Fig. 1

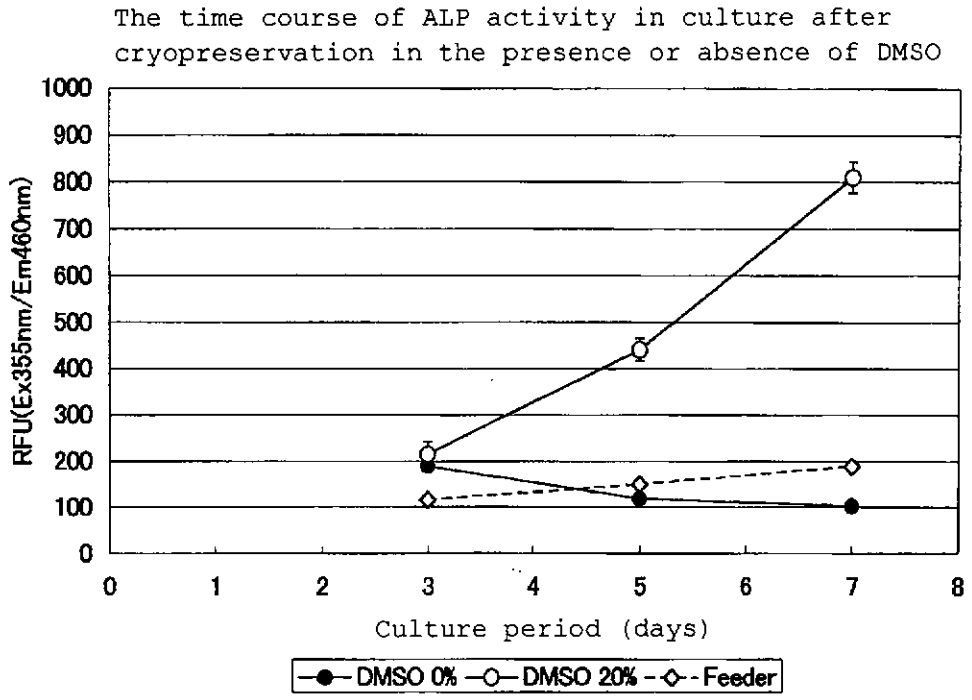

The time course of ALP activity in culture after cryopreservation in the presence or absence of DMSO
RFU(Ex355nm/Em460nm)
1000
900
800
700
600
500
400
300
200
100
0
0
1
2
3
4
5
6
7
8
Culture period (days)
DMSO 0%
DMSO 20%
Feeder

Fig. 2

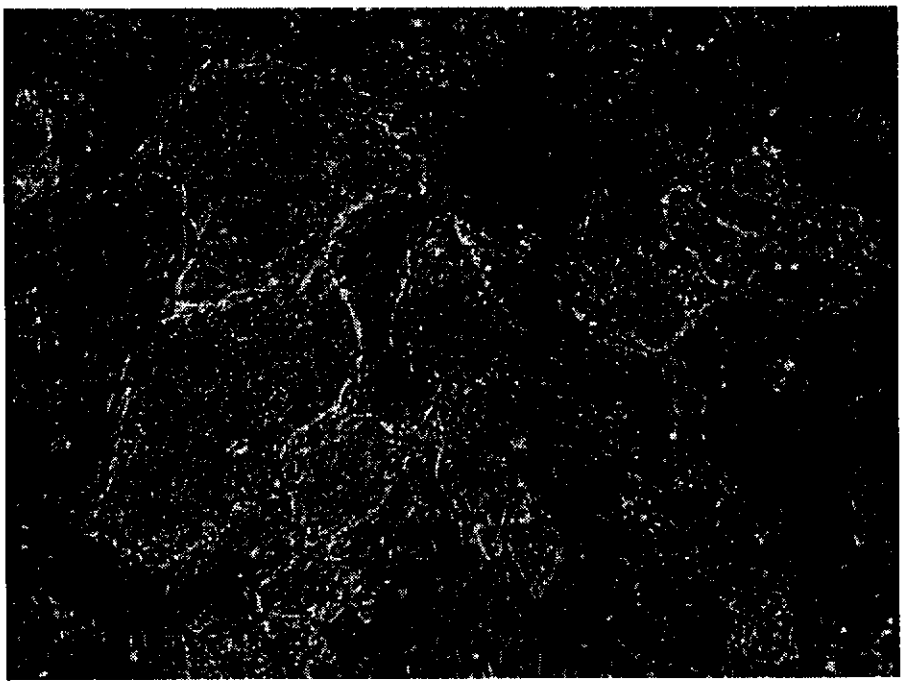


Fig.3

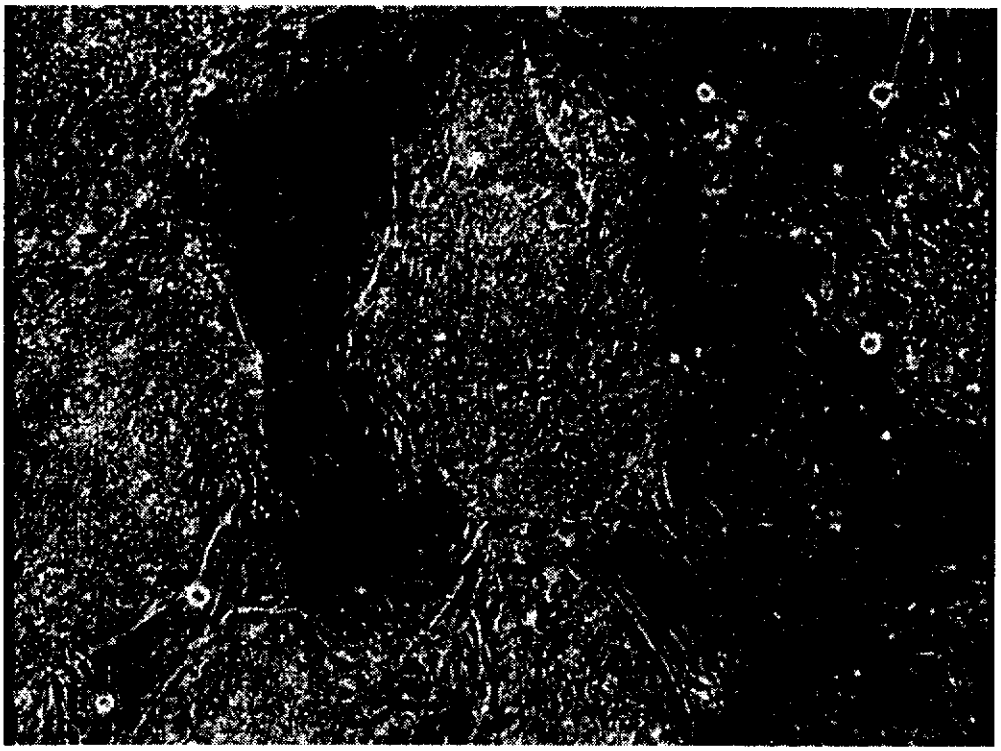


Fig.4

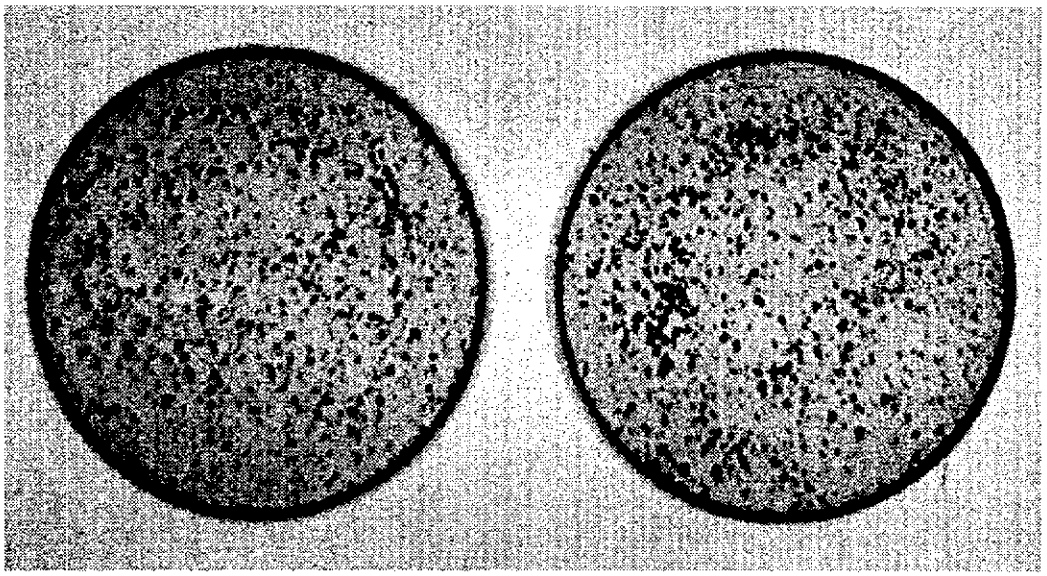


Fig.5

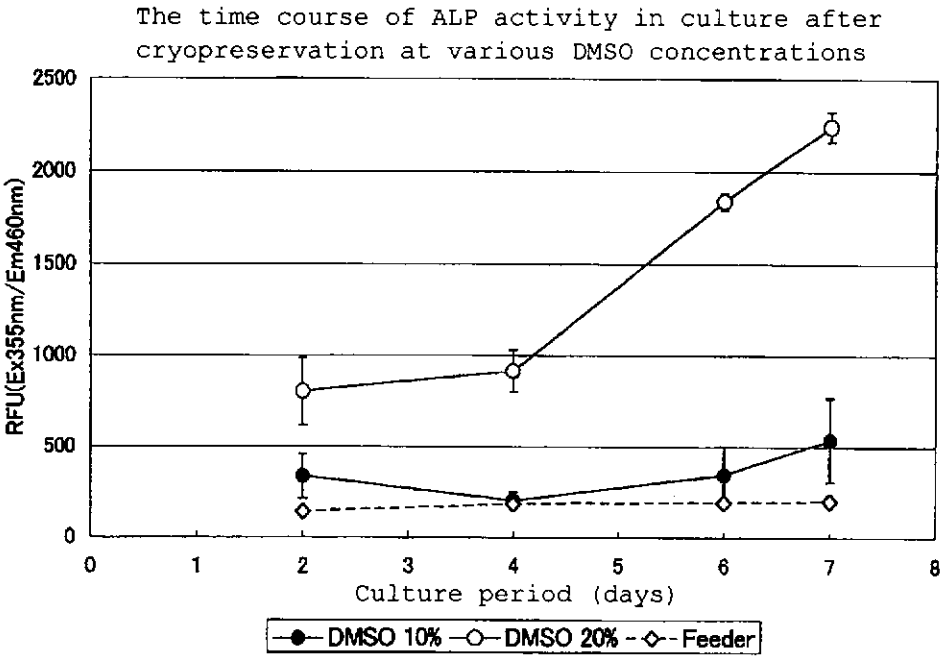

The time course of ALP activity in culture after cryopreservation at various DMSO concentrations
RFU(Ex355nm/Em460nm)
2500
2000
1500
1000
500
0
0
1
2
3
4
5
6
7
8
Culture period (days)
DMSO 10%
DMSO 20%
Feeder

Fig.6

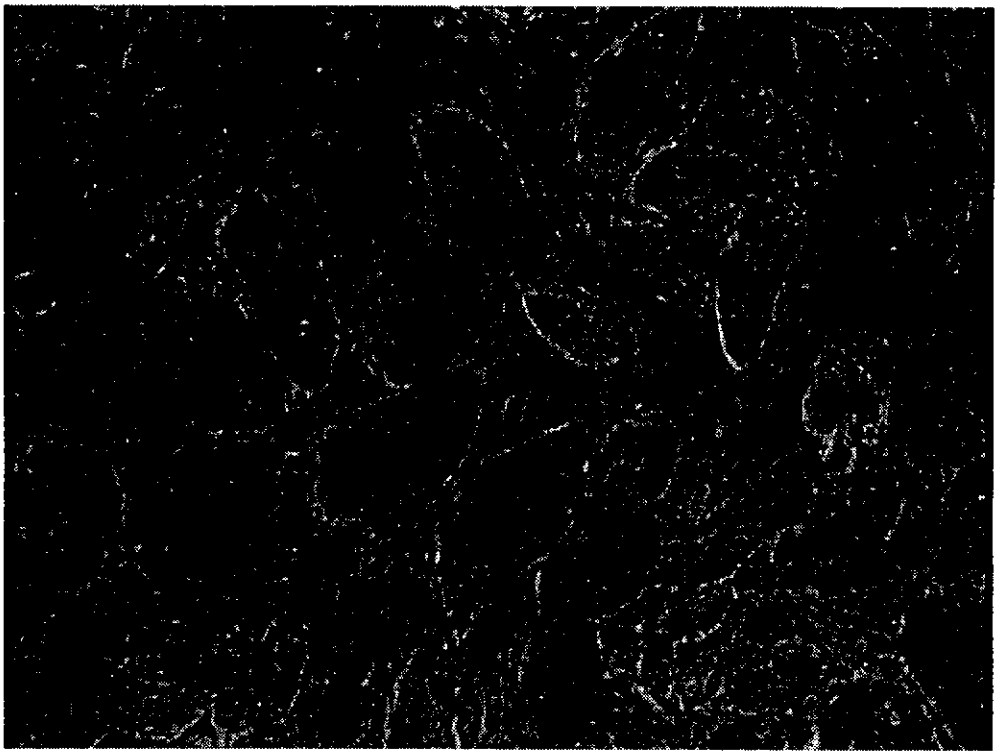


Fig.7

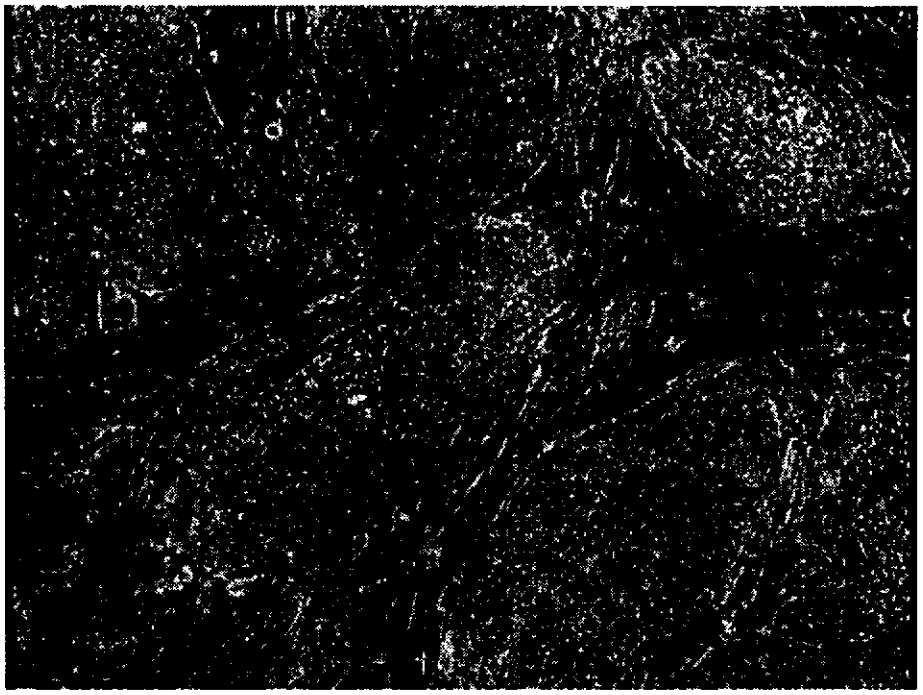


Fig.8

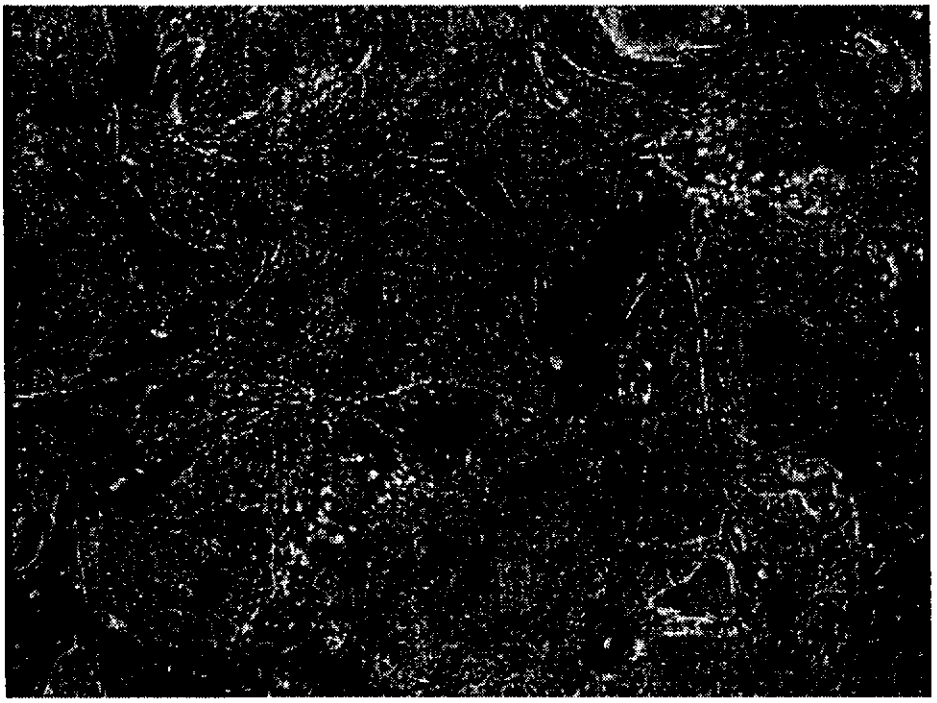


Fig. 9

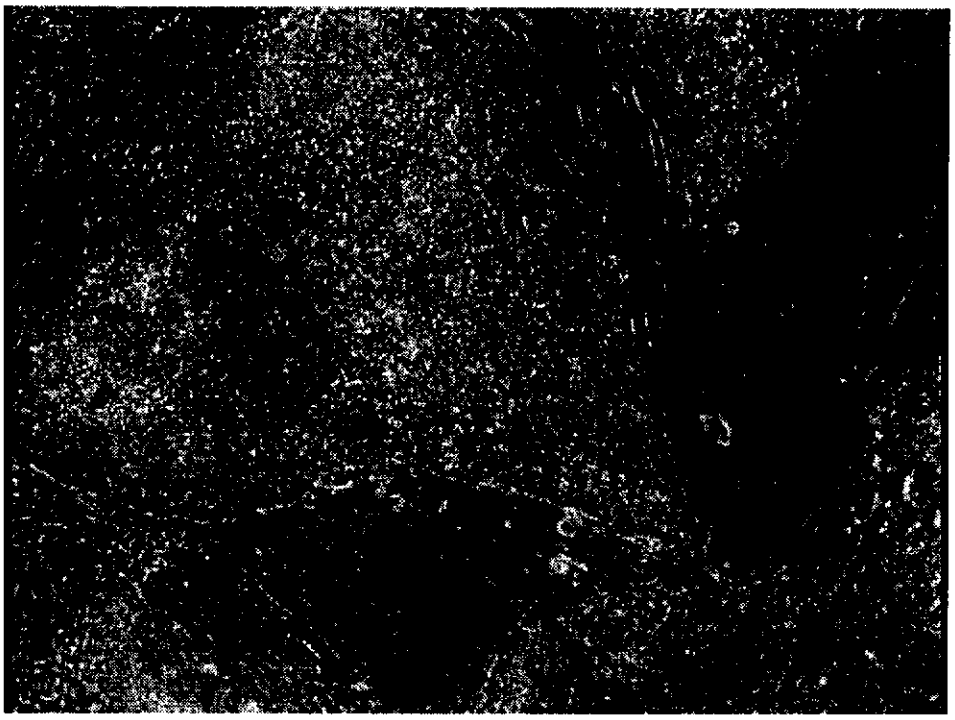


Fig. 10

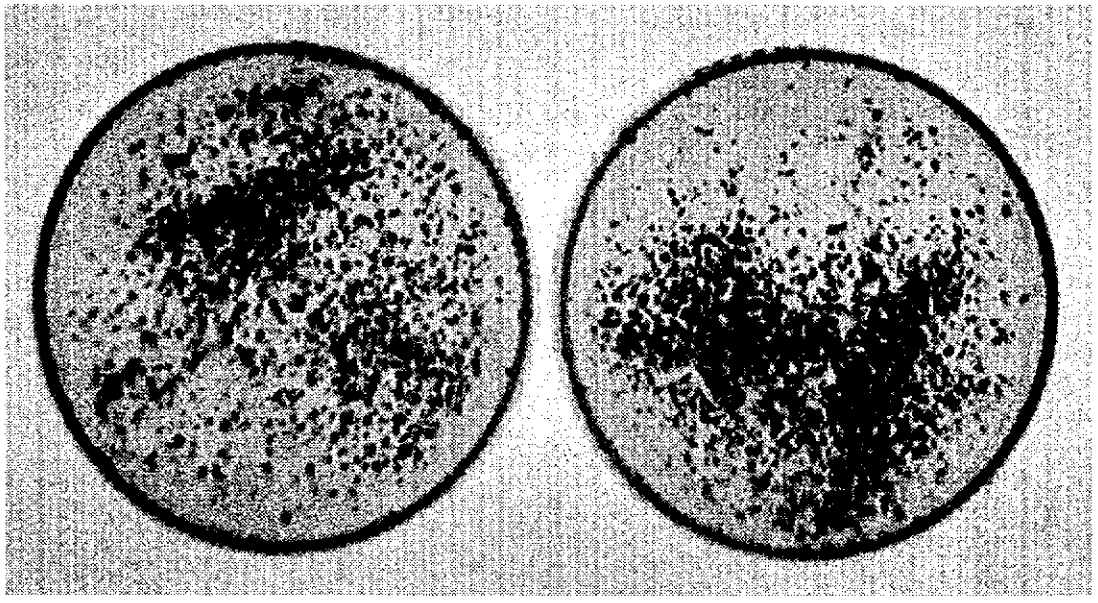


Fig. 11

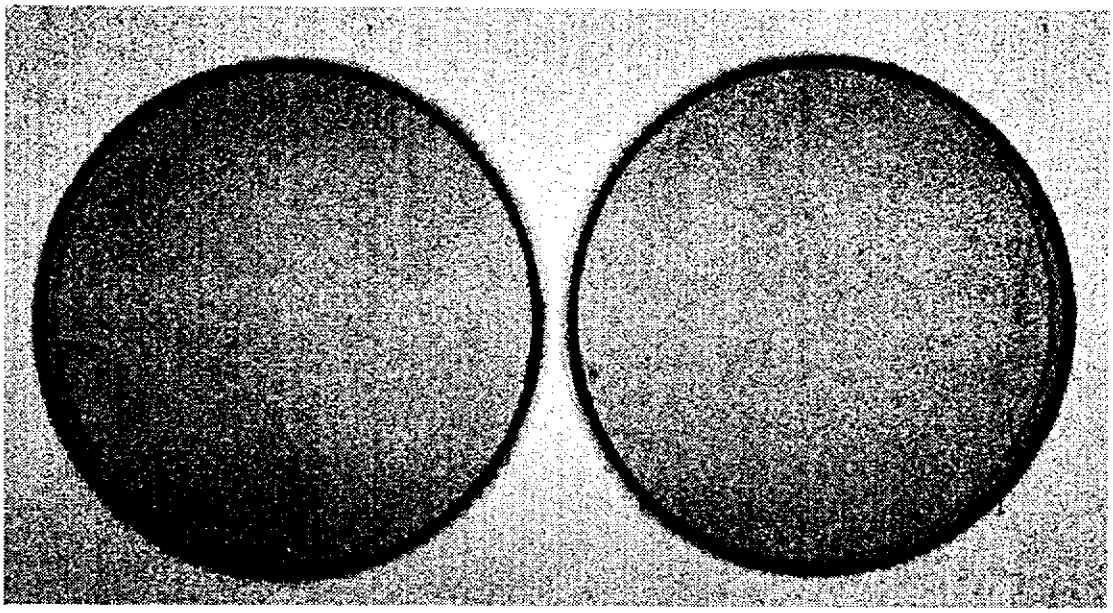


Fig. 12

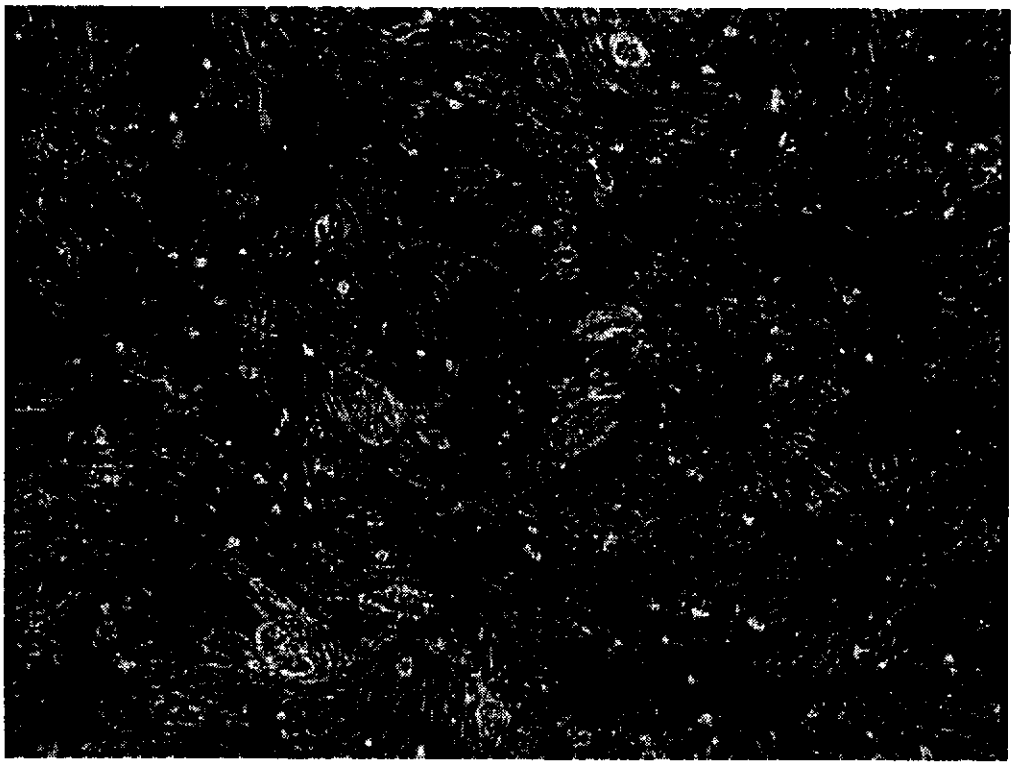


Fig. 13

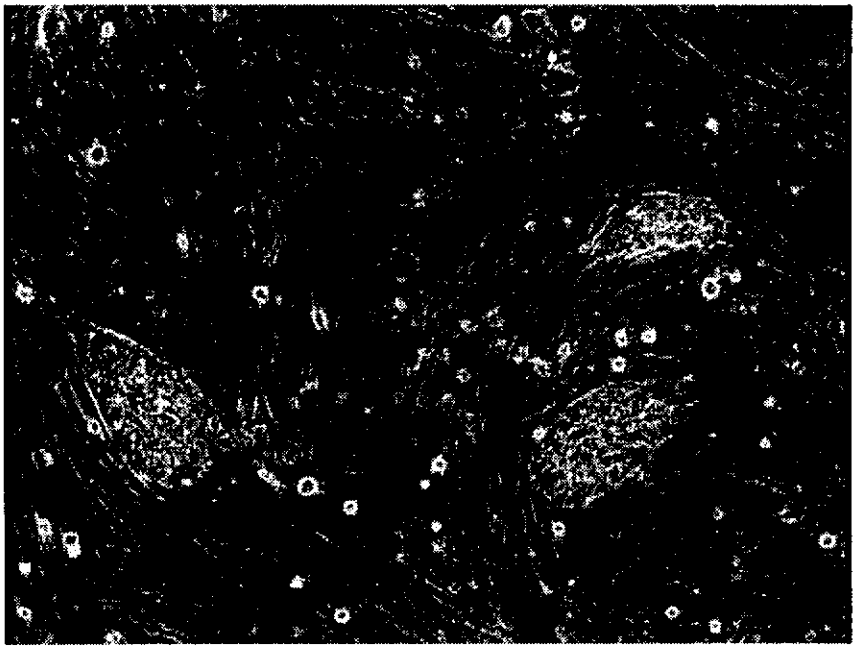


Fig. 14

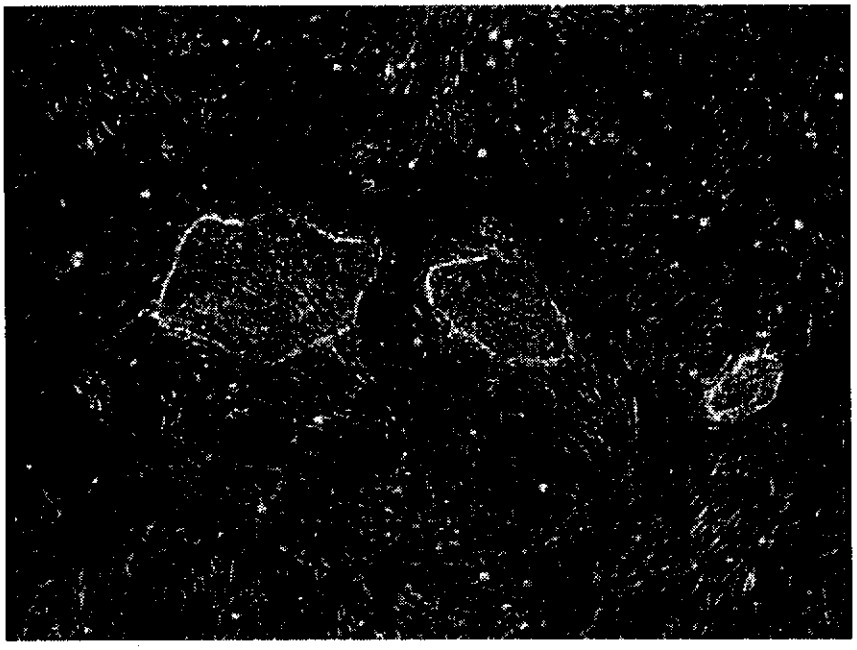


Fig. 15

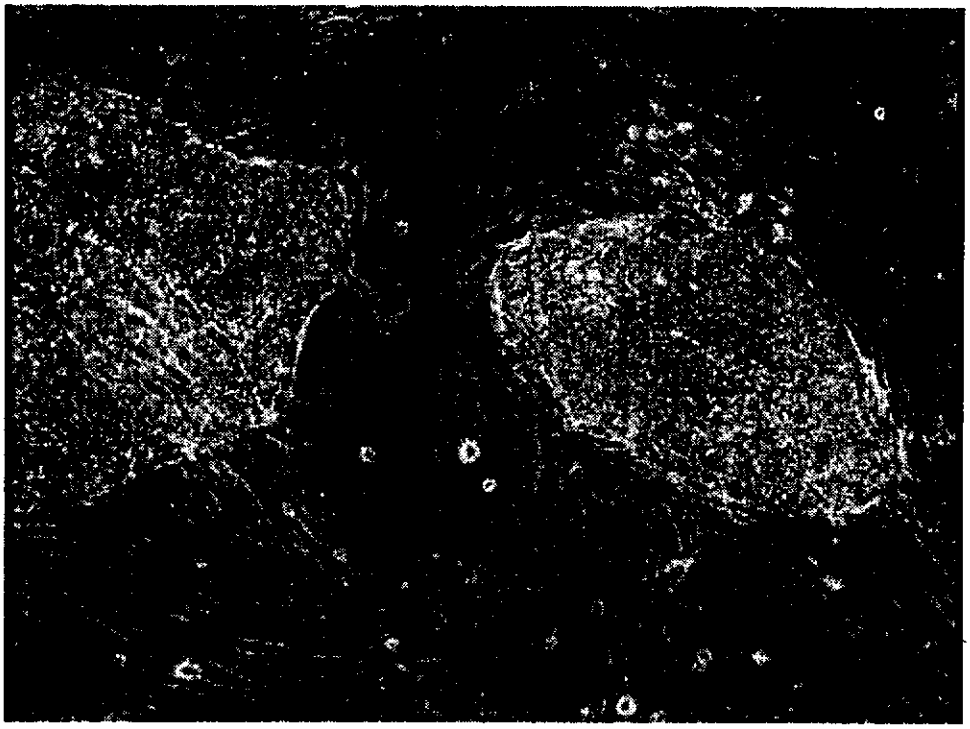


Fig. 16

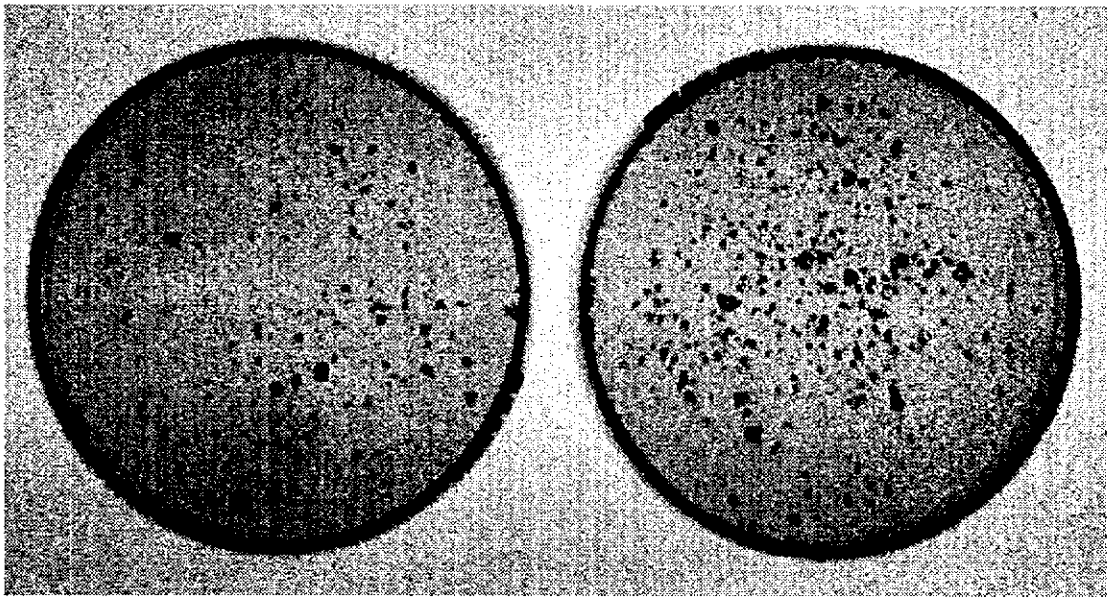


Fig. 17

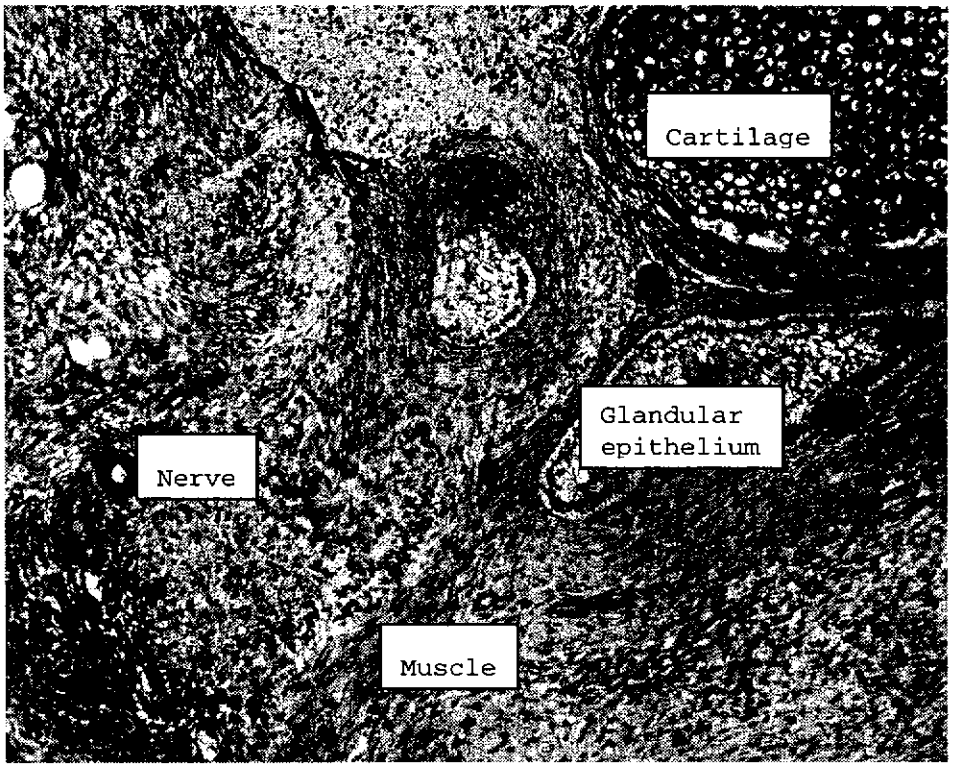
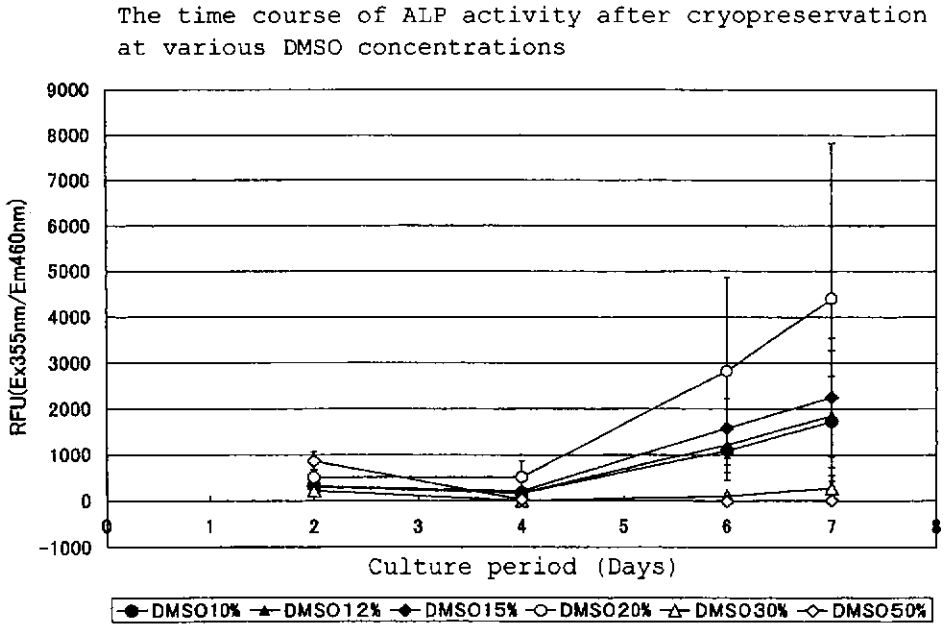
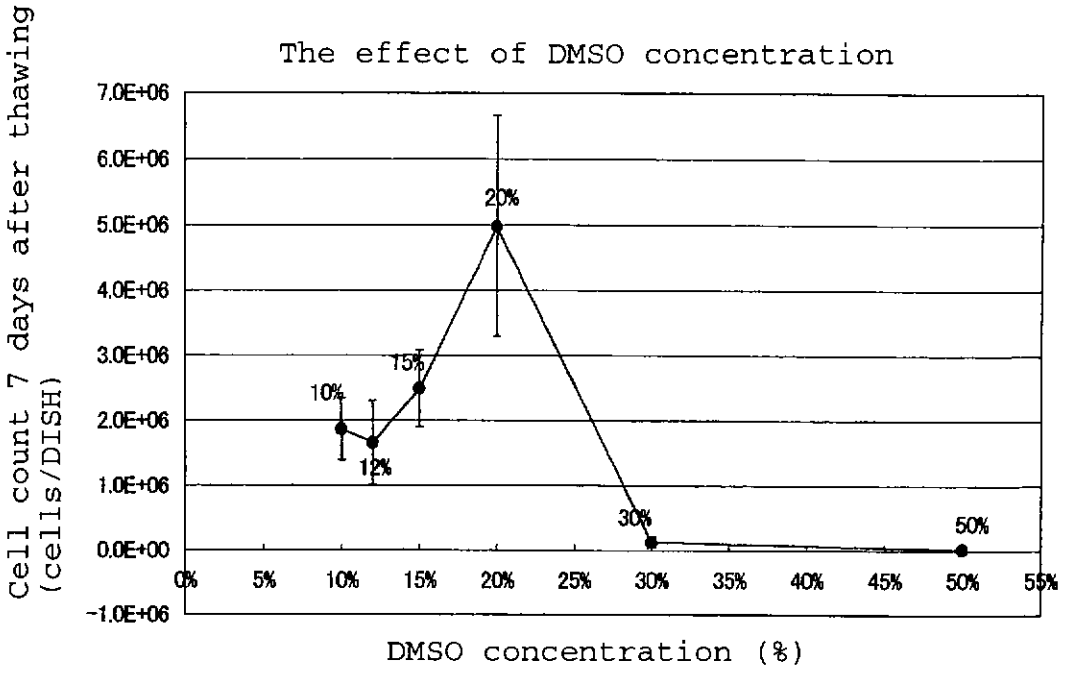

Cartilage
Glandular epithelium
Nerve
Muscle

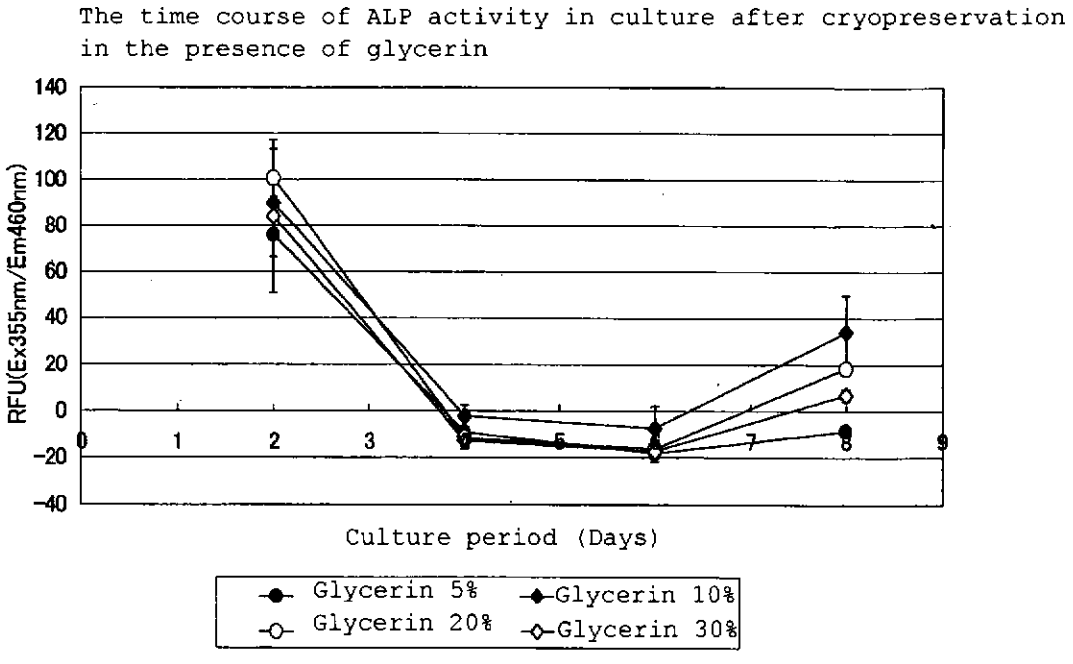
Fig. 18


The time course of ALP activity after cryopreservation at various DMSO concentrations
RFU(Ex355nm/Em460nm)
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
0
1
2
3
4
5
6
7
8
Culture period (Days)
DMSO10%
DMSO12%
DMSO15%
DMSO20%
DMSO30%
DMSO50%

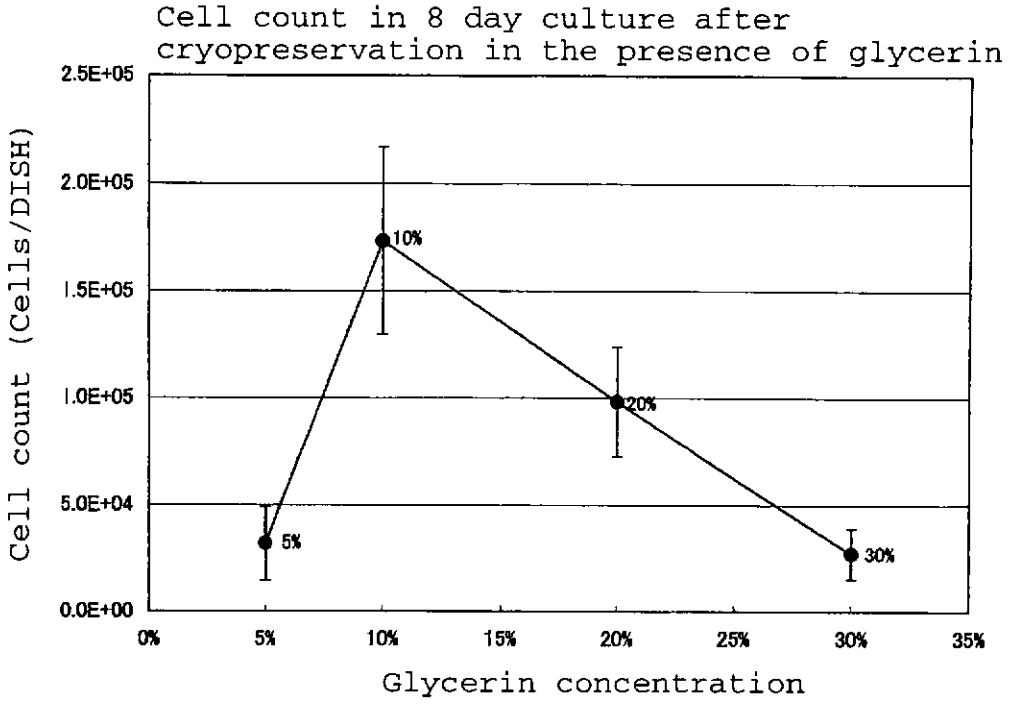
# Fig. 19


The effect of DMSO concentration
Cell count 7 days after thawing (cells/DISH)
7.0E+06
6.0E+06
5.0E+06
4.0E+06
3.0E+06
2.0E+06
1.0E+06
0.0E+00
-1.0E+06
0%
5%
10%
15%
20%
25%
30%
35%
40%
45%
50%
55%
10%
12%
15%
20%
30%
50%
DMSO concentration (%)

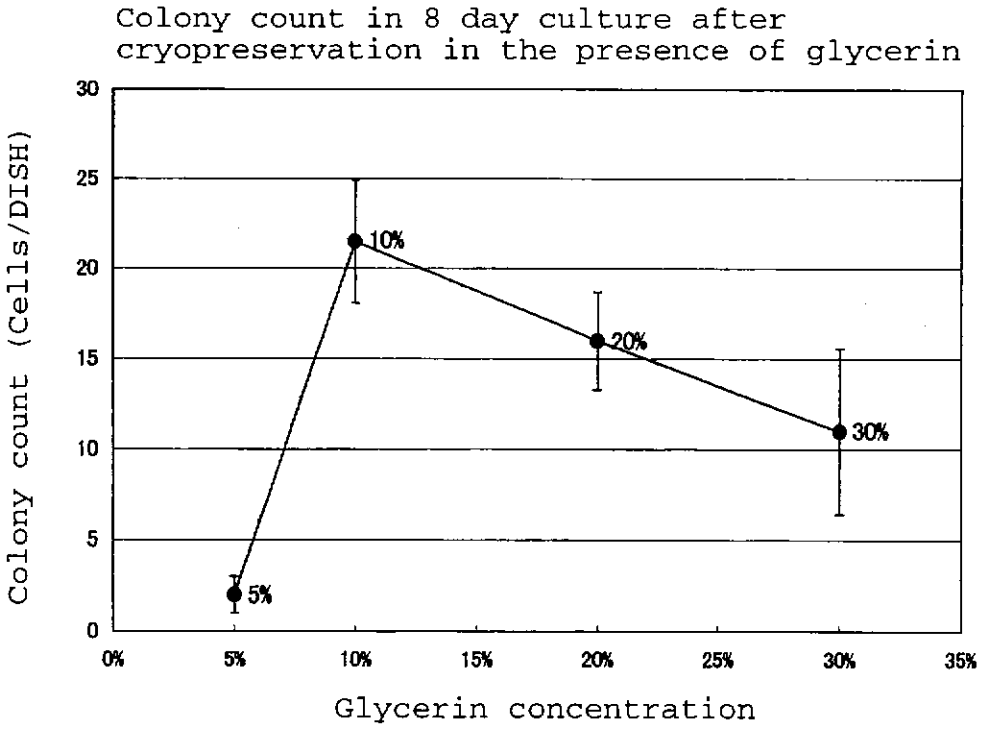
# Fig. 20


The time course of ALP activity in culture after cryopreservation in the presence of glycerin
RFU(Ex355nm/Em460nm)
140
120
100
80
60
40
20
0
-20
-40
0
1
2
3
4
5
6
7
8
9
Culture period (Days)
Glycerin 5%
Glycerin 10%
Glycerin 20%
Glycerin 30%

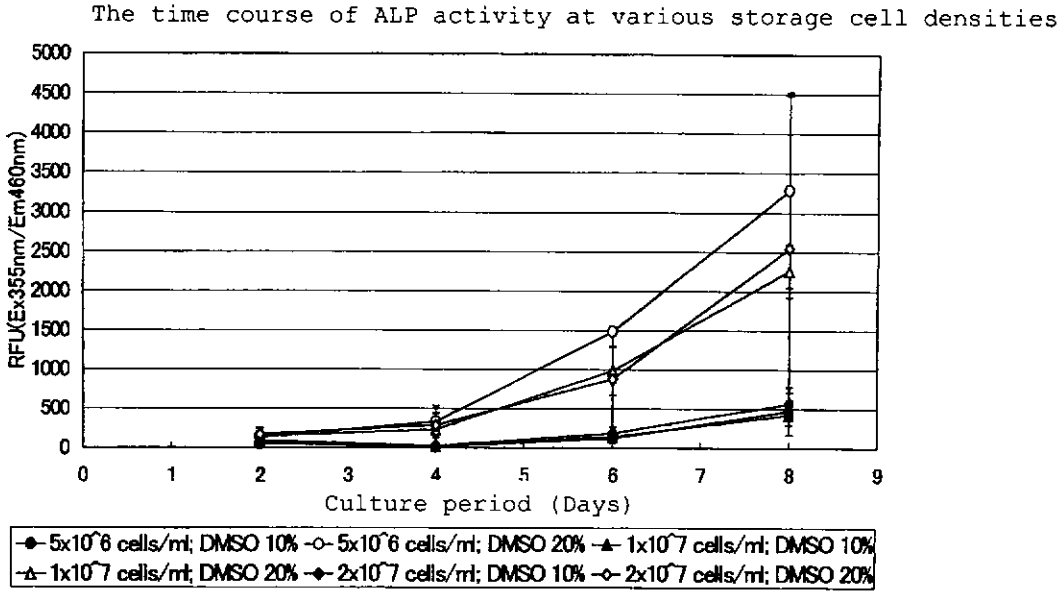
# Fig. 21


Cell count in 8 day culture after cryopreservation in the presence of glycerin
Cell count (Cells/DISH)
2.5E+05
2.0E+05
1.5E+05
1.0E+05
5.0E+04
0.0E+00
10%
20%
5%
30%
0%
5%
10%
15%
20%
25%
30%
35%
Glycerin concentration

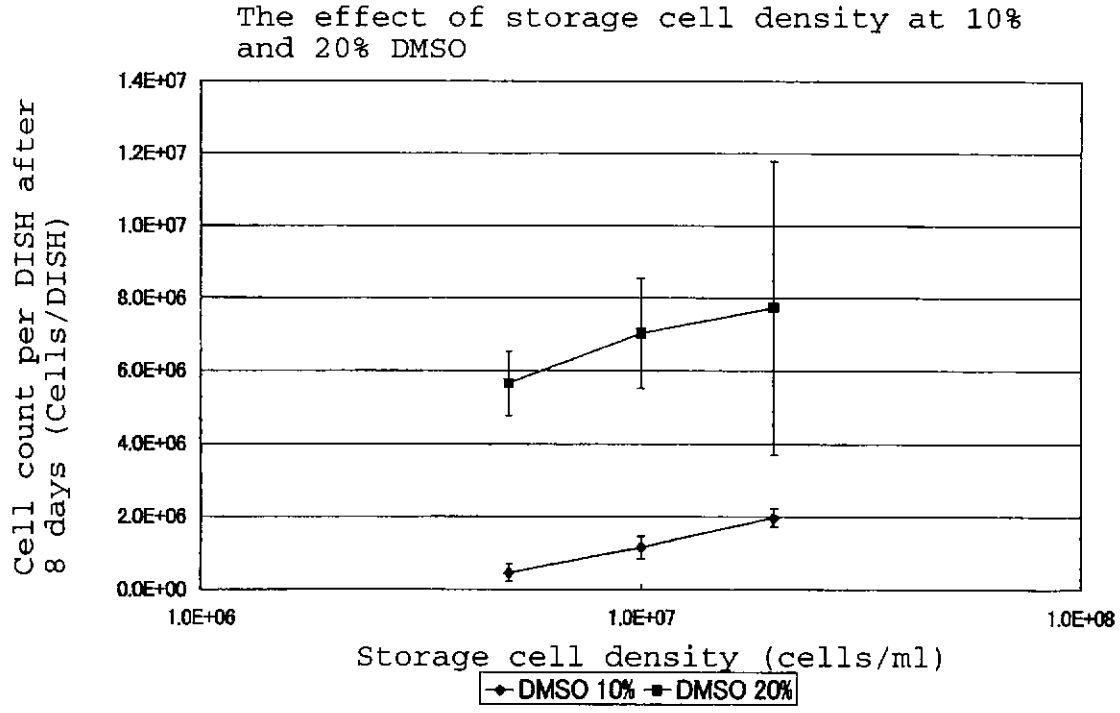
# Fig. 22


Colony count in 8 day culture after cryopreservation in the presence of glycerin
Colony count (Cells/DISH)
30
25
20
15
10
5
0
10%
20%
30%
5%
0%
5%
10%
15%
20%
25%
30%
35%
Glycerin concentration

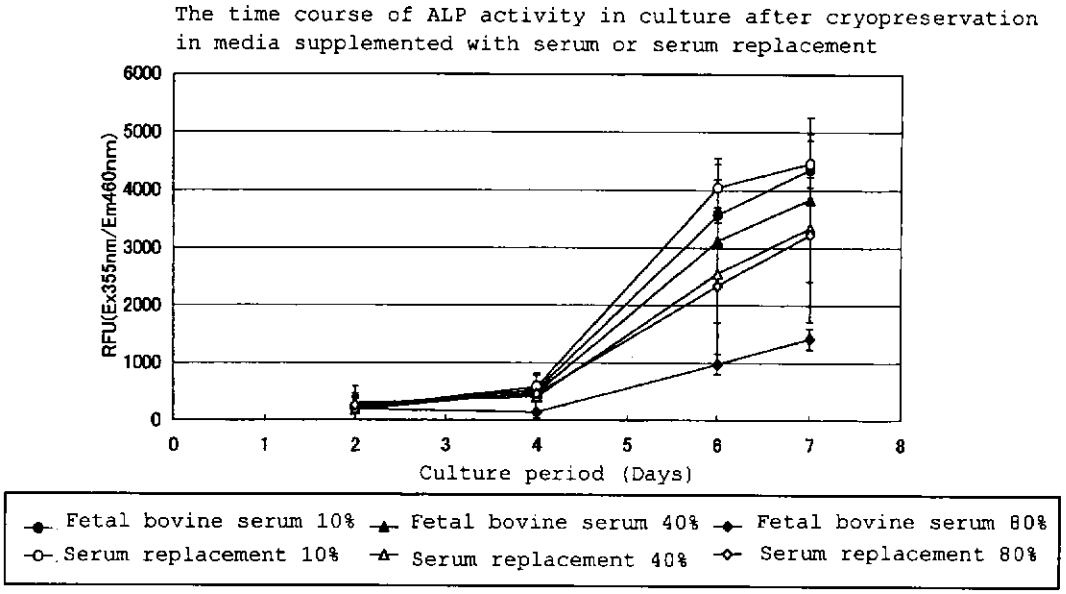
# Fig. 23


The time course of ALP activity at various storage cell densities
RFU(Ex355nm/Em460nm)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0 1 2 3 4 5 6 7 8 9
Culture period (Days)
5x10^6 cells/ml; DMSO 10%
5x10^6 cells/ml; DMSO 20%
1x10^7 cells/ml; DMSO 10%
1x10^7 cells/ml; DMSO 20%
2x10^7 cells/ml; DMSO 10%
2x10^7 cells/ml; DMSO 20%

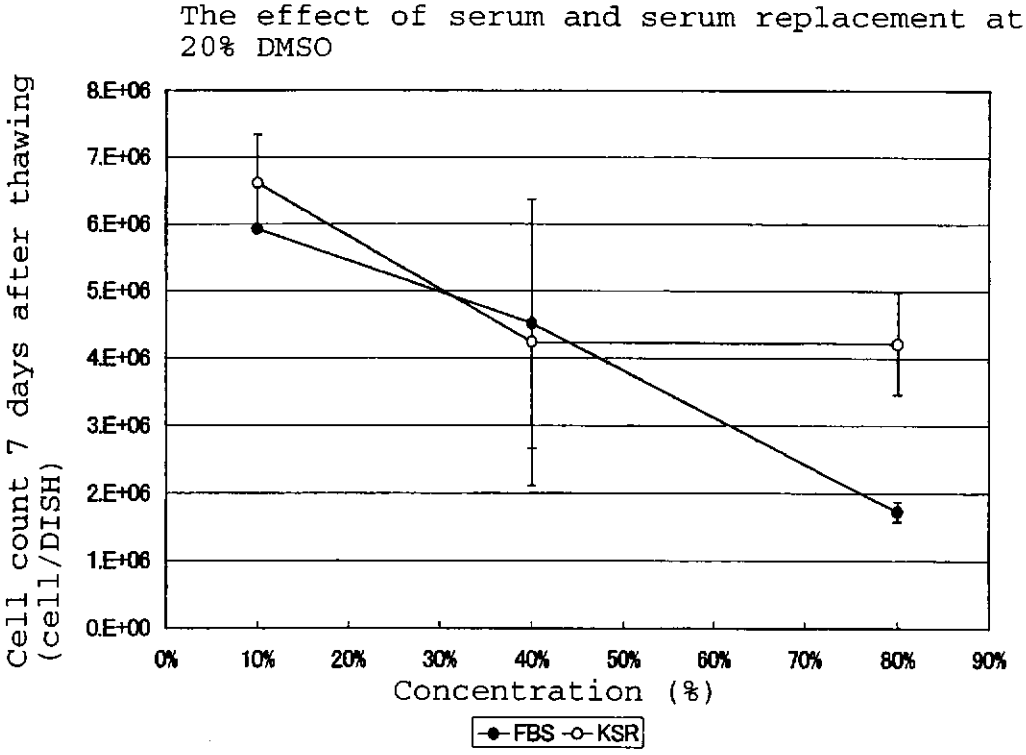
# Fig. 24


The effect of storage cell density at 10% and 20% DMSO
Cell count per DISH after 8 days (Cells/DISH)
1.4E+07
1.2E+07
1.0E+07
8.0E+06
6.0E+06
4.0E+06
2.0E+06
0.0E+00
1.0E+06
1.0E+07
1.0E+08
Storage cell density (cells/ml)
DMSO 10%
DMSO 20%

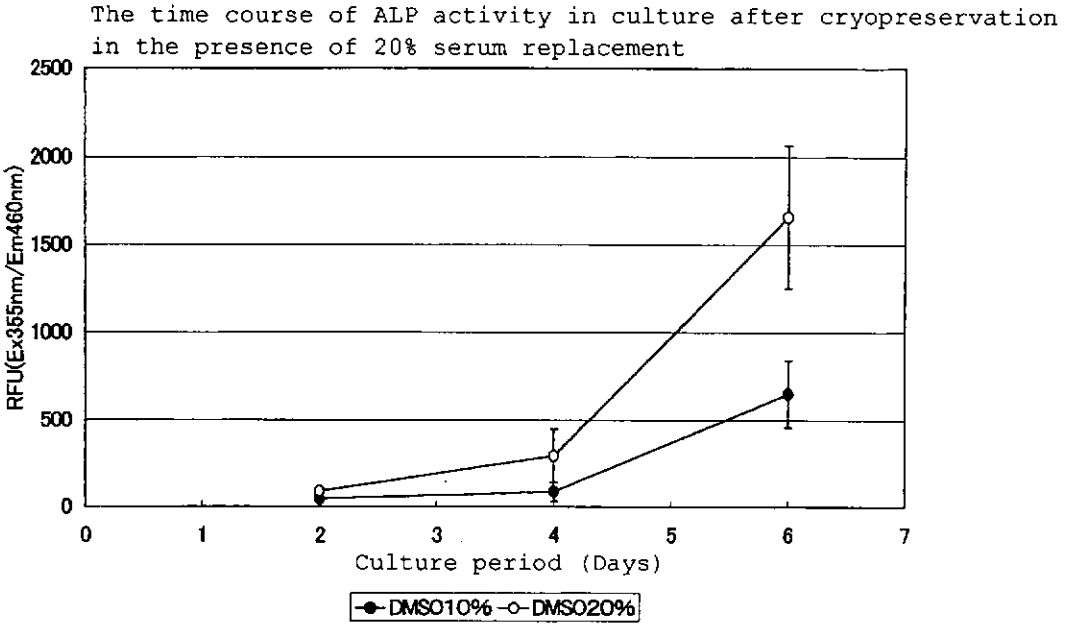
# Fig. 25


The time course of ALP activity in culture after cryopreservation in media supplemented with serum or serum replacement
RFU(Ex355nm/Em460nm)
6000
5000
4000
3000
2000
1000
0
0
1
2
3
4
5
6
7
8
Culture period (Days)
Fetal bovine serum 10%
Fetal bovine serum 40%
Fetal bovine serum 80%
Serum replacement 10%
Serum replacement 40%
Serum replacement 80%

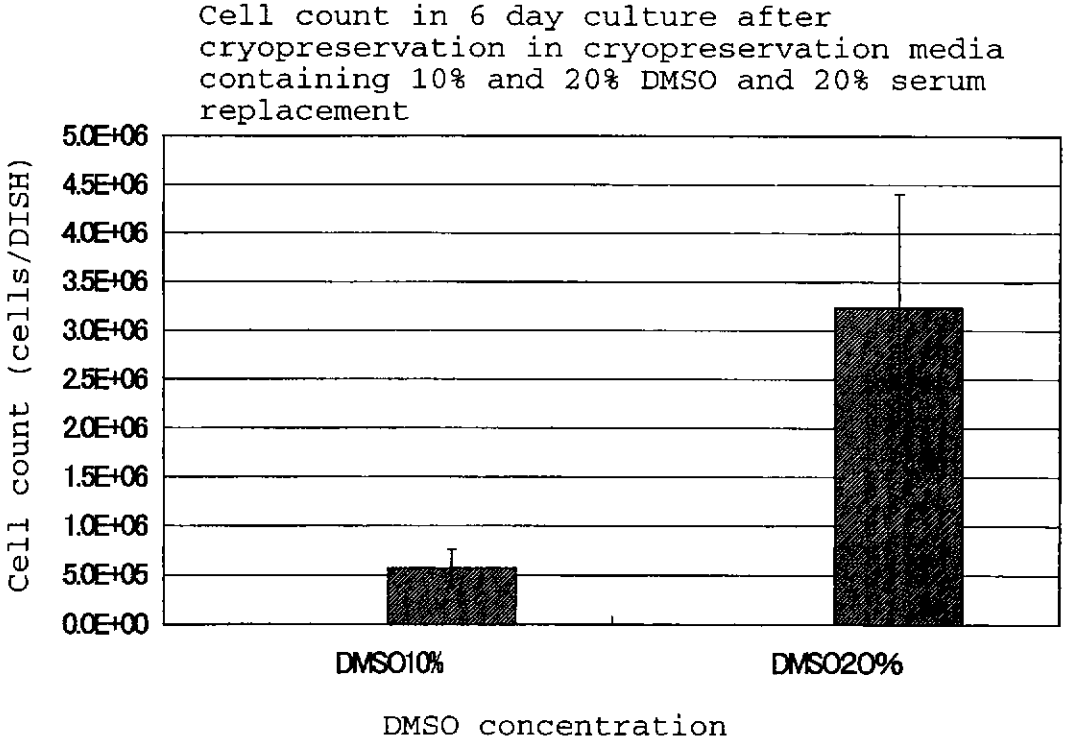
# Fig. 26


The effect of serum and serum replacement at 20% DMSO
Cell count 7 days after thawing (cell/DISH)
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%
10%
20%
30%
40%
50%
60%
70%
80%
90%
Concentration (%)
FBS
KSR

# Fig. 27


The time course of ALP activity in culture after cryopreservation in the presence of 20% serum replacement
RFU(Ex355nm/Em460nm)
2500
2000
1500
1000
500
0
0
1
2
3
4
5
6
7
Culture period (Days)
DMSO10%
DMSO20%

# Fig. 28


Cell count in 6 day culture after cryopreservation in cryopreservation media containing 10% and 20% DMSO and 20% serum replacement
Cell count (cells/DISH)
5.0E+06
4.5E+06
4.0E+06
3.5E+06
3.0E+06
2.5E+06
2.0E+06
1.5E+06
1.0E+06
5.0E+05
0.0E+00
DMSO10%
DMSO20%
DMSO concentration