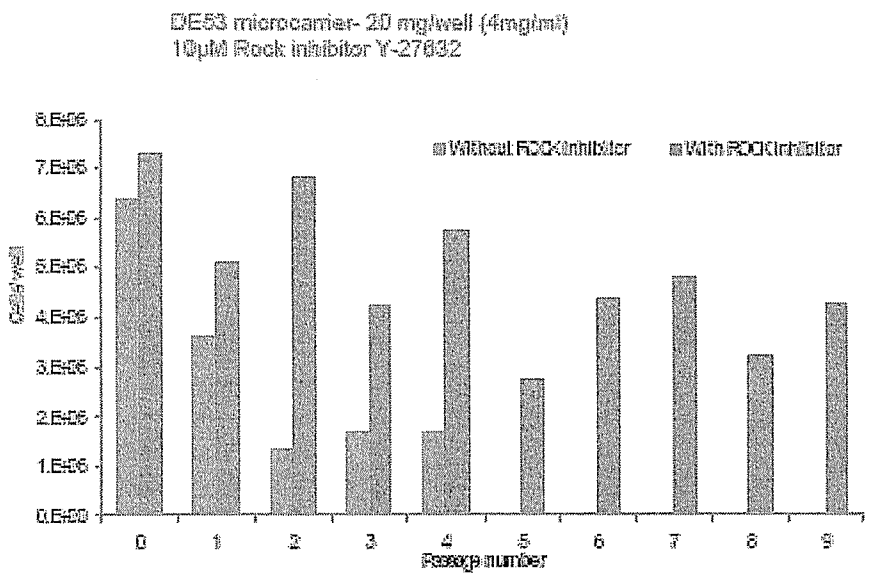
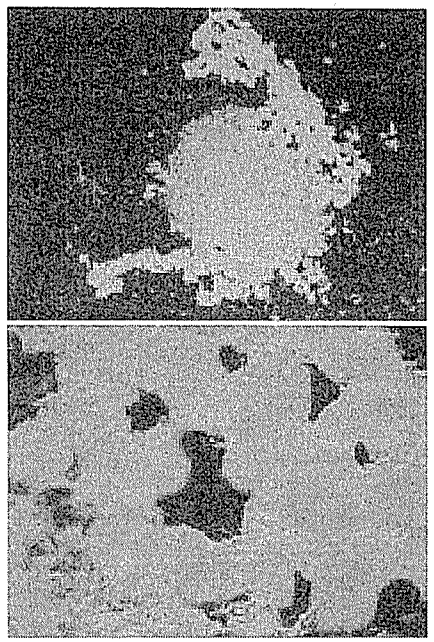
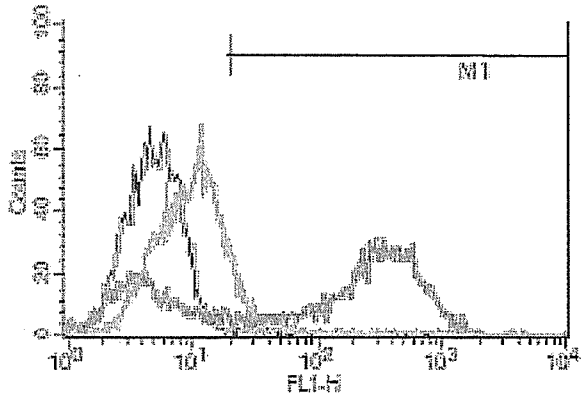




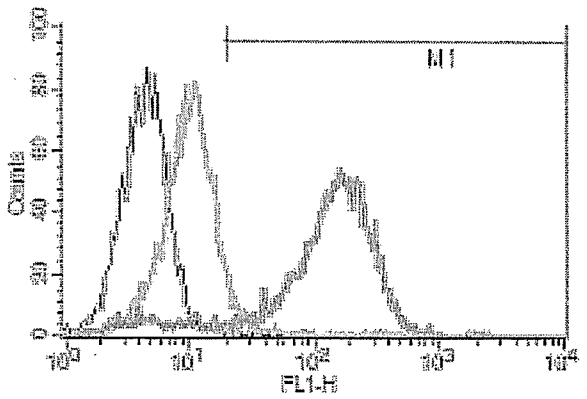
hESC 3 on uncoated DE53 microcarrier with ROCK inhibitor P6

Figure 1



Key	Name	Parameter	Gate	
	Data 010	CONTROL		
	Data 011	OCT 4		46.58%
	Data 012	MAB 84		66.18%

STATIC CULTURE
H3 P33KKP44 - P3
UNCOATED DE53
W/O ROCK INHIBITOR



Key	Name	Parameter	Gate	
	Data 013	CONTROL		
	Data 014	OCT 4		68.68%
	Data 015	MAB 84		92.29%

STATIC CULTURE
H3 P33KKP44 - P3
UNCOATED DE53
10uM ROCK INHIBITOR

Figure 2

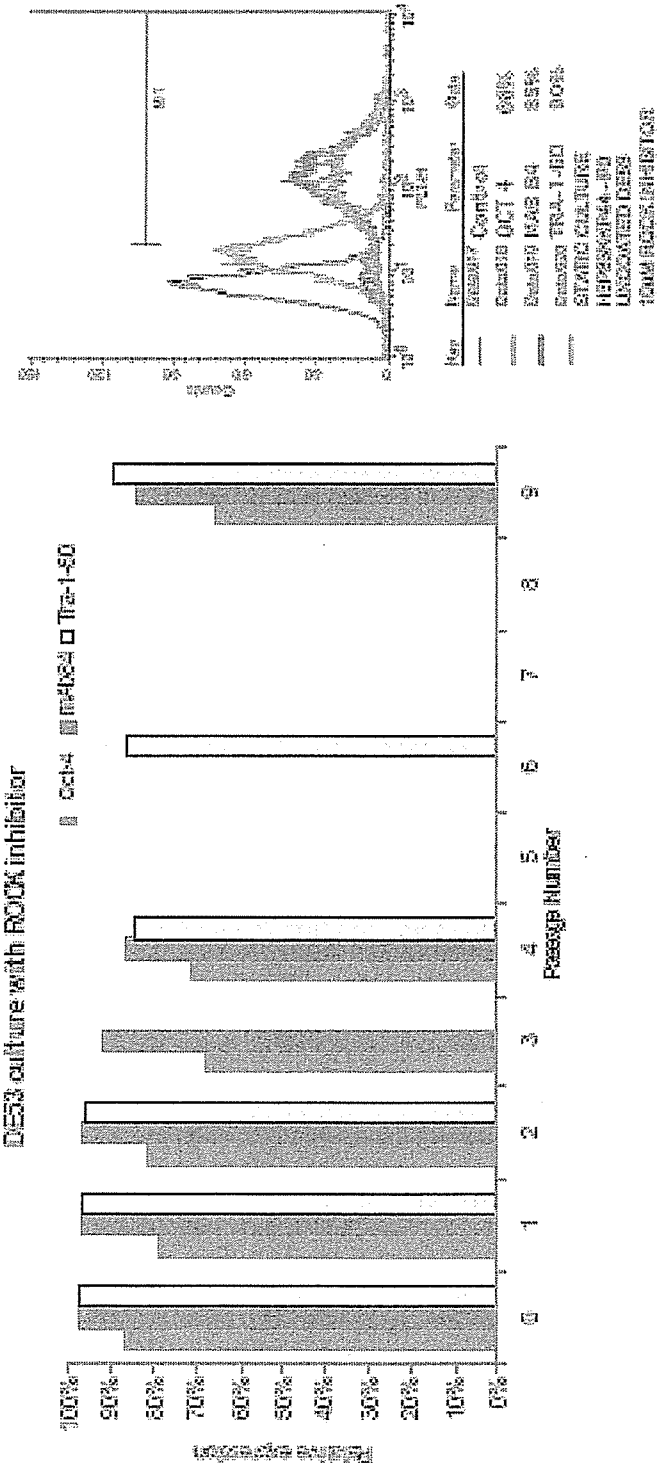
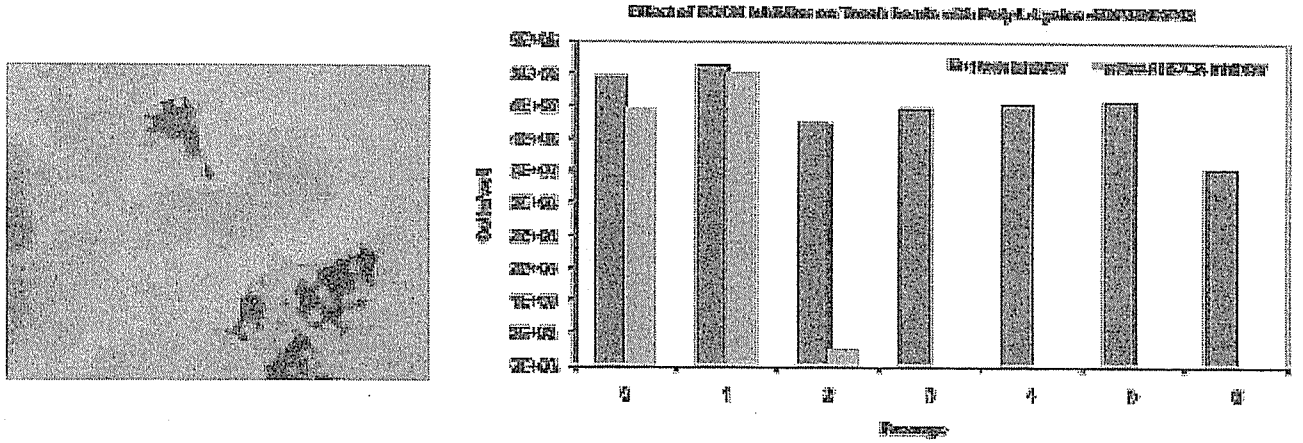
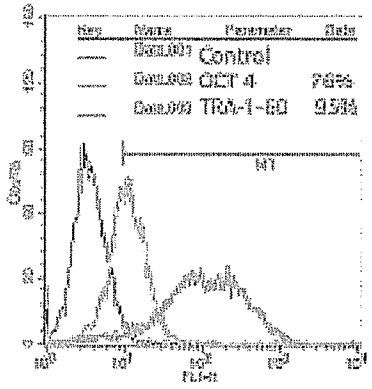

D53 culture with ROCK inhibitor
Oct-4
Tra-1-60
Passage Number
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
1
2
3
4
5
6
7
8

Figure 3



Poly-L-Lysine coated Tosoh beads with ROCK inhibitor P4



Poly-L-Lysine coated Tosoh beads without ROCK inhibitor P2

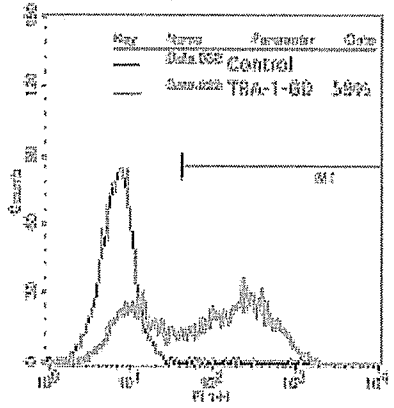


Figure 4

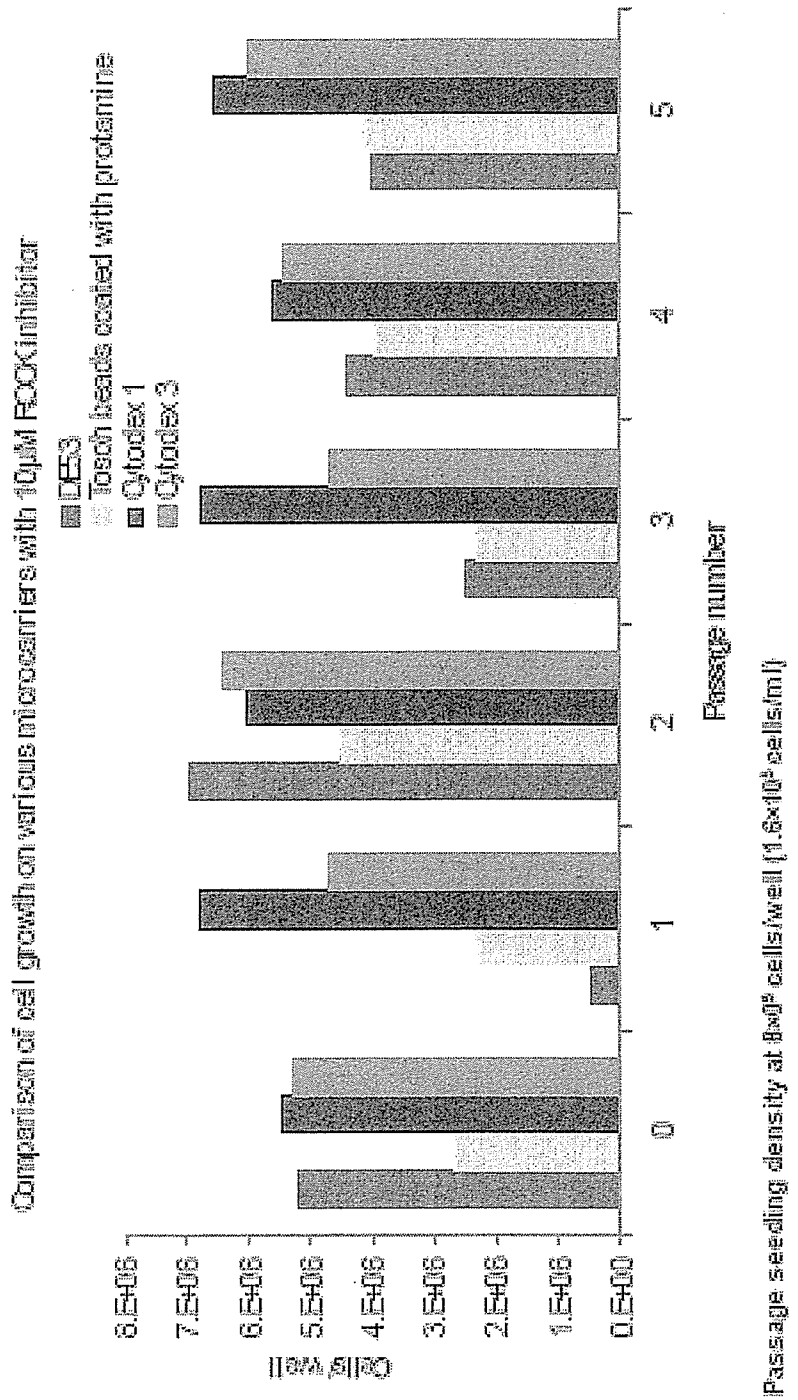

Comparison of cell growth on various microcarriers with 10µM ROCK inhibitor
DE3
Ibosch beads coated with protamine
Cytodex 1
Cytodex 3
Cells/well
8.E+06
7.E+06
6.E+06
5.E+06
4.E+06
3.E+06
2.E+06
1.E+06
0.E+00
0
1
2
3
4
5
Passage number
Passage seeding density at 8x10⁵ cells/well (1.6x10⁵ cells/ml)

Figure 5

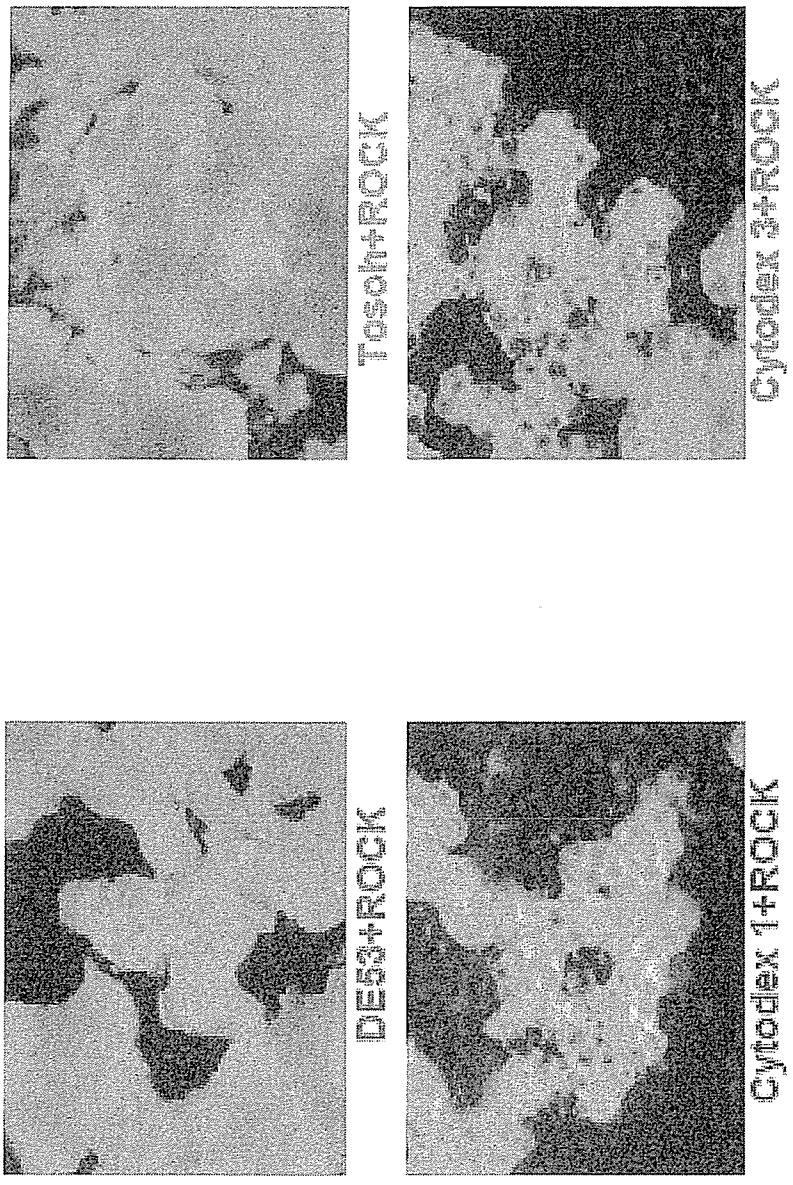


Figure 6

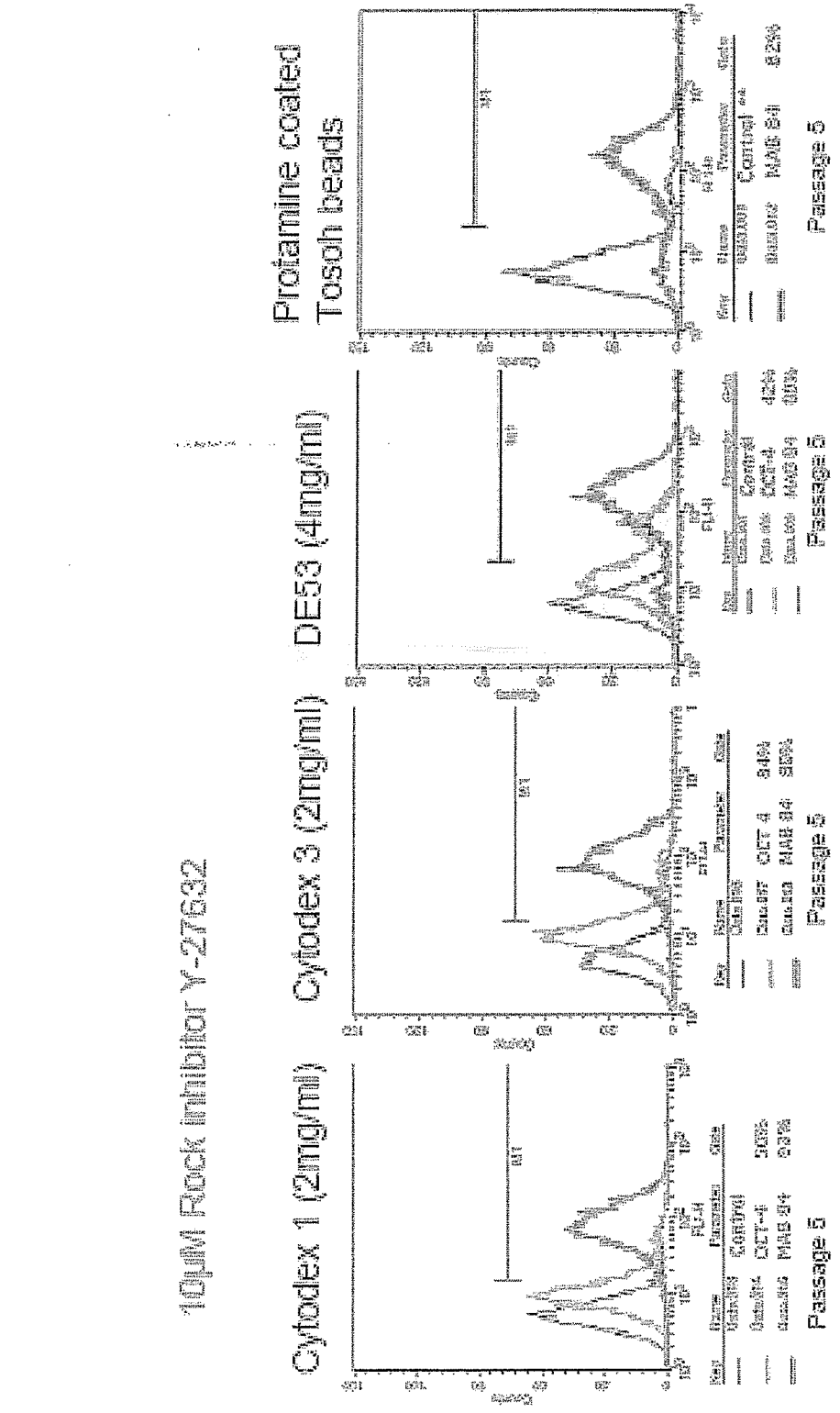

10μM Rock inhibitor Y-27632
Cytodex 1 (2mg/ml)
Cytodex 3 (2mg/ml)
DE53 (4mg/ml)
Protamine coated Tosoh beads
Passage 5
Passage 5
Passage 5
Passage 5

Figure 7

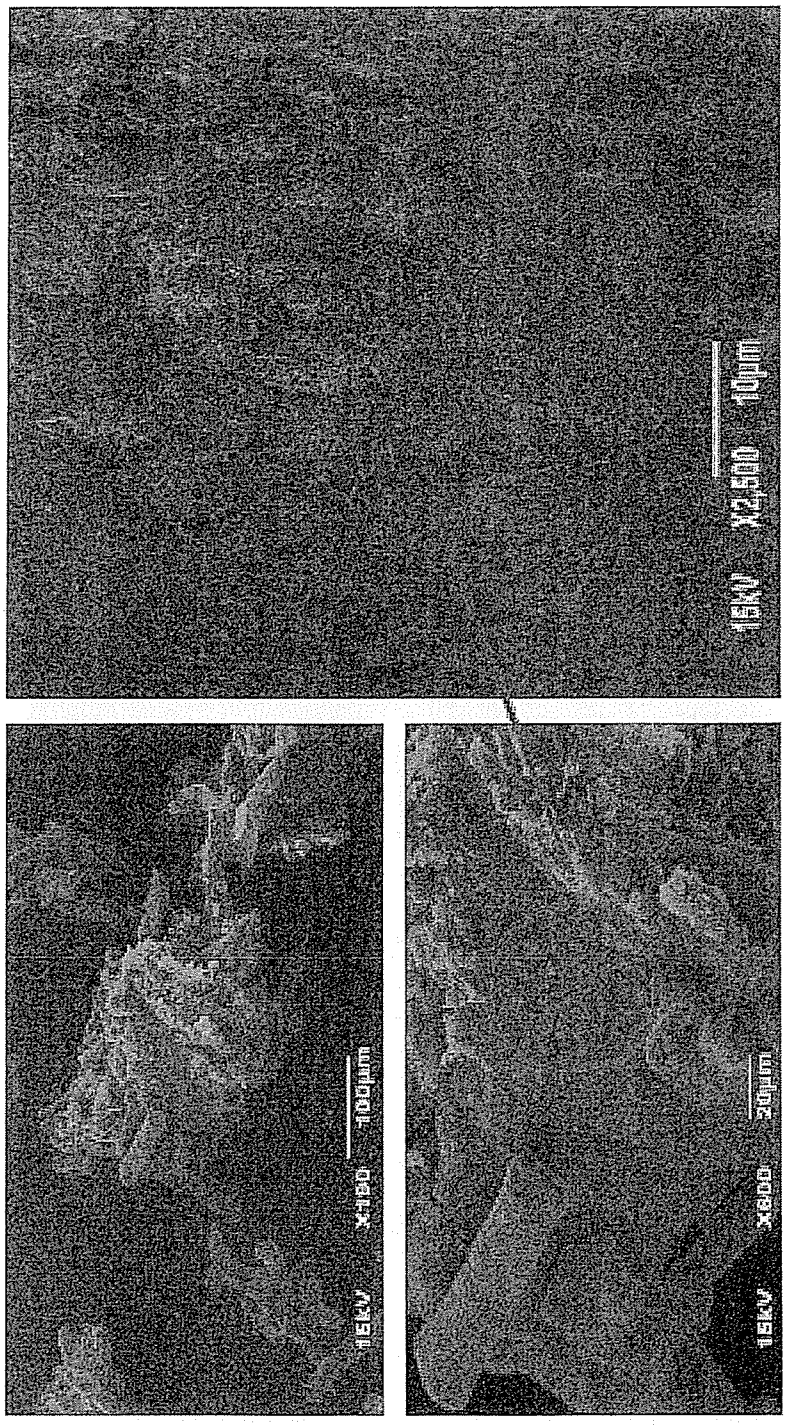


Figure 8

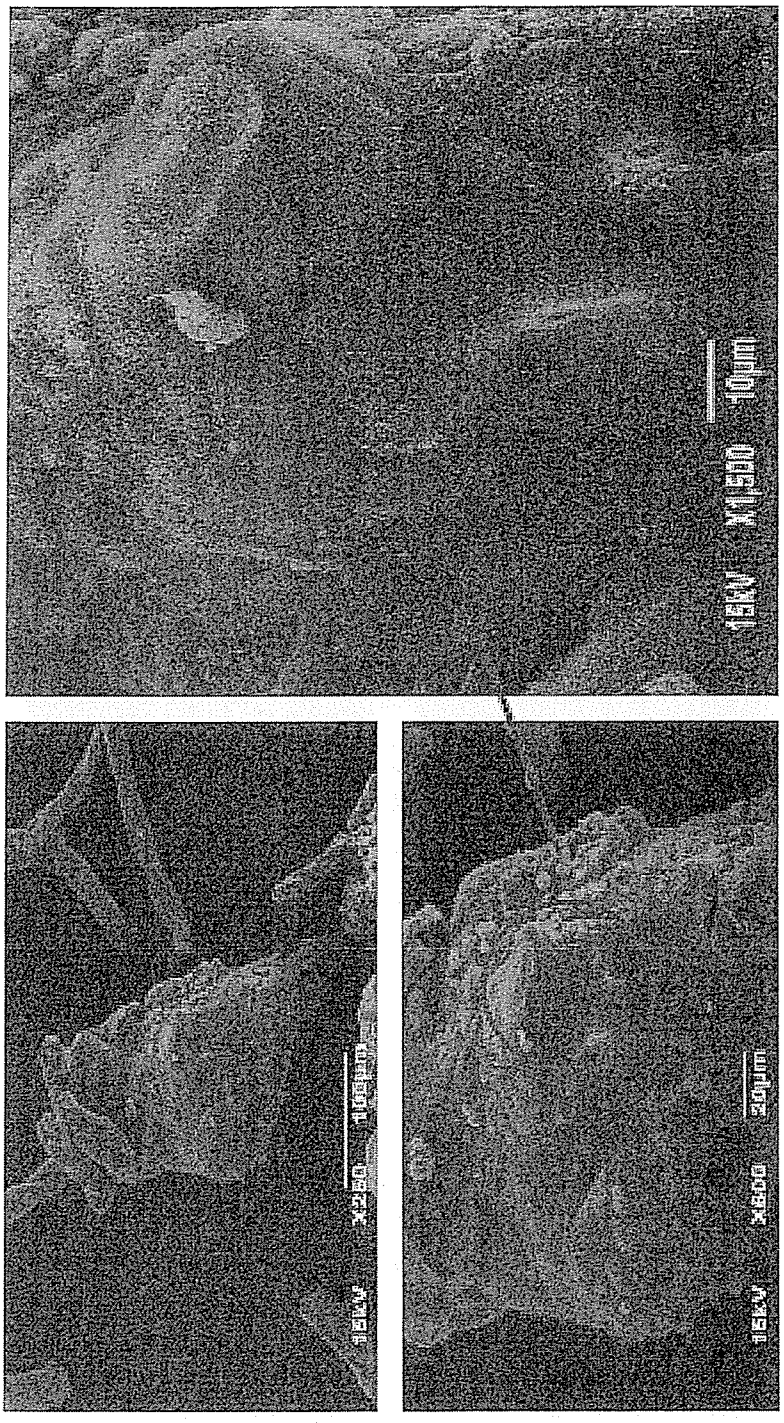


Figure 9

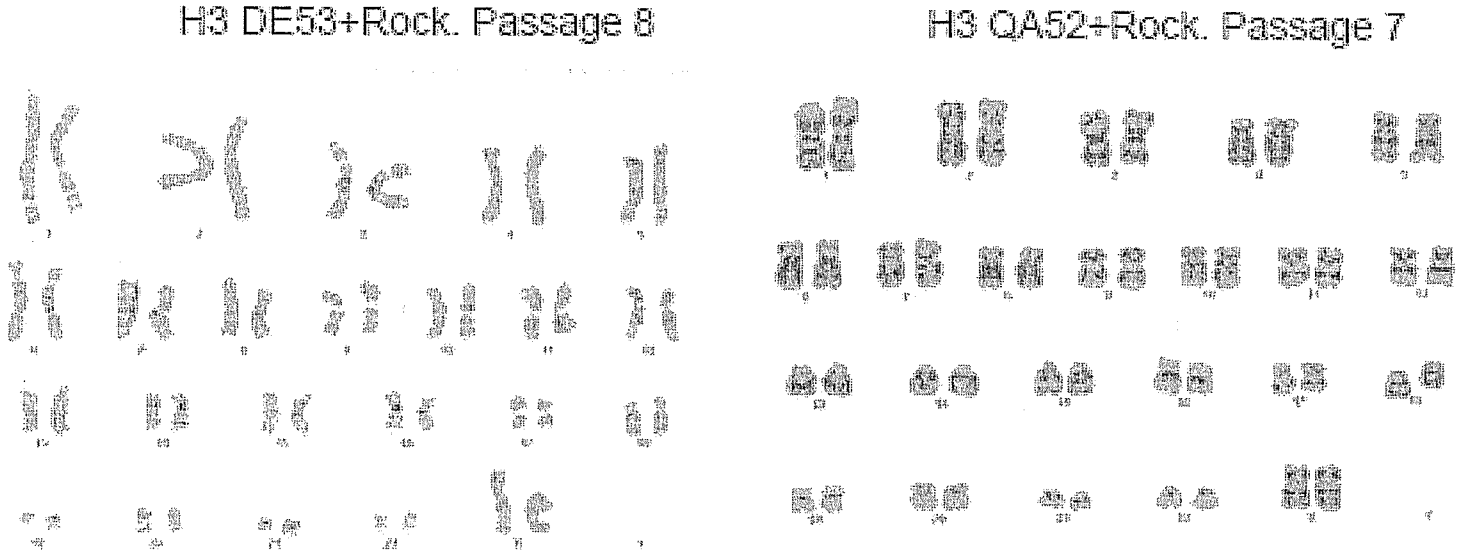


Figure 10

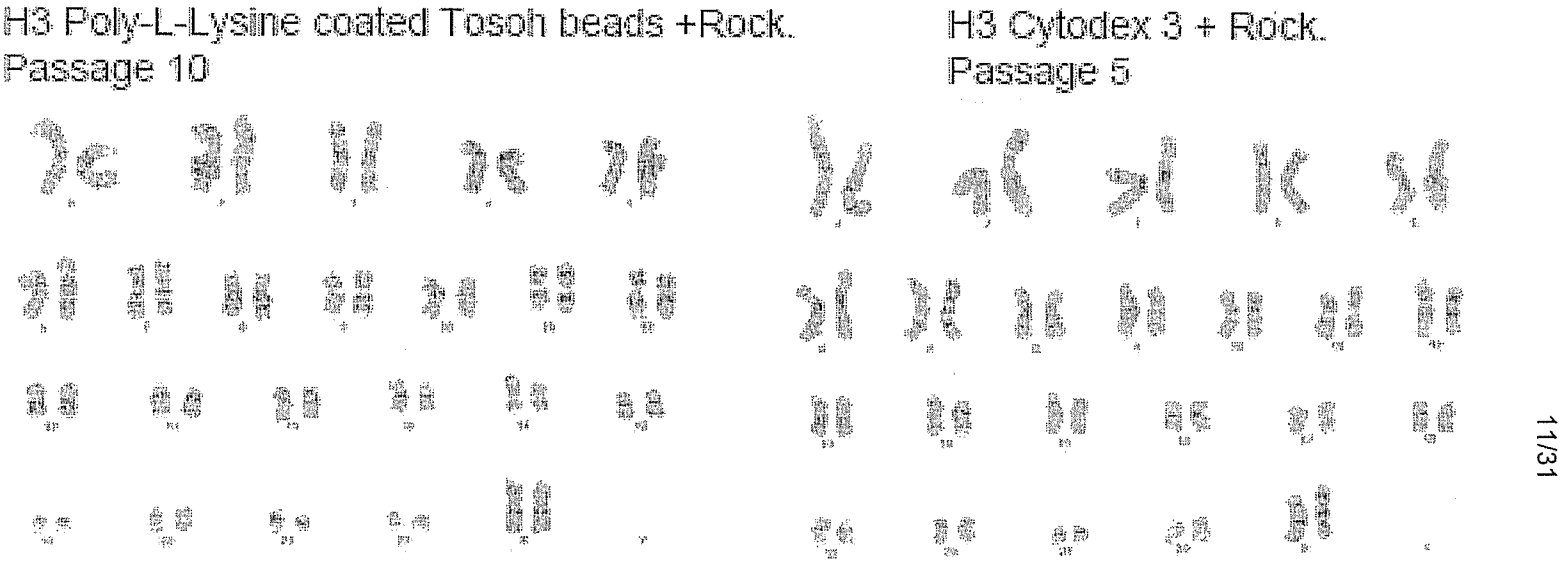

H3 Poly-L-Lysine coated Tosoh beads +Rock.
Passage 10
H3 Cytodex 3 + Rock.
Passage 5

Figure 11

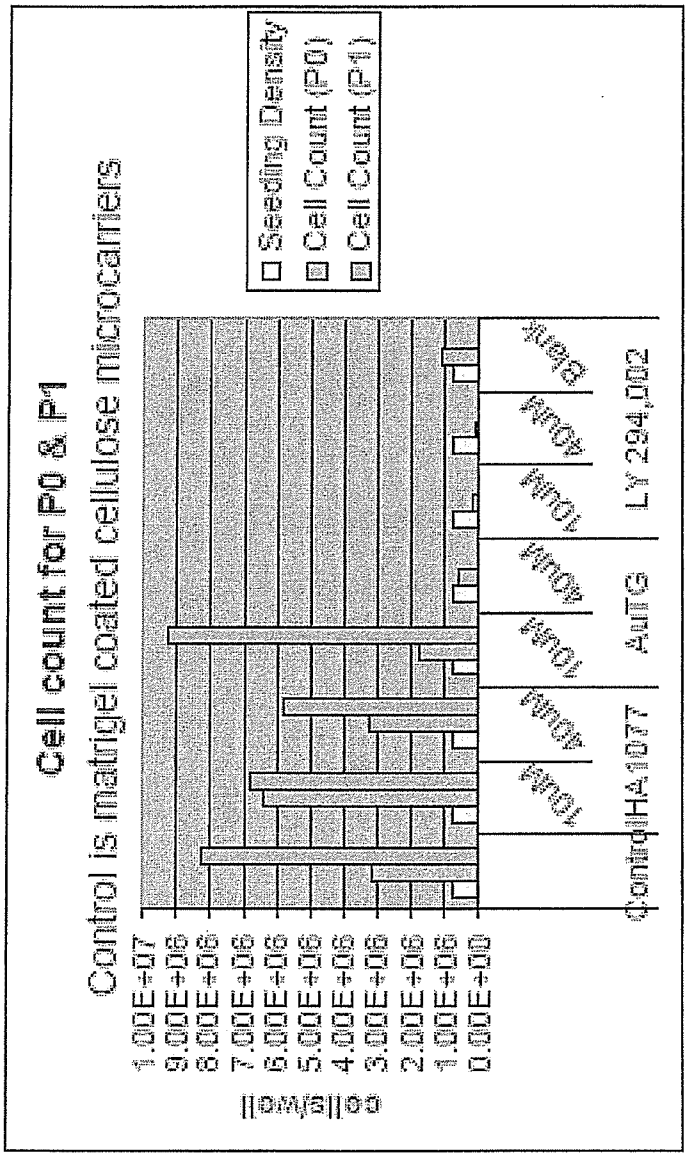

Cell count for P0 & P1
Control is matrigel coated cellulose microcarriers
Seeding Density
Cell Count (P0)
Cell Count (P1)
cells/well
1.00E+07
9.00E+06
8.00E+06
7.00E+06
6.00E+06
5.00E+06
4.00E+06
3.00E+06
2.00E+06
1.00E+06
0.00E+00
Control
HA 1077
AuTG
LY 294,002

Figure 12

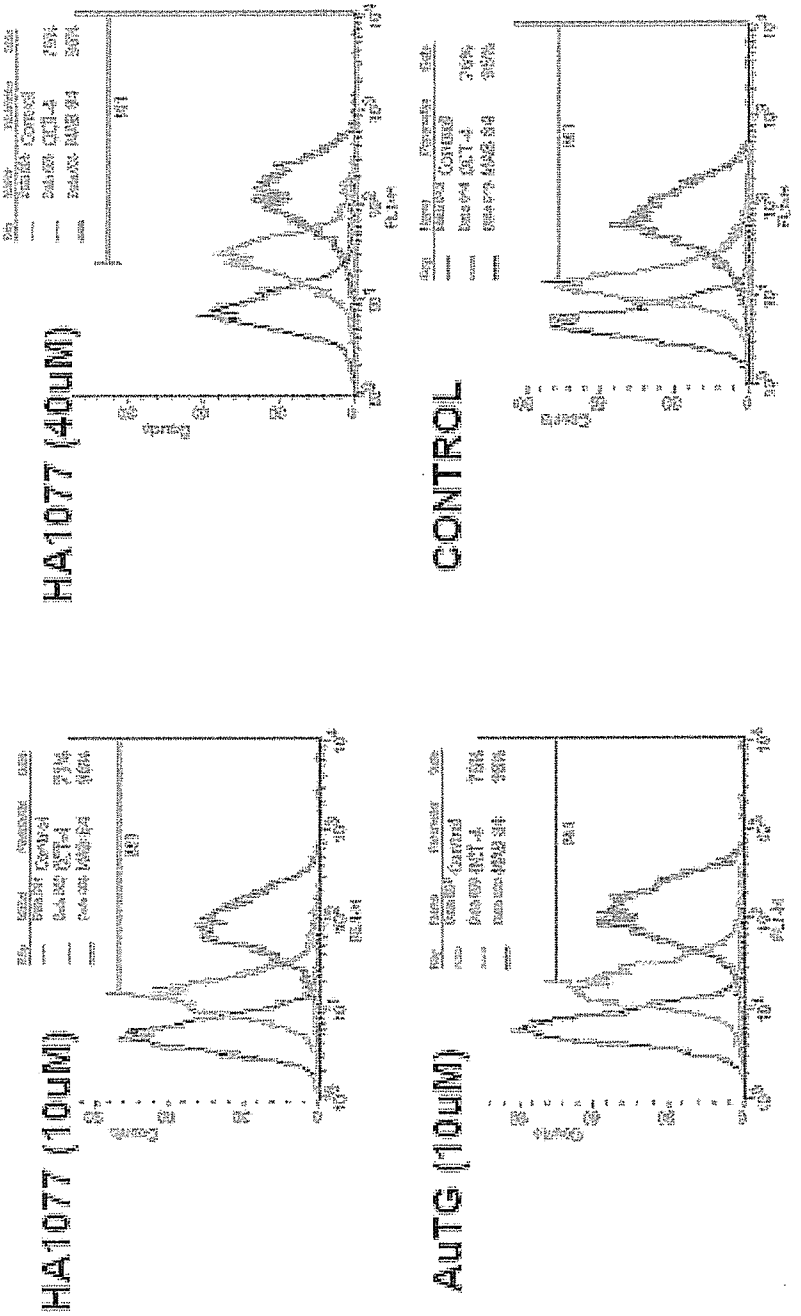


Figure 13

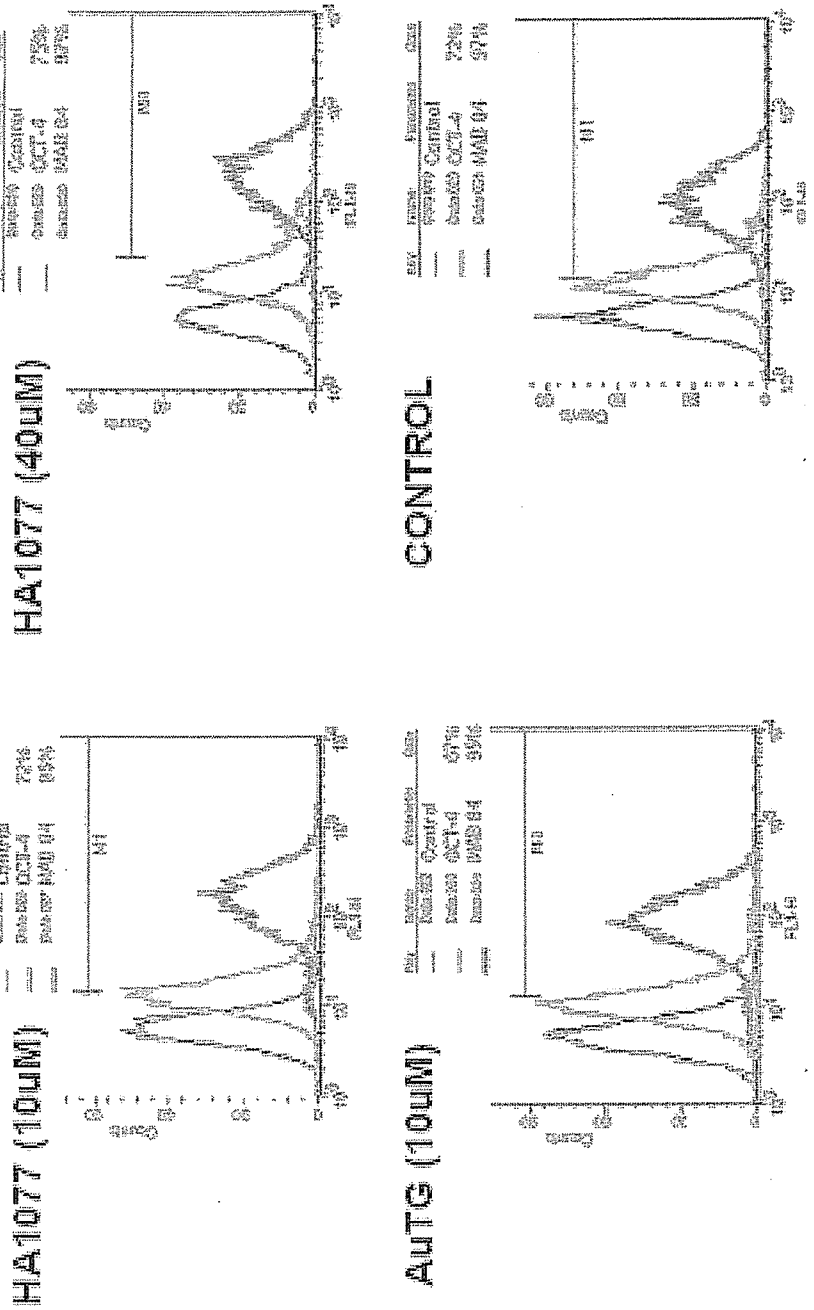


Figure 14

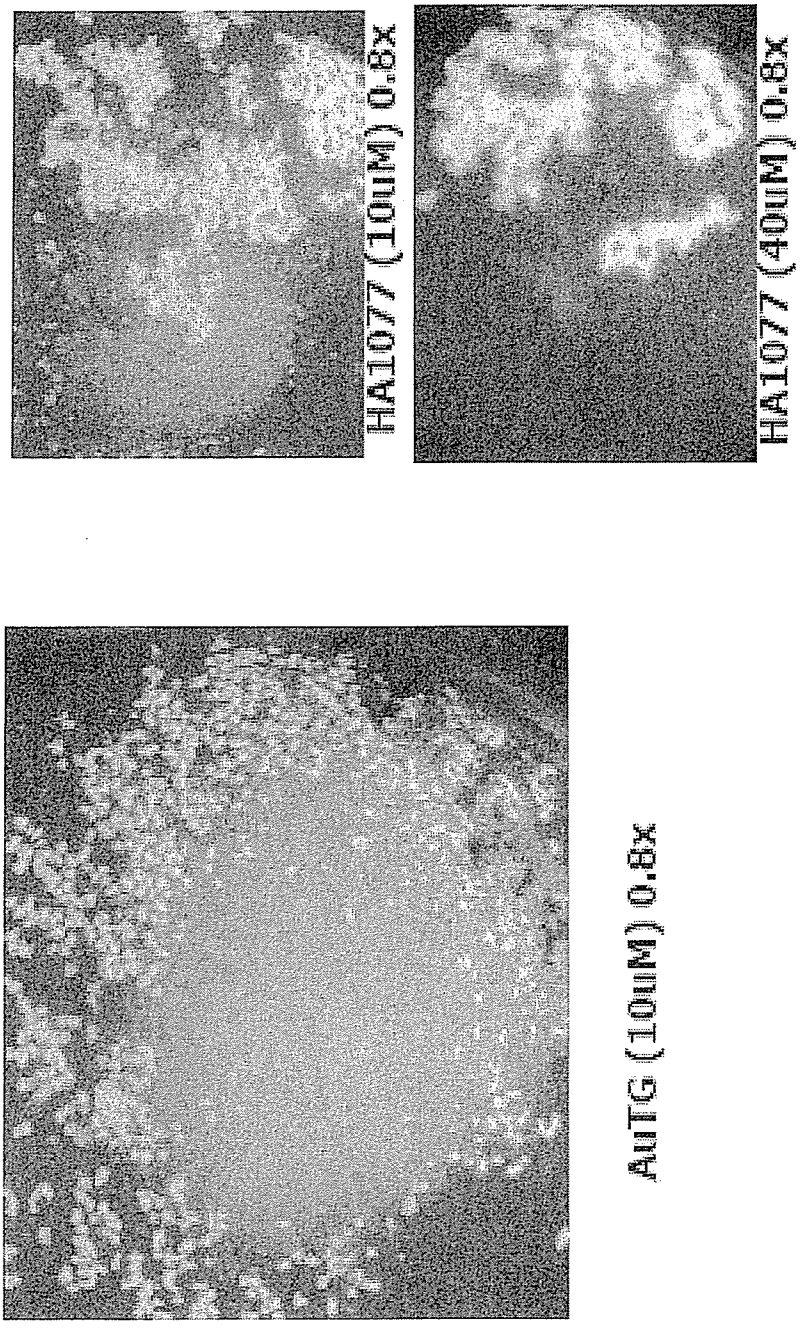


Figure 15

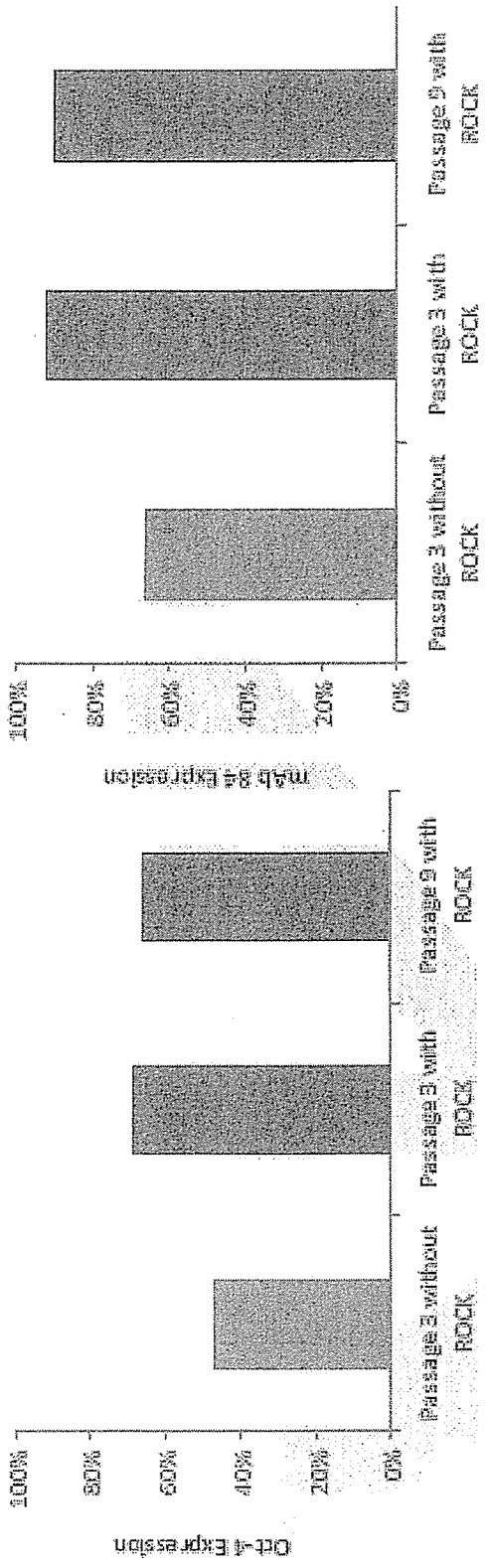

Oct-4 Expression
100%
80%
60%
40%
20%
0%
Passage 3 without ROCK
Passage 3 with ROCK
Passage 9 with ROCK
mSb 84 Expression
100%
80%
60%
40%
20%
0%
Passage 3 without ROCK
Passage 3 with ROCK
Passage 9 with ROCK

Figure 16

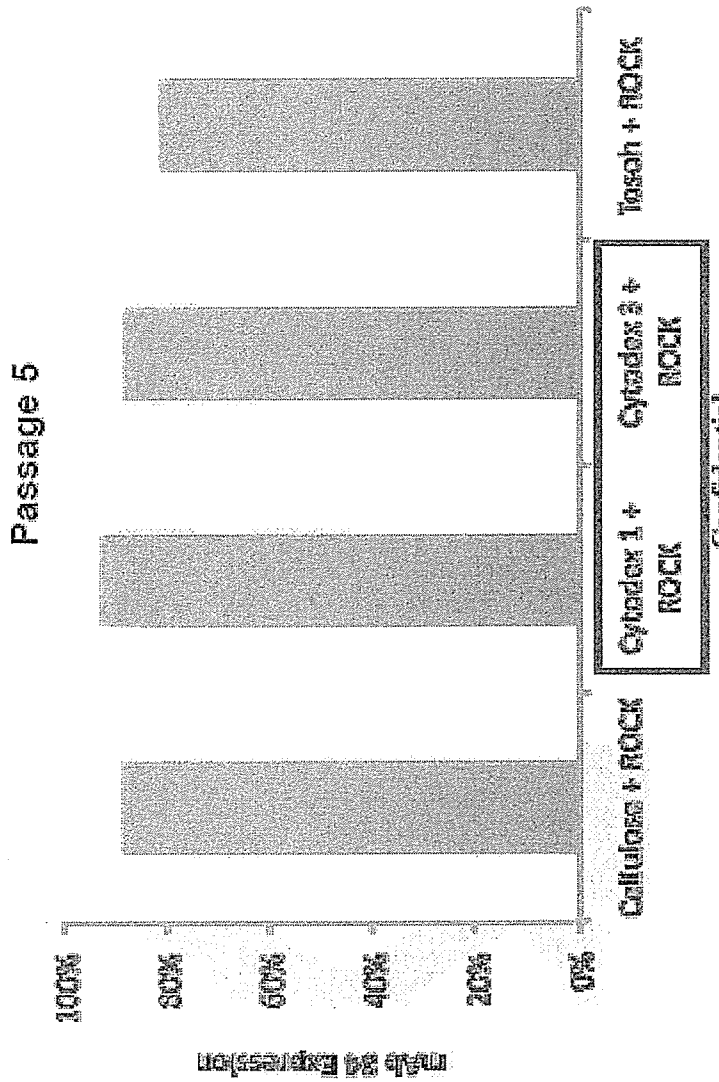

Passage 5
100%
80%
60%
40%
20%
0%
Cellulose + ROCK
Cytodex 1 + ROCK
Cytodex 3 + ROCK
Tasin + ROCK
Confidential

Figure 17

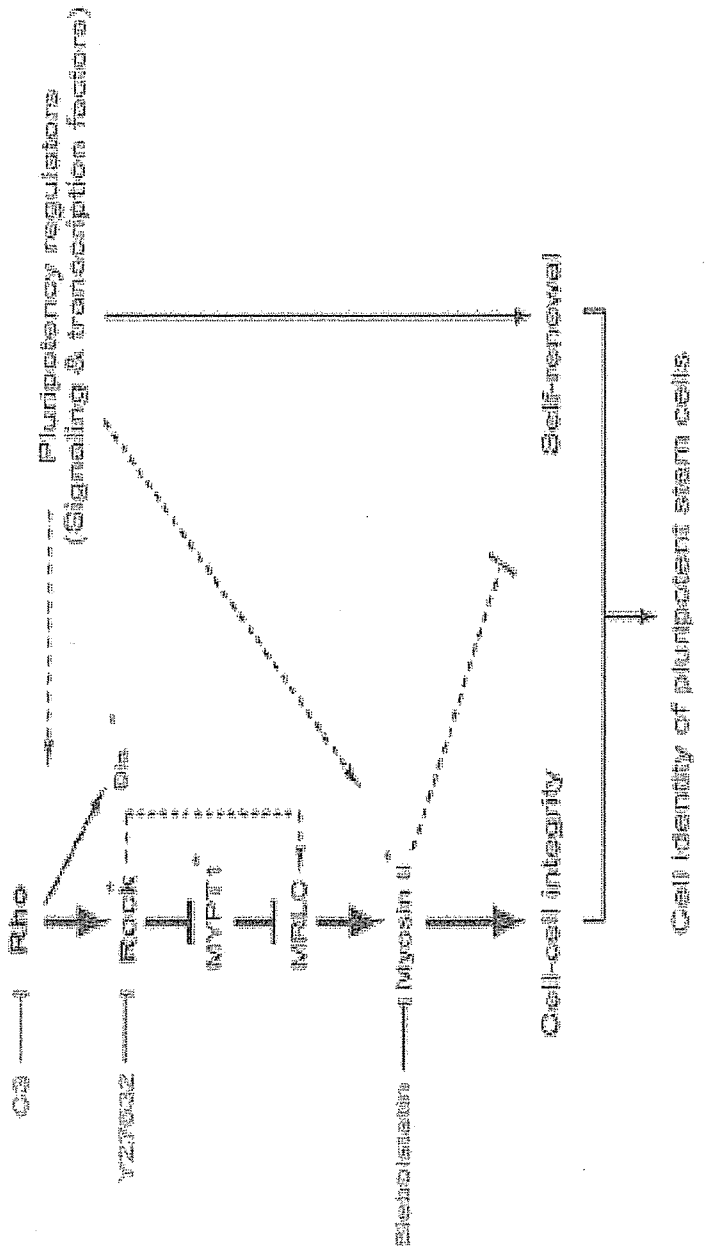

C3
Rho
Y27632
Rock
MYPT1
MRLC
Blebbistatin
Myosin II
Cell-cell integrity
Pluripotency regulators
(Signaling & transcription factors)
Self-renewal
Cell identity of pluripotent stem cells

Figure 18

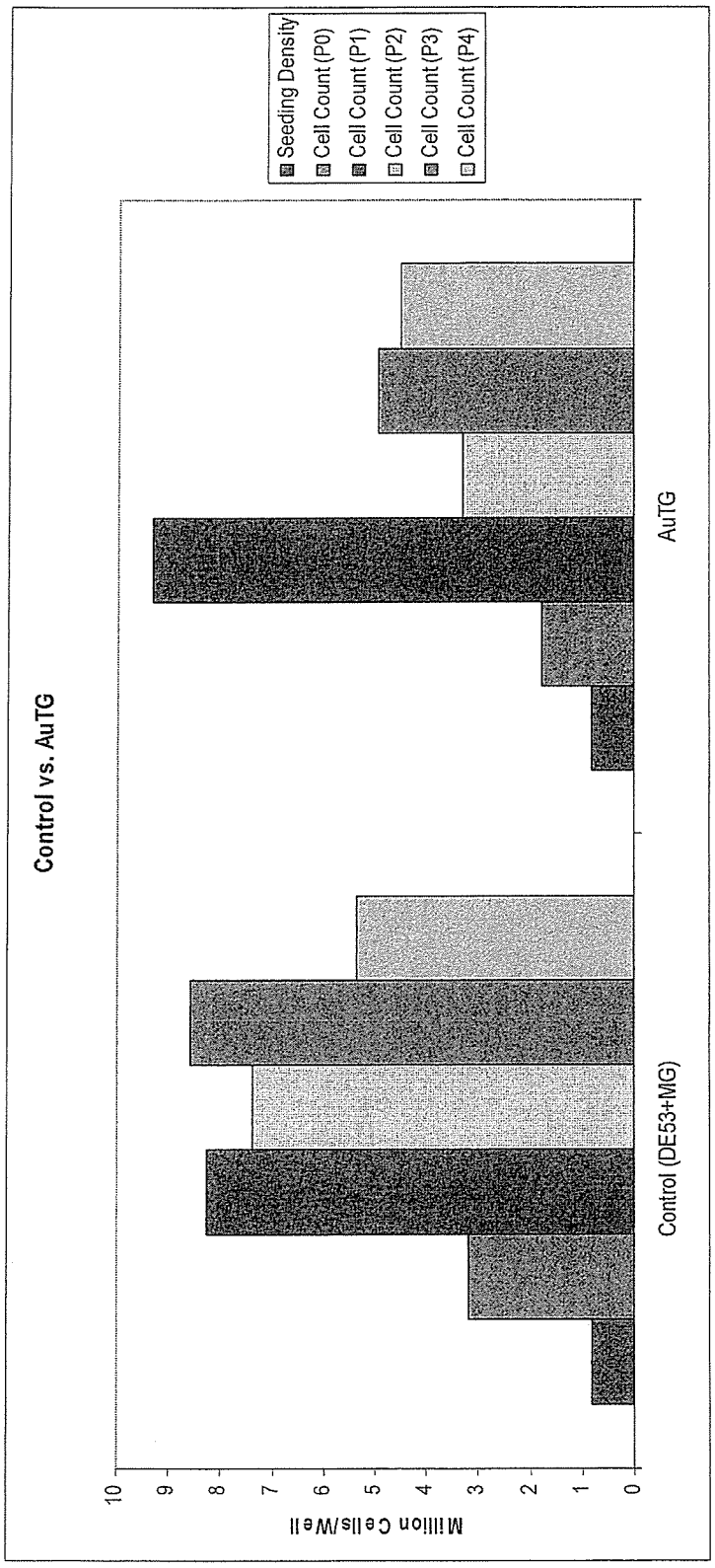
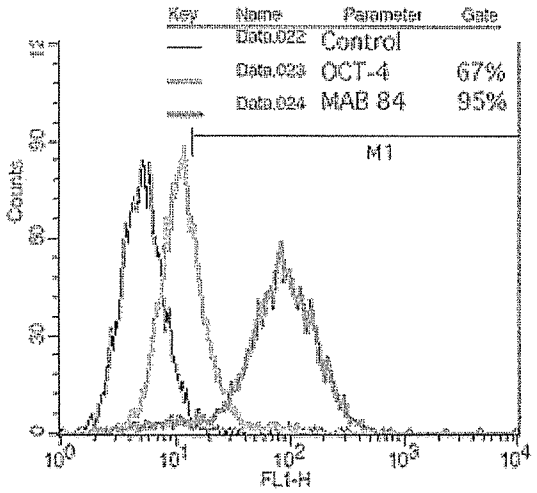
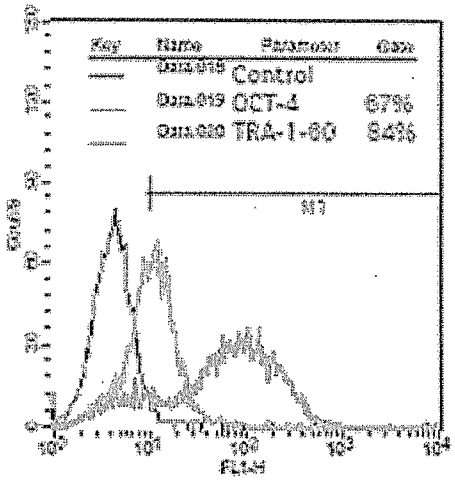

Control vs. AuTG
Seeding Density
Cell Count (P0)
Cell Count (P1)
Cell Count (P2)
Cell Count (P3)
Cell Count (P4)
Million Cells/Well
0
1
2
3
4
5
6
7
8
9
10
Control (DE53+MG)
AuTG

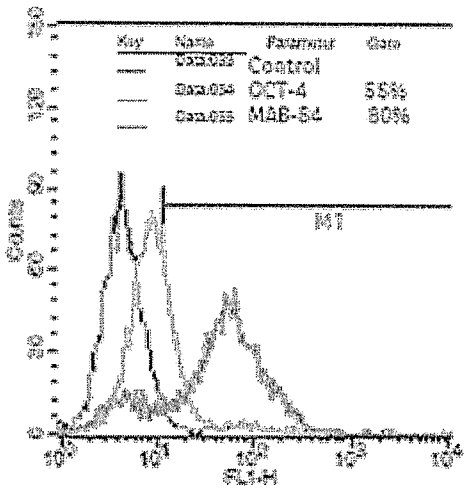
Figure 19



P1 (19 Feb 09)



P3 (5 Mar 09)



P4 (13 Mar 09)

Figure 20

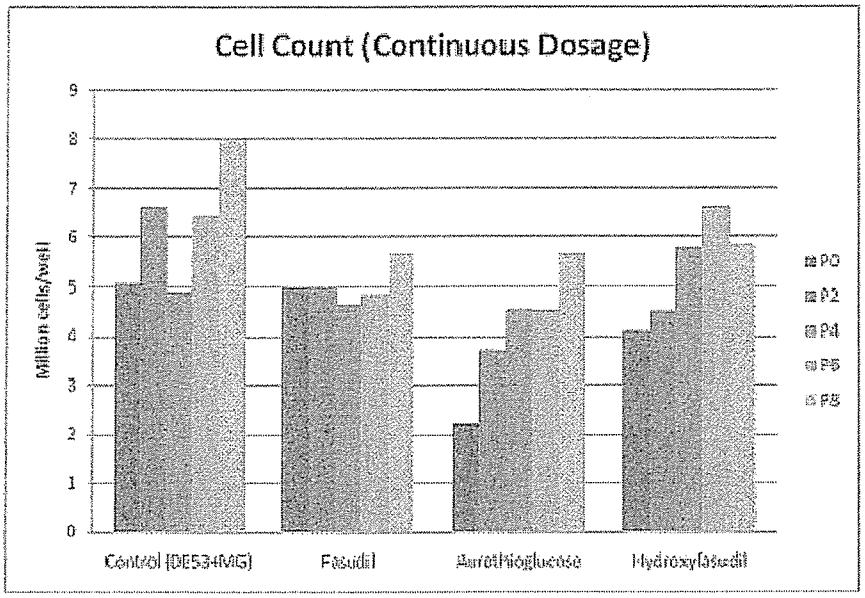


Figure 21A

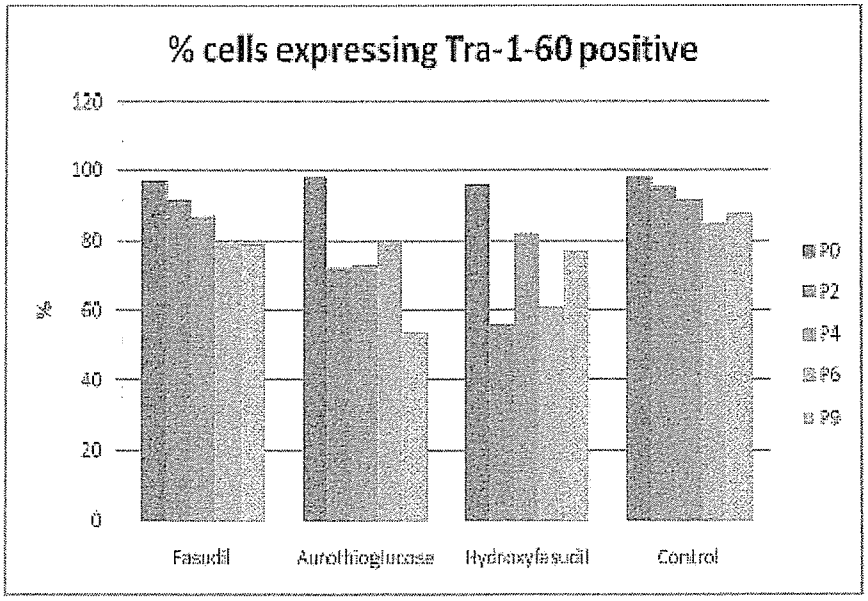


Figure 21B

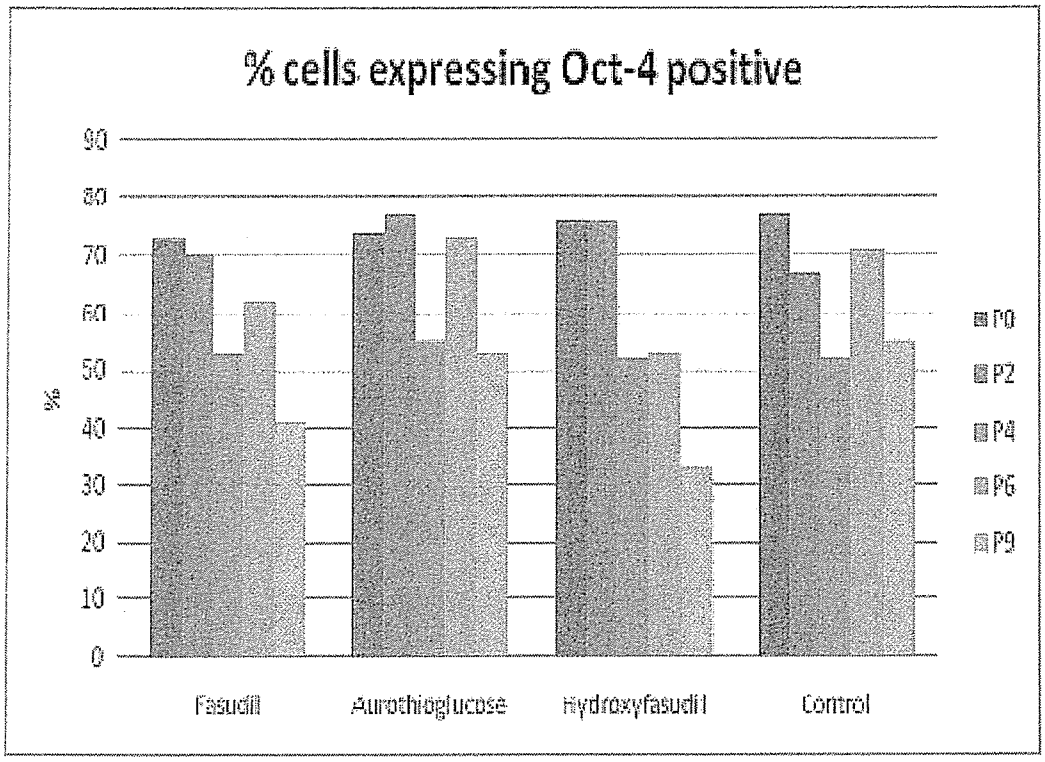

% cells expressing Oct-4 positive
90
80
70
60
50
40
30
20
10
0
%
Fasudil
Aurothioglucose
Hydroxyfasudil
Control
P0
P2
P4
P6
P9

Figure 21C

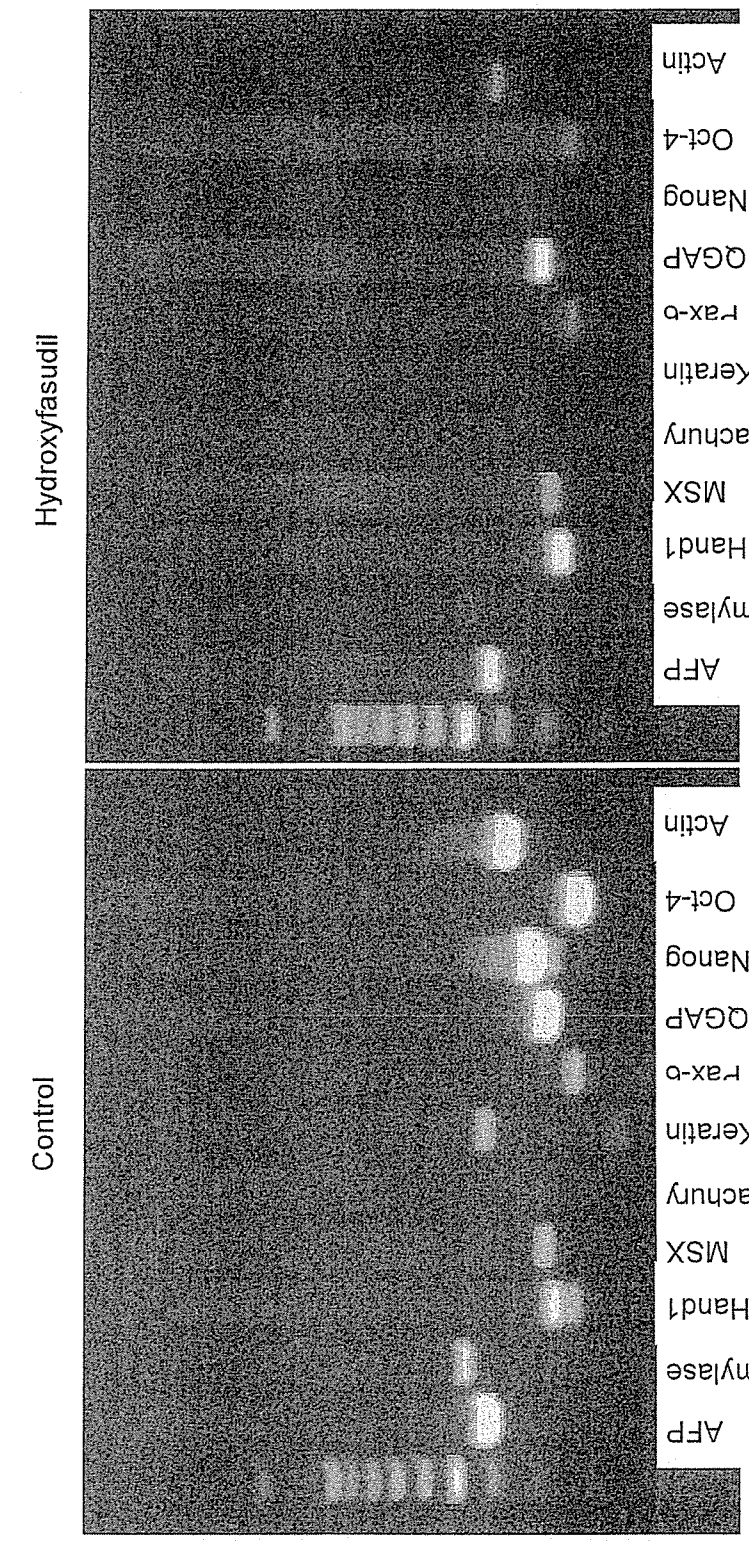

Hydroxyfasudil
Control
AFP
Amylase
Hand1
MSX
Brachury
Keratin
Rax-b
IQGAP
Nanog
Oct-4
Actin
AFP
Amylase
Hand1
MSX
Brachury
Keratin
Rax-b
IQGAP
Nanog
Oct-4
Actin
After EB Formation

Figure 22

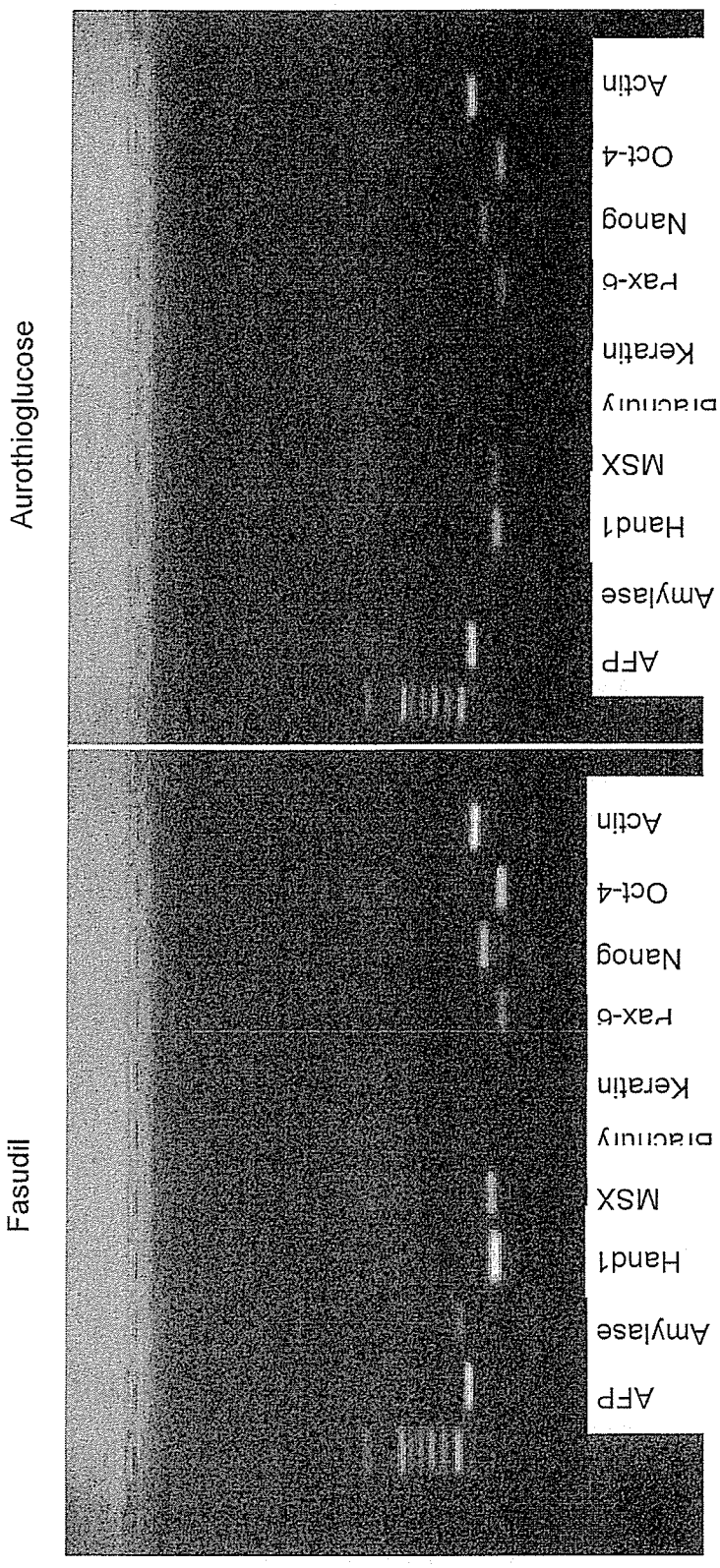

Fasudil
Aurothioglucose
AFP
Amylase
Hand1
MSX
Brachury
Keratin
Pax-6
Nanog
Oct-4
Actin
AFP
Amylase
Hand1
MSX
Brachury
Keratin
Pax-6
Nanog
Oct-4
Actin
After EB Formation

Figure 23

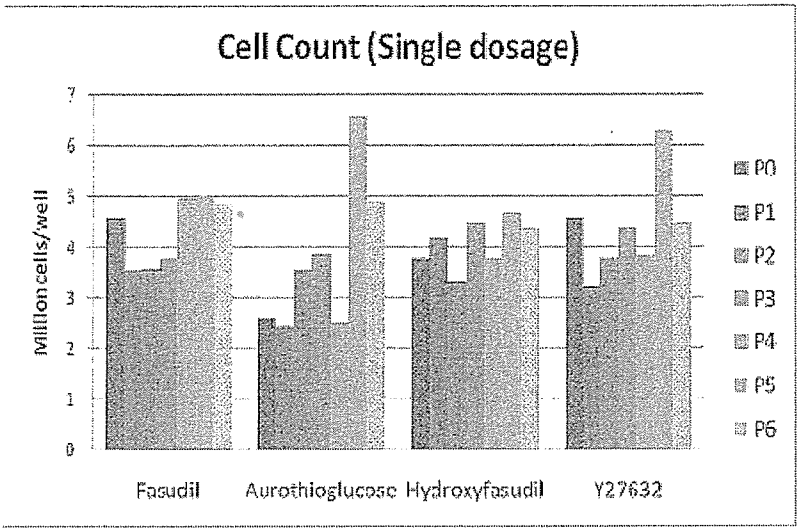


Figure 24A

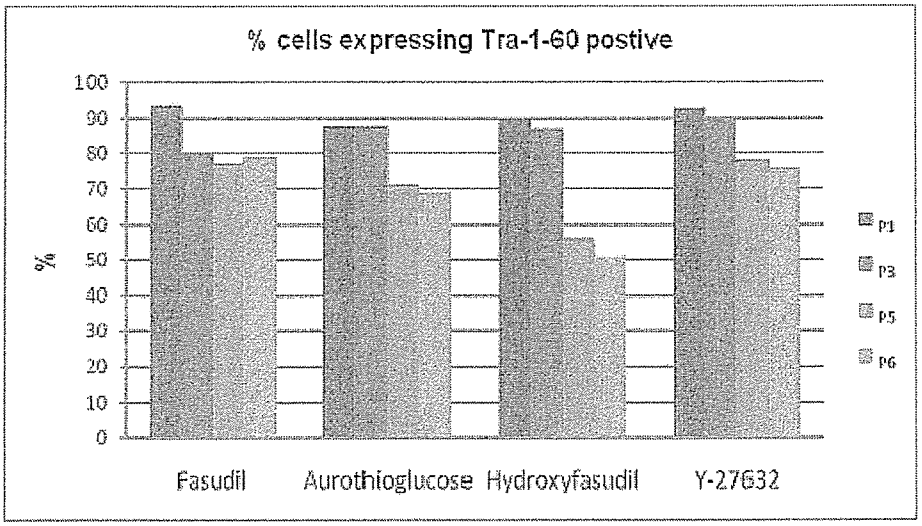


Figure 24B

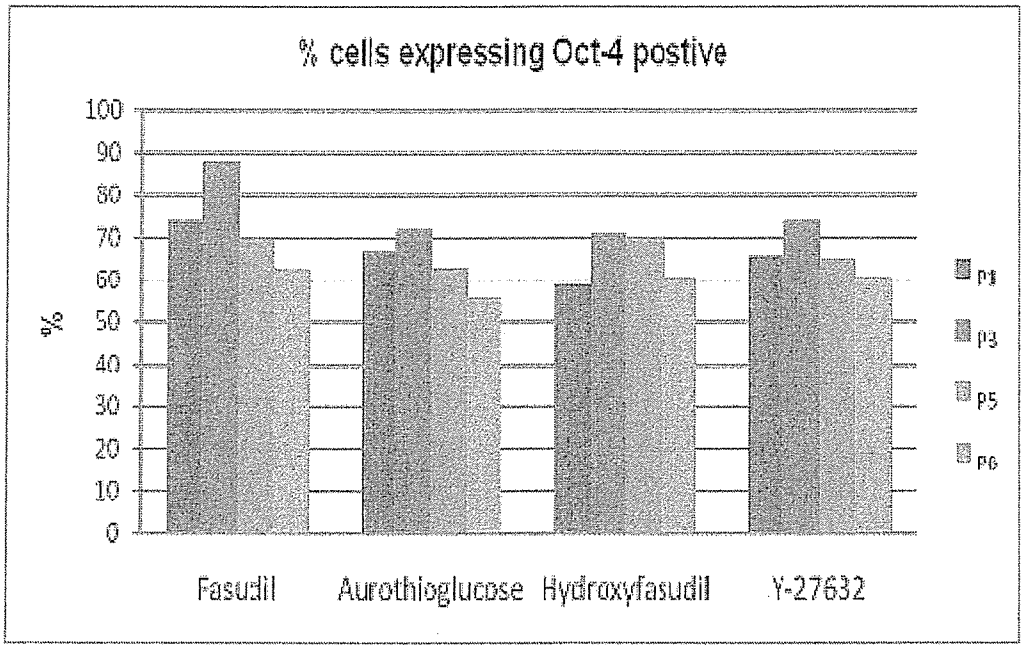

% cells expressing Oct-4 postive
100
90
80
70
60
50
40
30
20
10
0
%
Fasudil
Aurothioglucose
Hydroxyfasudil
Y-27632
p3
p5
p6

Figure 25

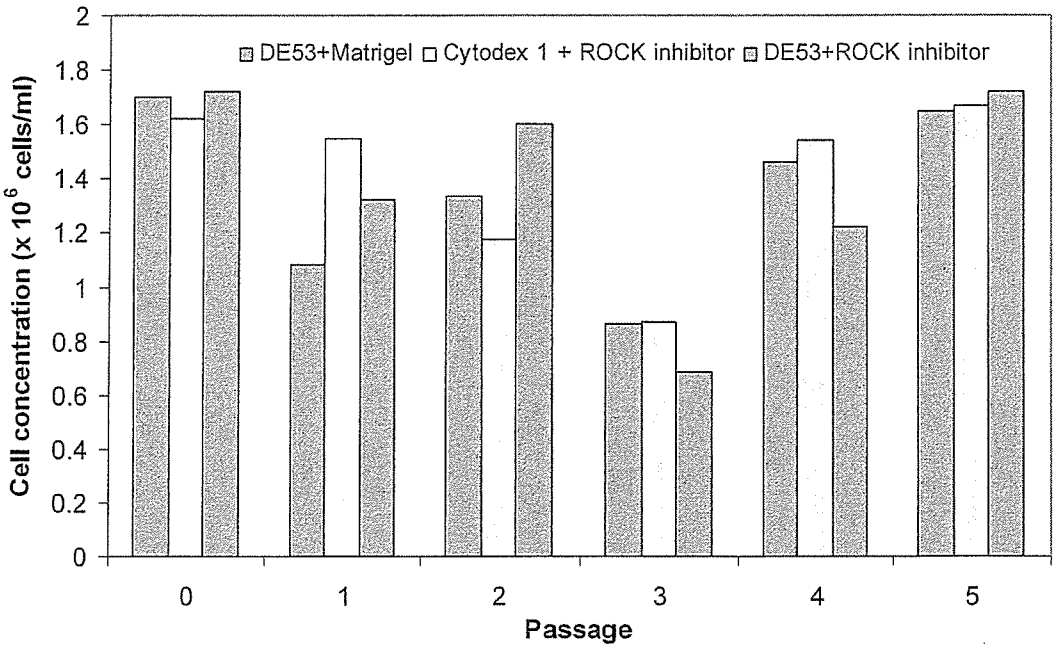
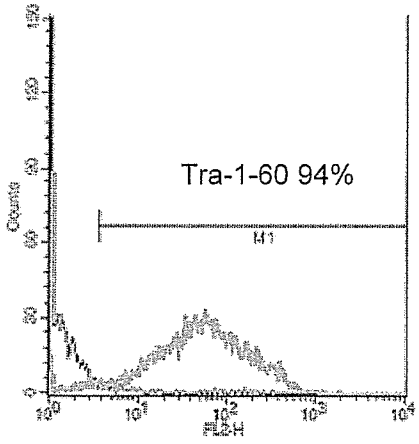
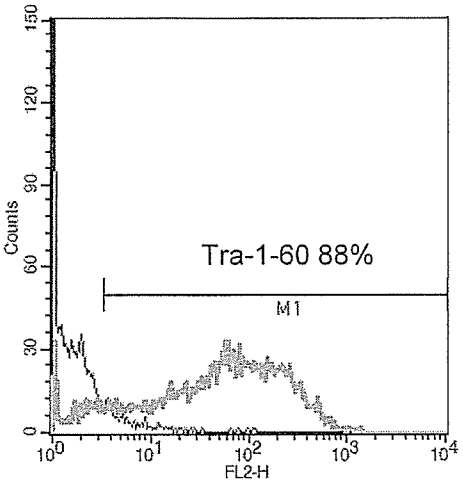

DE53+Matrigel
Cytodex 1 + ROCK inhibitor
DE53+ROCK inhibitor
Cell concentration (x 10^6 cells/ml)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
1
2
3
4
5
Passage

Figure 26



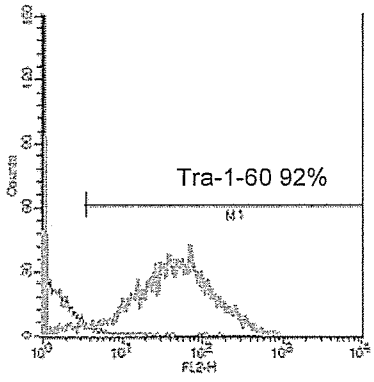
Cytodex 1 + ROCK P5

Figure 27A



DE53+Matrigel P5

Figure 27B



DE53+ROCK P5

Figure 27C

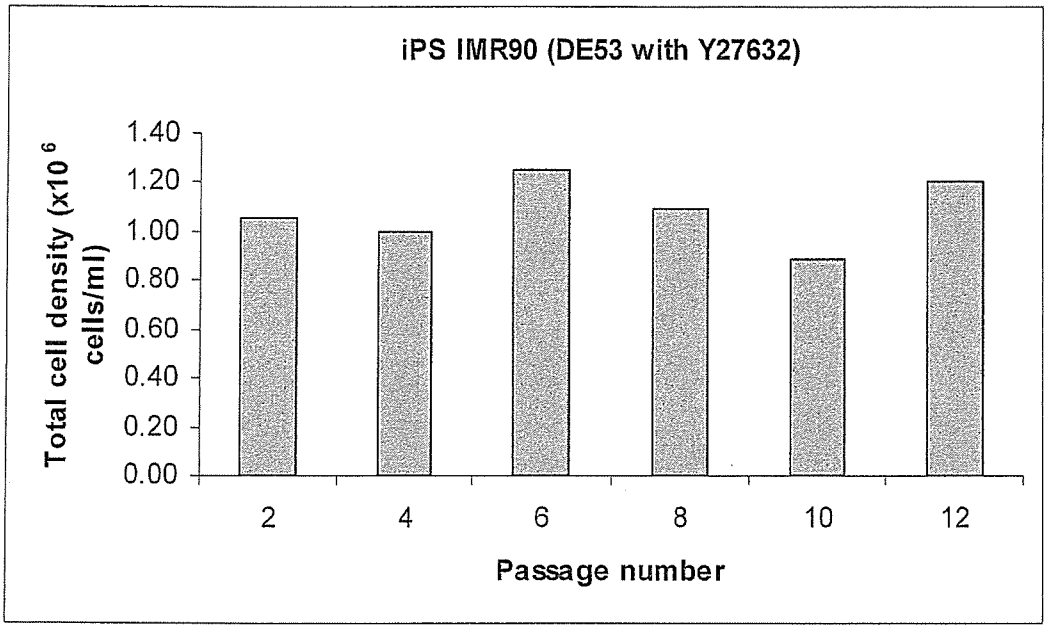

iPS IMR90 (DE53 with Y27632)
Total cell density (x10 6 cells/ml)
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
2
4
6
8
10
12
Passage number

Figure 28

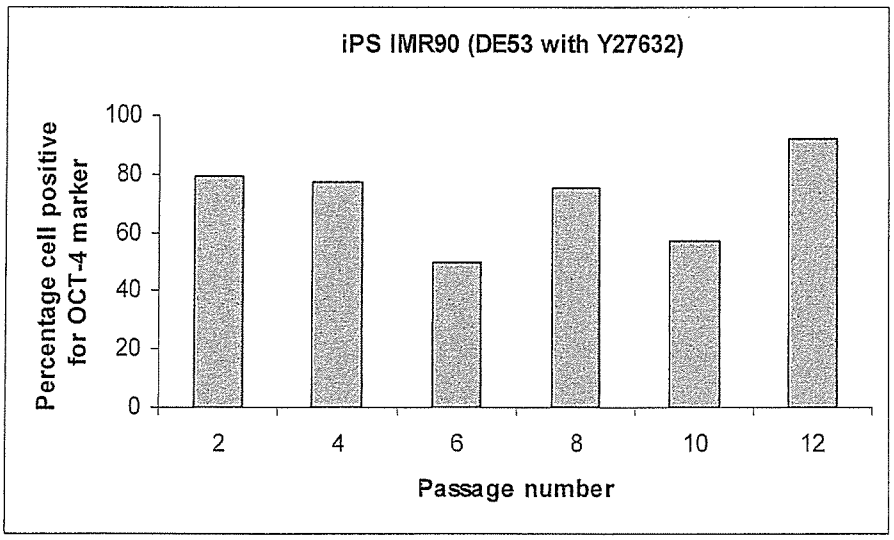


Figure 29A

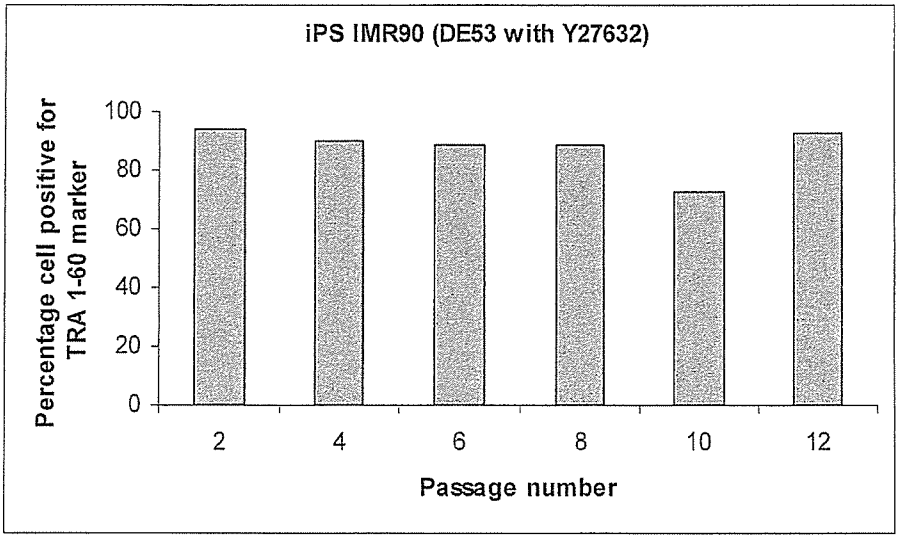
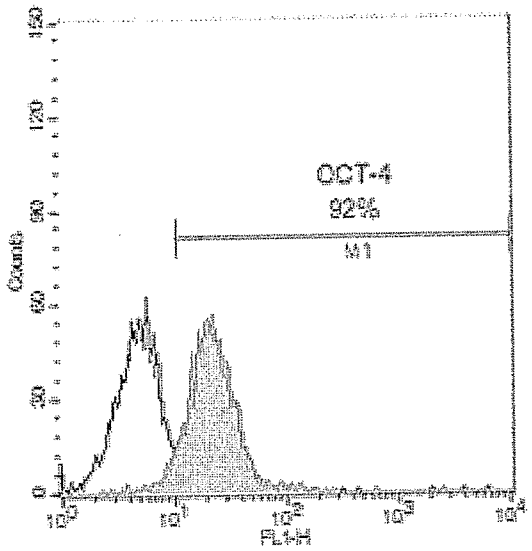


Figure 29B



P12

Figure 30A

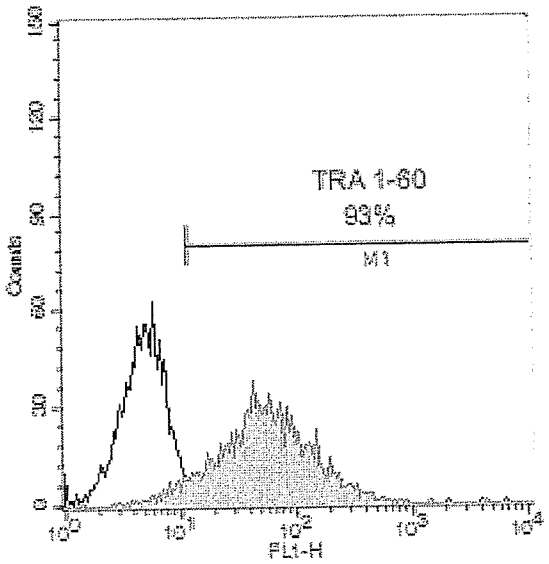


Figure 30B