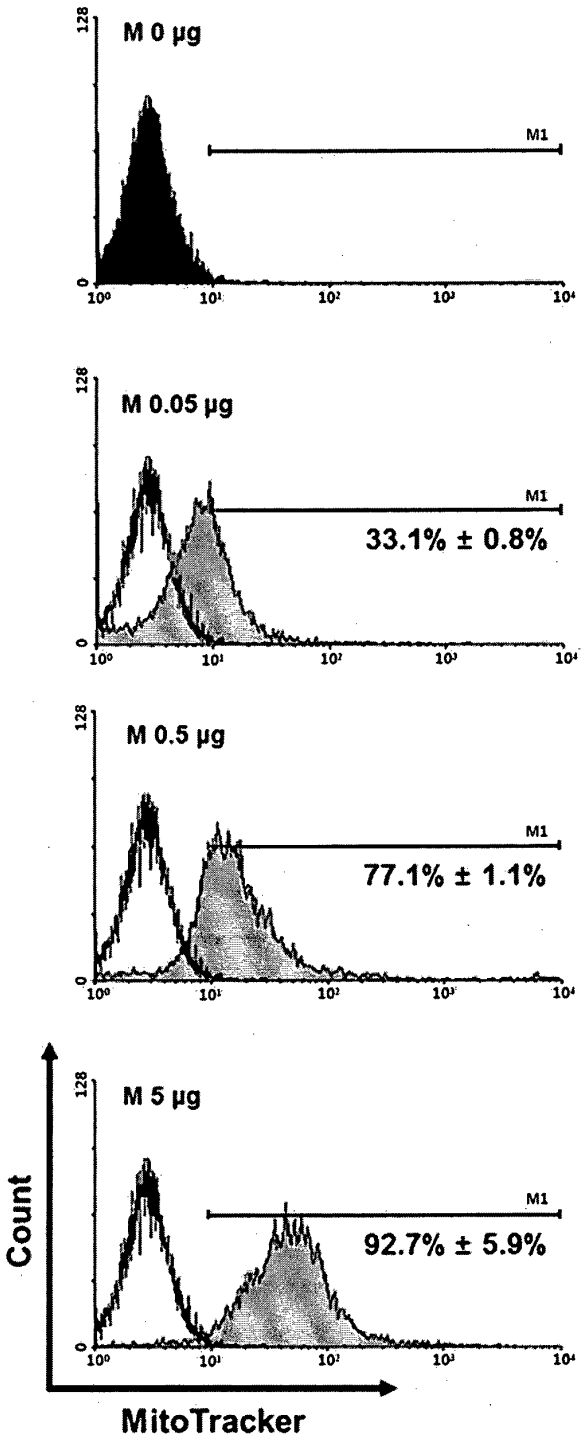
【도 7h】



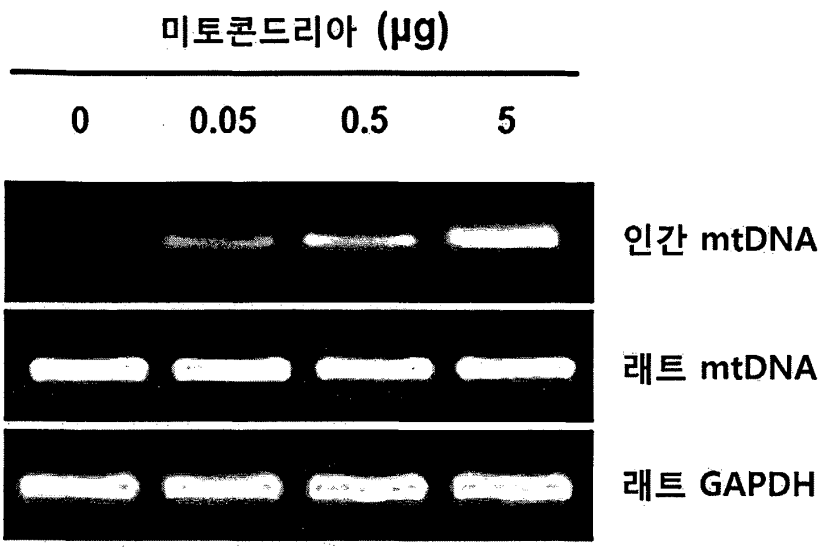
【도 7i】



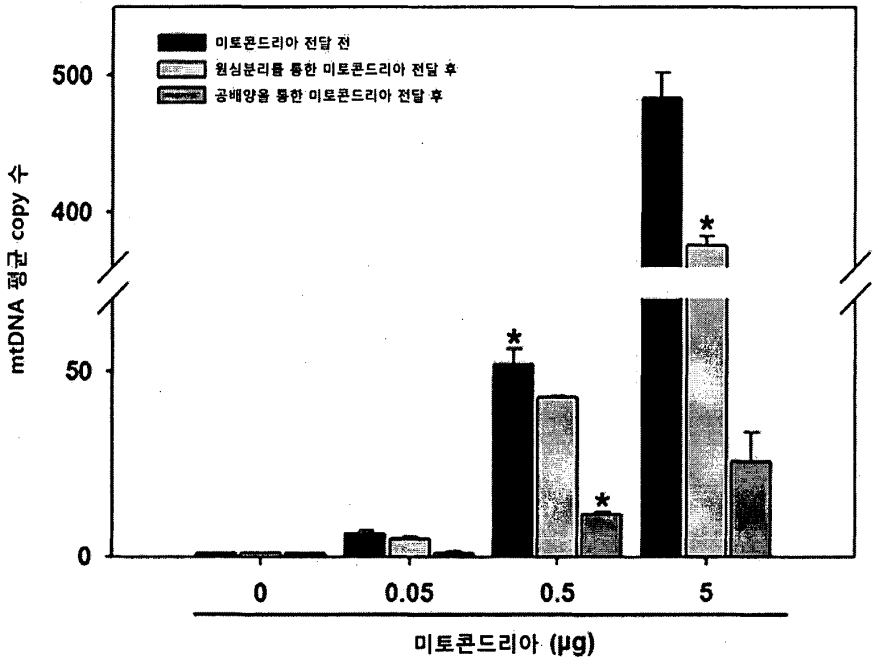
【도 8】


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

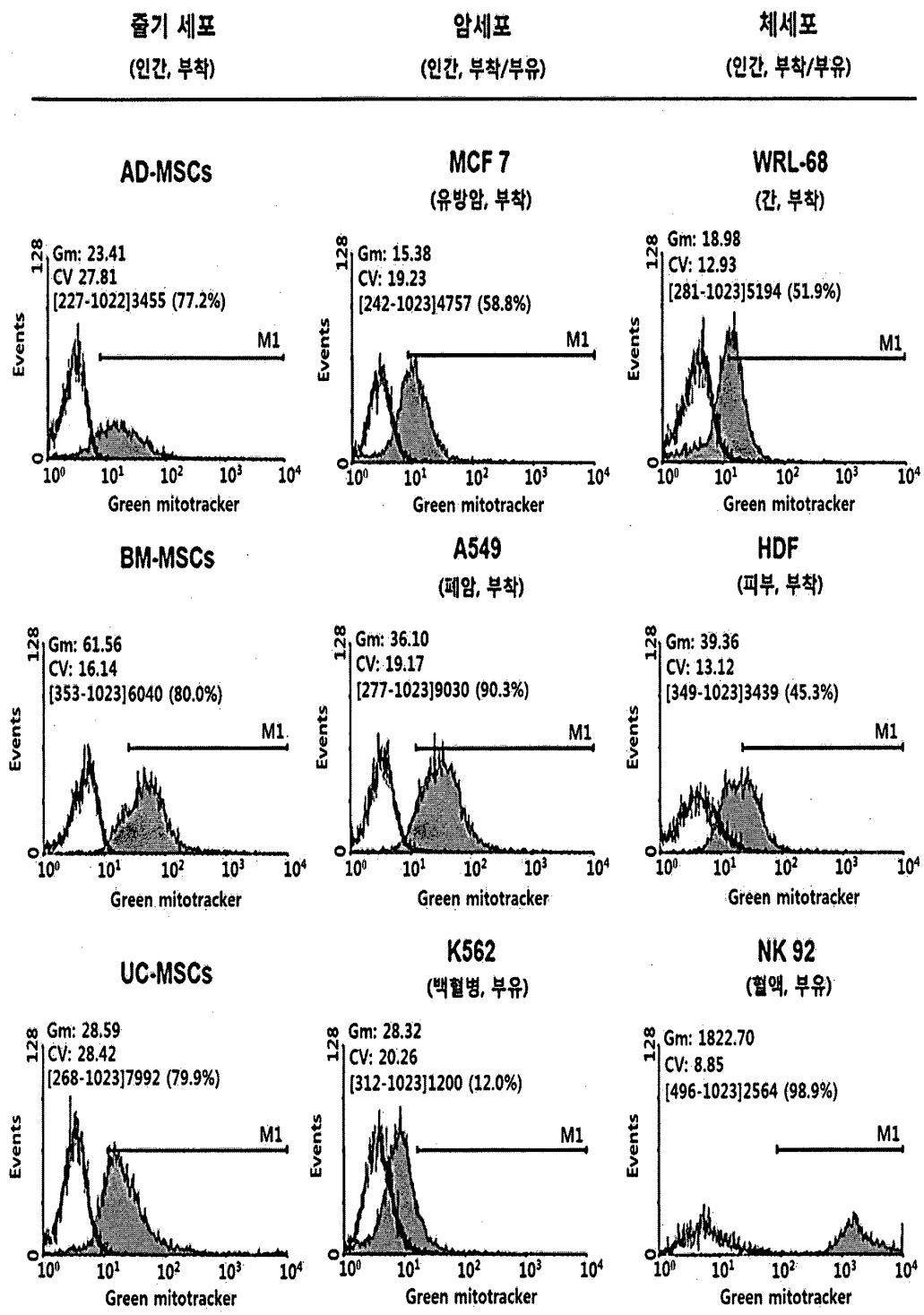
【도 9】


미토콘드리아 (μg)
0
0.05
0.5
5
인간 mtDNA
래트 mtDNA
래트 GAPDH

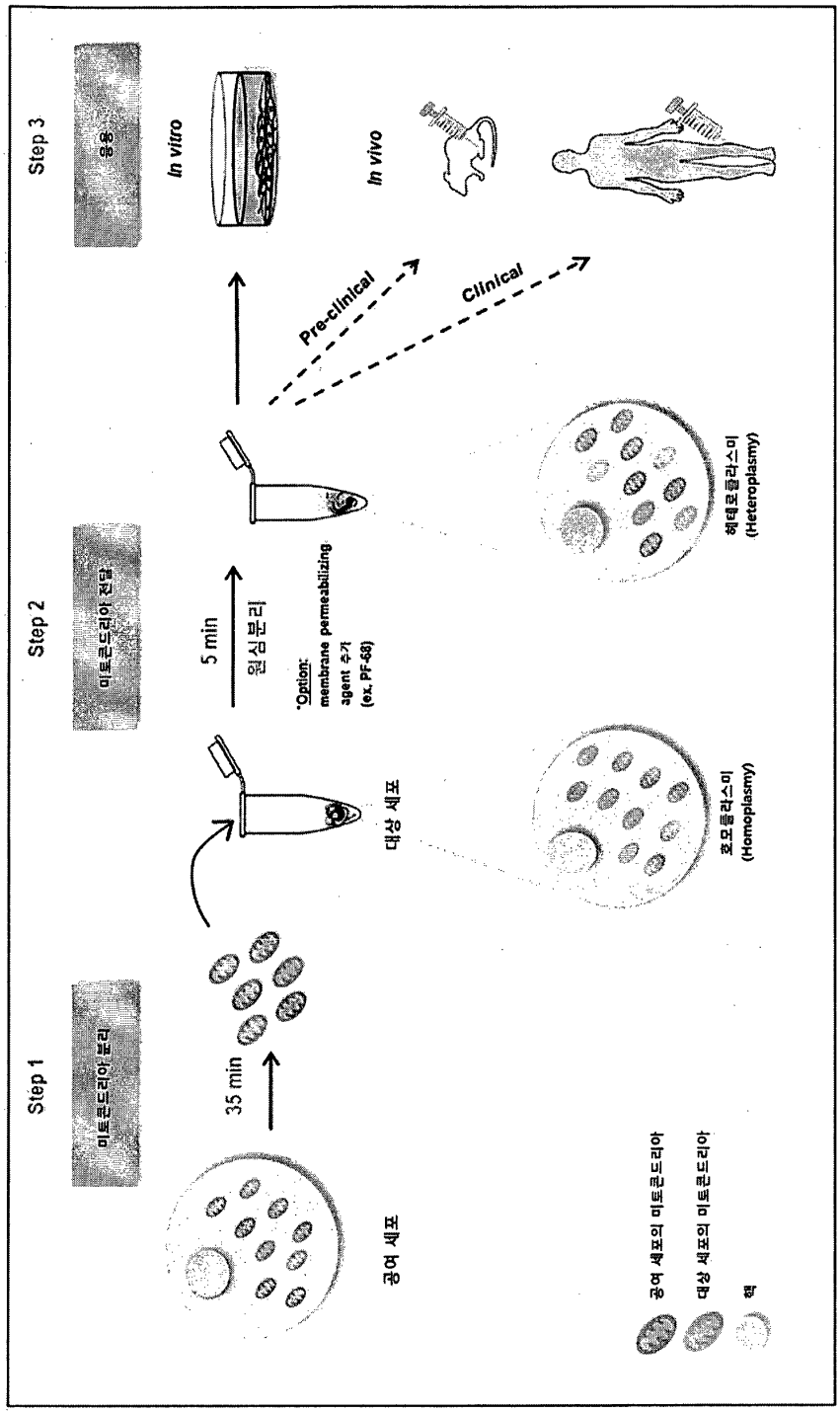
【도 10】


미토콘드리아 전달 전
원심분리를 통한 미토콘드리아 전달 후
공배양을 통한 미토콘드리아 전달 후
mtDNA 평균 copy 수
500
400
50
0
*
*
*
0
0.05
0.5
5
미토콘드리아 (μg)

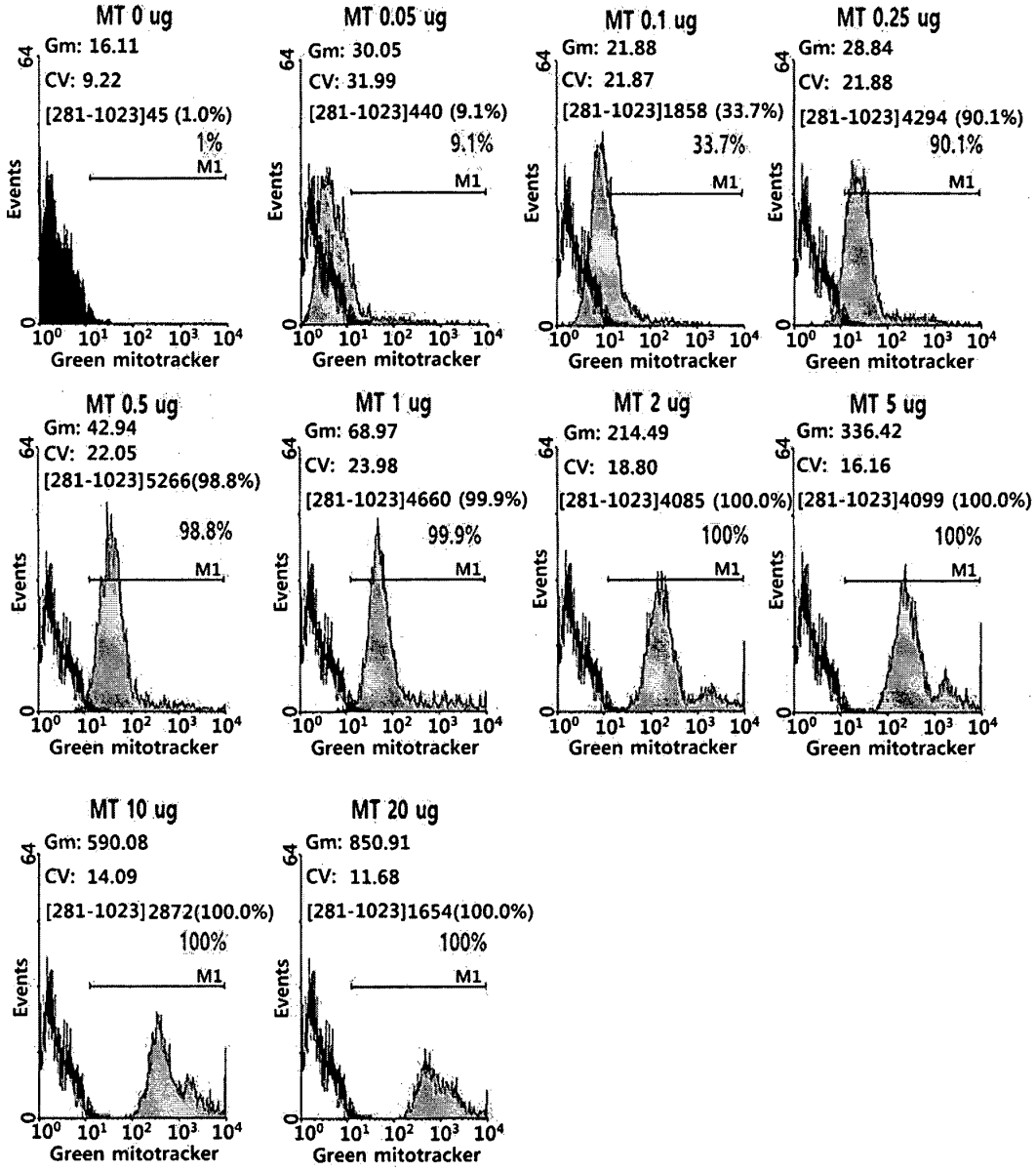
【도 11】


줄기 세포
(인간, 부착)
암세포
(인간, 부착/부유)
체세포
(인간, 부착/부유)
AD-MSCs
Gm: 23.41
CV 27.81
[227-1022]3455 (77.2%)
MCF 7
(유방암, 부착)
Gm: 15.38
CV: 19.23
[242-1023]4757 (58.8%)
WRL-68
(간, 부착)
Gm: 18.98
CV: 12.93
[281-1023]5194 (51.9%)
BM-MSCs
Gm: 61.56
CV: 16.14
[353-1023]6040 (80.0%)
A549
(폐암, 부착)
Gm: 36.10
CV: 19.17
[277-1023]9030 (90.3%)
HDF
(피부, 부착)
Gm: 39.36
CV: 13.12
[349-1023]3439 (45.3%)
UC-MSCs
Gm: 28.59
CV: 28.42
[268-1023]7992 (79.9%)
K562
(백혈병, 부유)
Gm: 28.32
CV: 20.26
[312-1023]1200 (12.0%)
NK 92
(혈액, 부유)
Gm: 1822.70
CV: 8.85
[496-1023]2564 (98.9%)
M1
Events
Green mitotracker

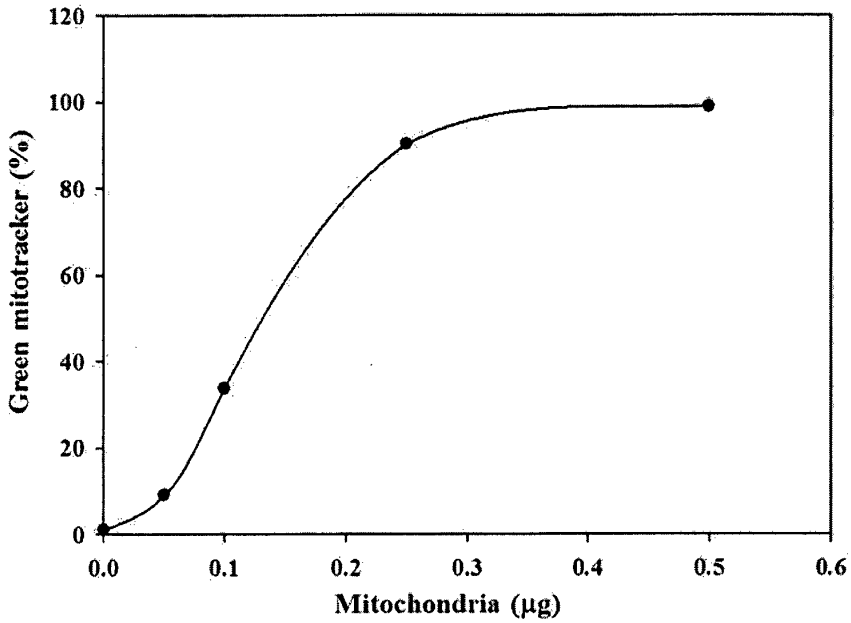
【도 12】


Step 1
미토콘드리아 분리
35 min
공여 세포
Step 2
미토콘드리아 전달
5 min
원심분리
*Option:
membrane permeabilizing
agent 추가
(ex. PF-68)
대상 세포
Step 3
응용
In vitro
In vivo
Pre-clinical
Clinical
호모플라스미
(Homoplasmy)
헤테로플라스미
(Heteroplasmy)
공여 세포의 미토콘드리아
대상 세포의 미토콘드리아
핵

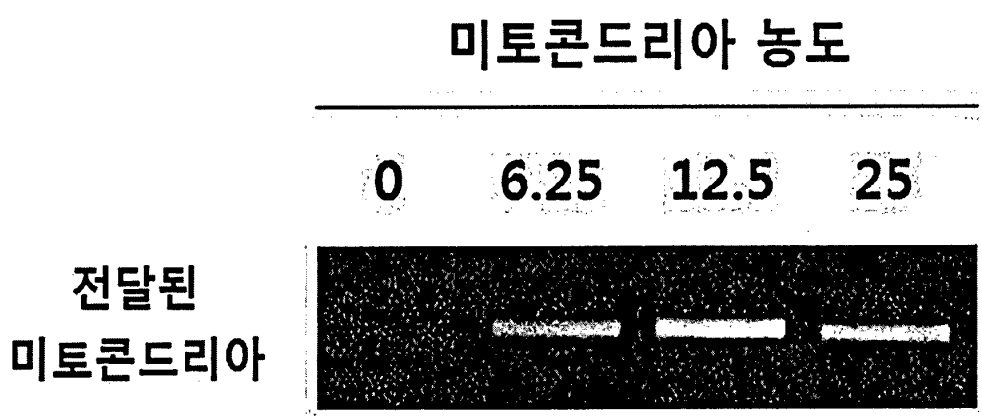
【도 13】


MT 0 ug
Gm: 16.11
CV: 9.22
[281-1023]45 (1.0%)
1%
M1
MT 0.05 ug
Gm: 30.05
CV: 31.99
[281-1023]440 (9.1%)
9.1%
M1
MT 0.1 ug
Gm: 21.88
CV: 21.87
[281-1023]1858 (33.7%)
33.7%
M1
MT 0.25 ug
Gm: 28.84
CV: 21.88
[281-1023]4294 (90.1%)
90.1%
M1
MT 0.5 ug
Gm: 42.94
CV: 22.05
[281-1023]5266(98.8%)
98.8%
M1
MT 1 ug
Gm: 68.97
CV: 23.98
[281-1023]4660 (99.9%)
99.9%
M1
MT 2 ug
Gm: 214.49
CV: 18.80
[281-1023]4085 (100.0%)
100%
M1
MT 5 ug
Gm: 336.42
CV: 16.16
[281-1023]4099 (100.0%)
100%
M1
MT 10 ug
Gm: 590.08
CV: 14.09
[281-1023]2872(100.0%)
100%
M1
MT 20 ug
Gm: 850.91
CV: 11.68
[281-1023]1654(100.0%)
100%
M1
Events
Green mitotracker

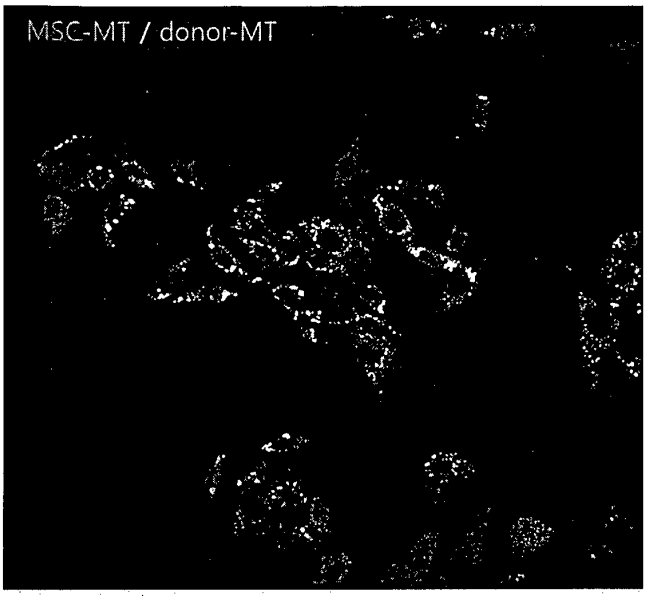
【도 14】


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)

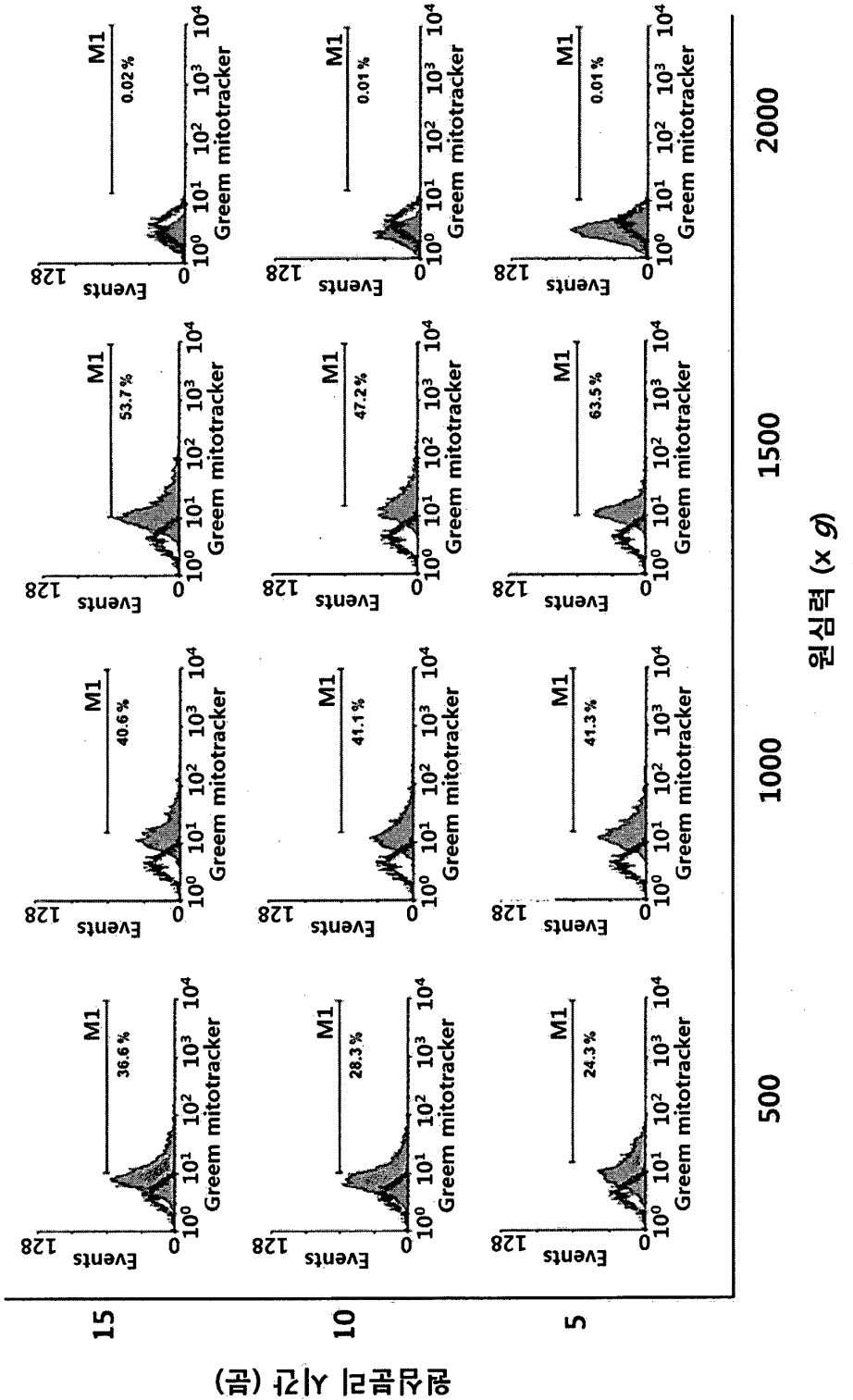
【도 15】


미토콘드리아 농도
0
6.25
12.5
25
전달된
미토콘드리아

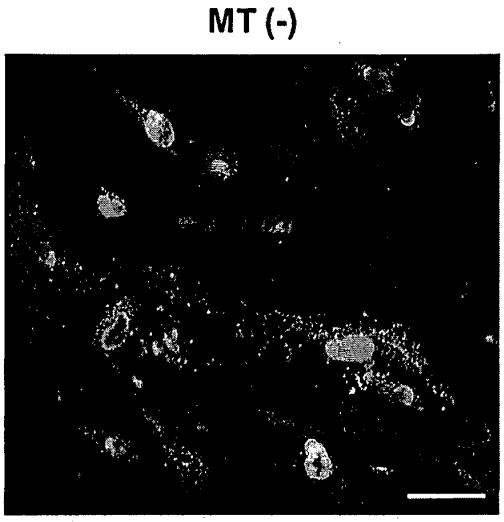
【도 16】



【도 17】


원심분리 시간 (분)
15
10
5
원심력 (x g)
500
1000
1500
2000
M1
0.02 %
0.01 %
0.01 %
53.7 %
47.2 %
63.5 %
40.6 %
41.1 %
41.3 %
36.6 %
28.3 %
24.3 %
Events
Greem mitotracker

【도 18a】



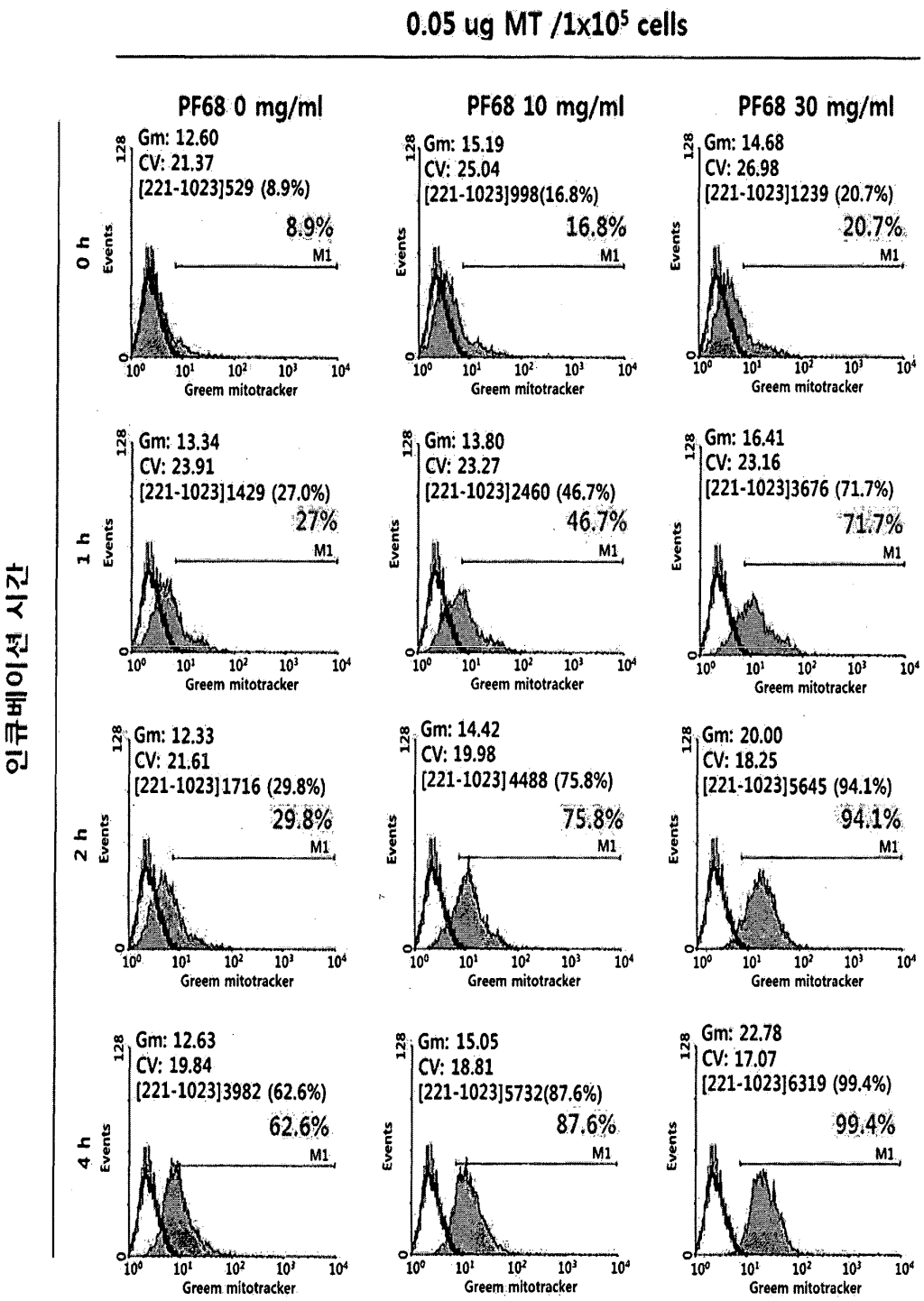
대상 세포: L6 muscle
MT 공여세포: 인간 UC-MSCs

【도 18b】

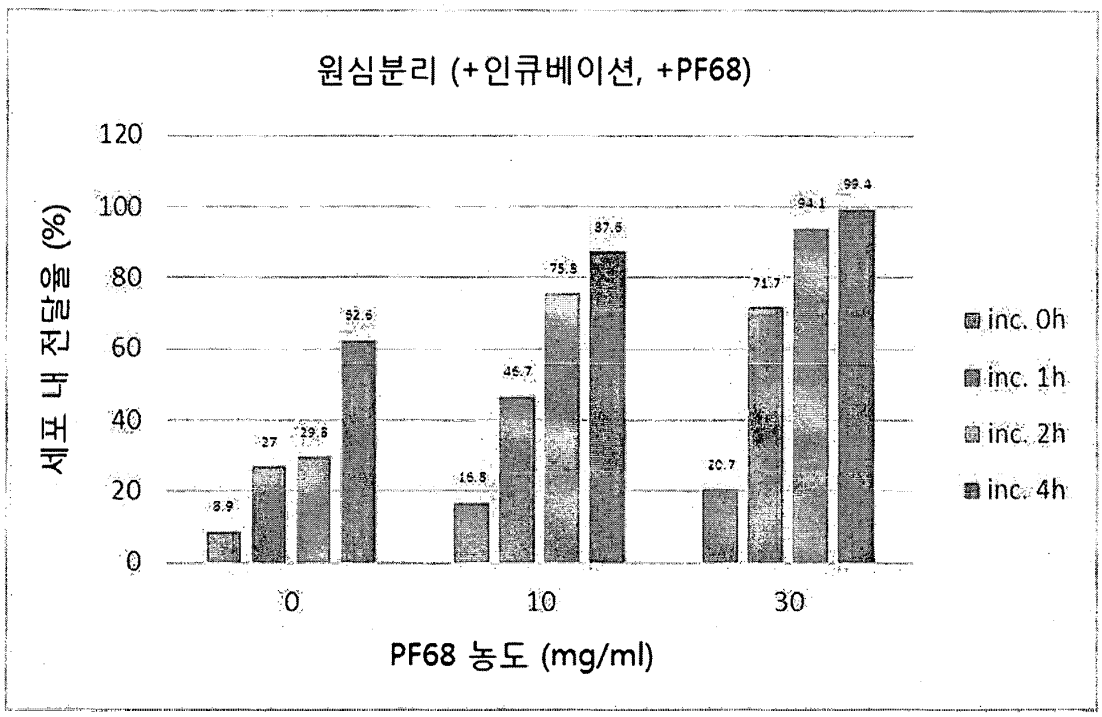


대상 세포: L6 muscle
MT 공여세포: 인간 UC-MSCs

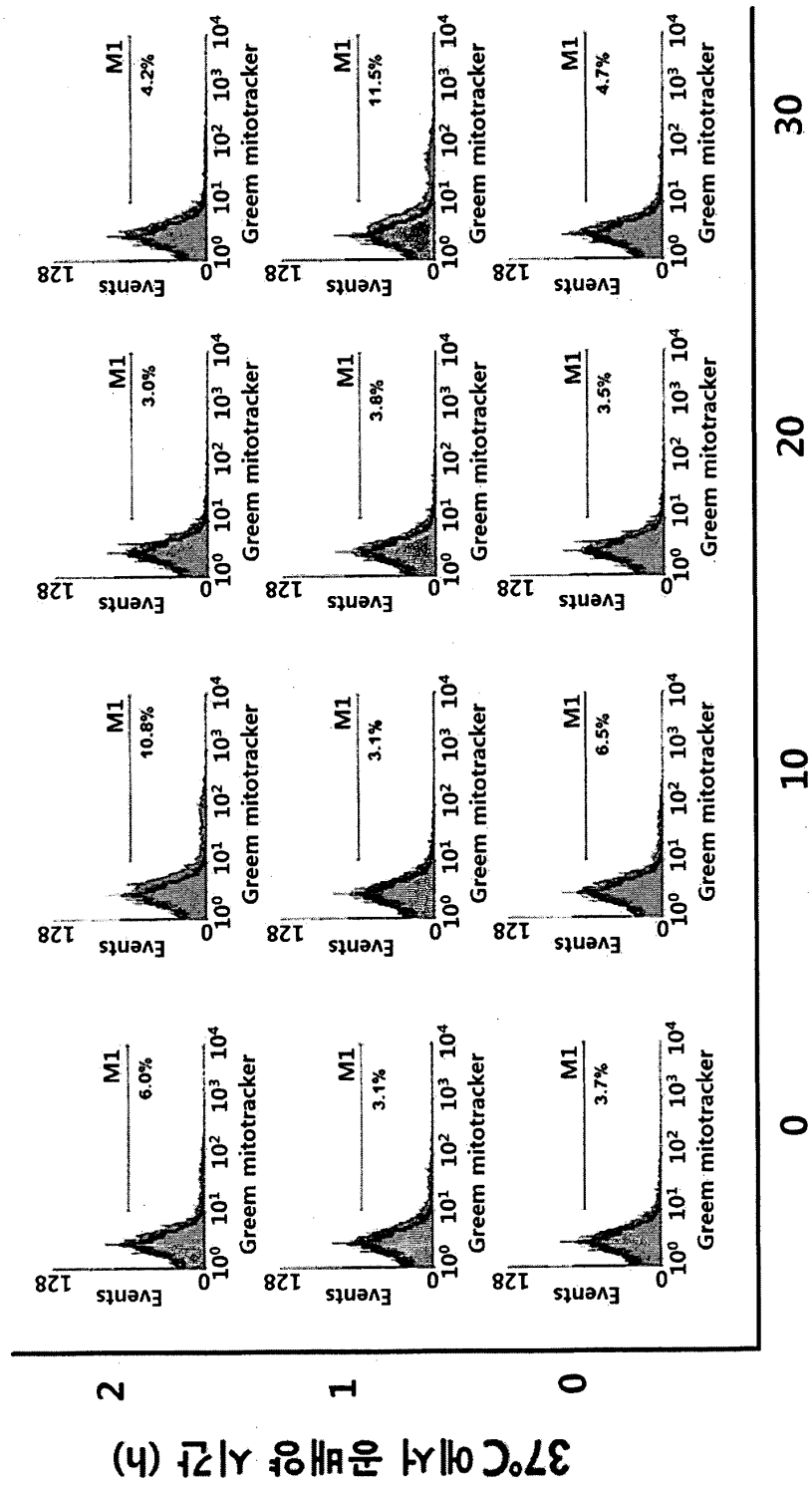
【도 19】


0.05 ug MT /1x10^5 cells
PF68 0 mg/ml
PF68 10 mg/ml
PF68 30 mg/ml
인큐베이션 시간
0 h
1 h
2 h
4 h
Gm: 12.60
CV: 21.37
[221-1023]529 (8.9%)
8.9%
Gm: 15.19
CV: 25.04
[221-1023]998(16.8%)
16.8%
Gm: 14.68
CV: 26.98
[221-1023]1239 (20.7%)
20.7%
Gm: 13.34
CV: 23.91
[221-1023]1429 (27.0%)
27%
Gm: 13.80
CV: 23.27
[221-1023]2460 (46.7%)
46.7%
Gm: 16.41
CV: 23.16
[221-1023]3676 (71.7%)
71.7%
Gm: 12.33
CV: 21.61
[221-1023]1716 (29.8%)
29.8%
Gm: 14.42
CV: 19.98
[221-1023]4488 (75.8%)
75.8%
Gm: 20.00
CV: 18.25
[221-1023]5645 (94.1%)
94.1%
Gm: 12.63
CV: 19.84
[221-1023]3982 (62.6%)
62.6%
Gm: 15.05
CV: 18.81
[221-1023]5732(87.6%)
87.6%
Gm: 22.78
CV: 17.07
[221-1023]6319 (99.4%)
99.4%
M1
Events
Greem mitotracker

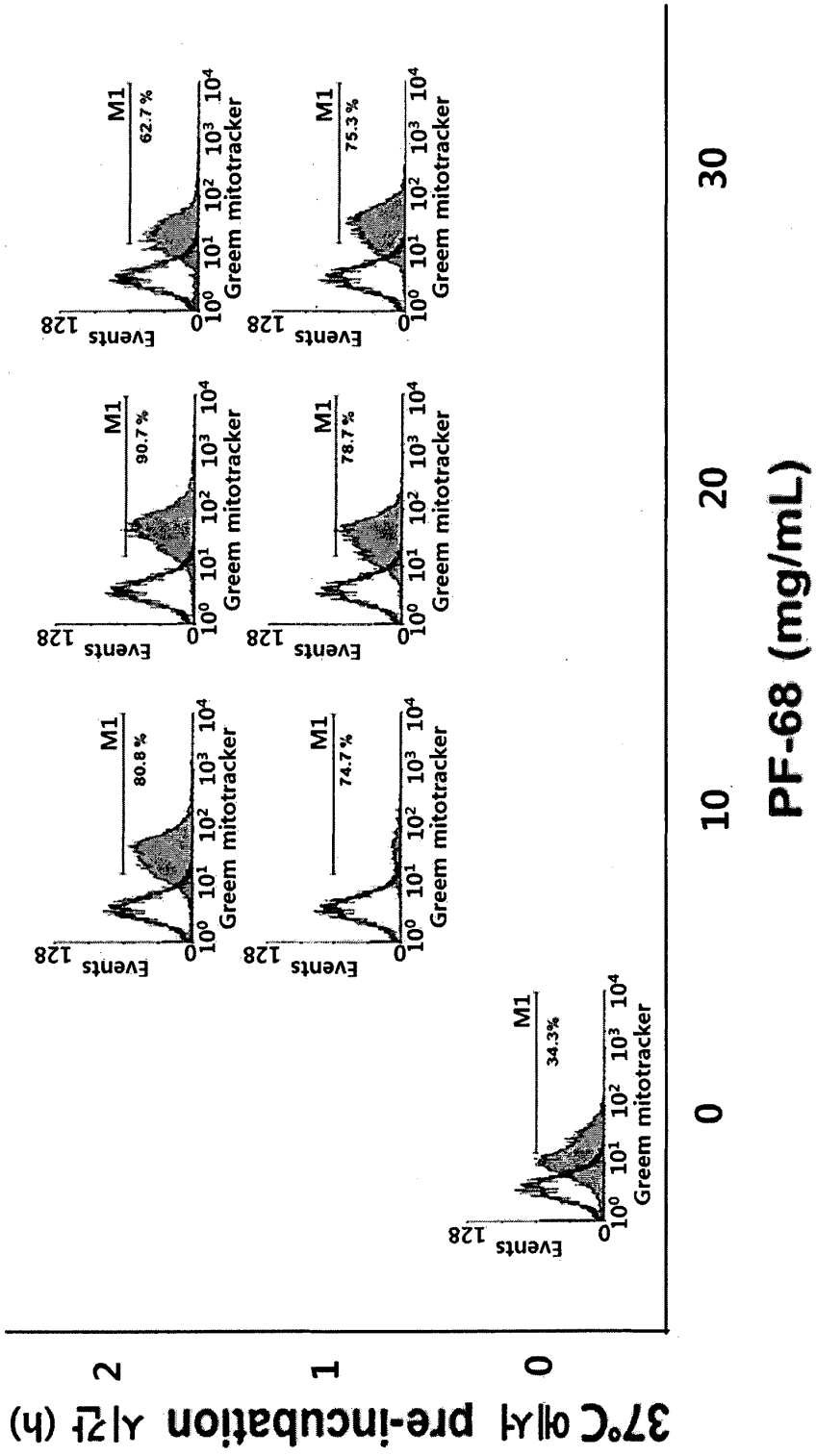
【도 20】


원심분리 (+인큐베이션, +PF68)
세포 내 전달율 (%)
120
100
80
60
40
20
0
8.9
27
29.6
62.6
16.8
46.7
75.8
87.6
20.7
71.7
94.1
99.4
0
10
30
PF68 농도 (mg/ml)
inc. 0h
inc. 1h
inc. 2h
inc. 4h

【도 21】


37℃에서 배양 시간 (h)
2
1
0
0
10
20
30
PF-68 (mg/mL)
M1
4.2%
11.5%
4.7%
3.0%
3.8%
3.5%
10.8%
3.1%
6.5%
6.0%
3.1%
3.7%
Greem mitotracker
Events
128
0

【도 22】


37℃에서 pre-incubation 시간 (h)
2
1
0
0
10
20
30
PF-68 (mg/mL)
M1
34.3%
62.7%
75.3%
80.8%
74.7%
90.7%
78.7%
Events
128
0
Greem mitotracker