NIPPON Genetics EUROPE

Application

Comparison of cryopreservation efficency of human iPS cells / human ES cells

Product

Bambanker hRM (product number: BBH0#)
Serum-free cell cryopreservation solution for regenerative medicine research

Manufacturer

Lymphotec Corporation

The following feedback was published due to the kindness of Mr. Takamichi Miyazaki, embryonic stem cell research field, Institute for Frontier Medical Sciences, Kyoto University, Japan.

Experimental Method

For cryopreservation of human iPS cells and human ES cells maintained in culture in our laboratory, we used Bambanker hRM (BBH0#) and Bambanker (BB0#) and compared cryopreservation efficiency.

Method	Preservative	Feature
Slow freezing	Bambanker hRM	Serum-free, Xeno-free
Slow freezing	Bambanker	Serum-free

● Feeder-free culture

① Human iPS cells (iPS (IMR 90)-1 strain)

[Frozen] Preparation of 1 x 10^6 cells / tube after single dispersion peeling of human iPS cells (IMR 90-1 strain) (subcultured on mTeSR1 medium, Matrigel) with EDTA/DPBS and TrypLE select ※. The cell suspension was dispersed and centrifuged (300 x g, 3 minutes). The cells were suspended in cryoperservation solution (0.5 ml), transferred to a freezing tube, placed in Mr. Frosty, cooled overnight at -80°C, transferred to a -150°C freezer and stored.

※ Details of distribution method:
Miyazaki T, et al. Genesis. 2014 Jan;52（1）:49-55. doi: 10.1002/dvg.22725

[Thawing and Survival Rate Measurement] One month later, frozen cells were thawed in a 37 ° C warm bath, 5 volumes of mTeSR medium was added and centrifuged (500 x g, 5 min). Cells were suspended in an appropriate amount of medium, then the number of viable cells was counted by trypan blue staining method, and the average value of 3 thawed samples was evaluated as survival rate.

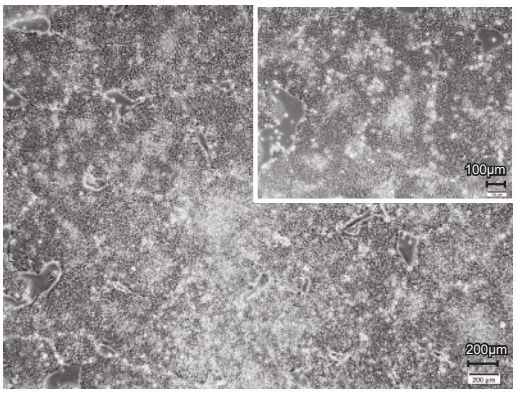
[Cultivation after thawing] The number of seeds was adjusted so that the seeding density of viable cells would be 1 x 10^5 cells / cm^2, seeded on an iMatrix-511 coated dish and cell survival was confirmed 24 hours later.

② Human ES cells (KhES-1 strain)

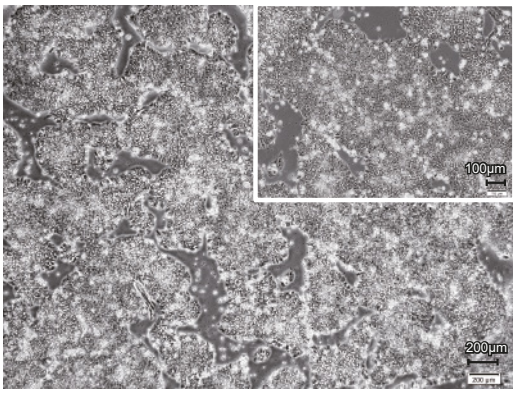
[Freezing] The same operation as above for human iPS cells was performed.

[Thawing and Survial Measurement] The same operation as above ① .

[Culture of viable after thawing] The same operation as above ①.



Human ES cells（KhES-1 strain）3 days after sowing Bambanker hRM



Human iPS cells（iPS（IMR90）-1 strain）4 days after sowing Bambanker hRM

● On feeder culture

③ Human ES cells (KhES-1 strain)

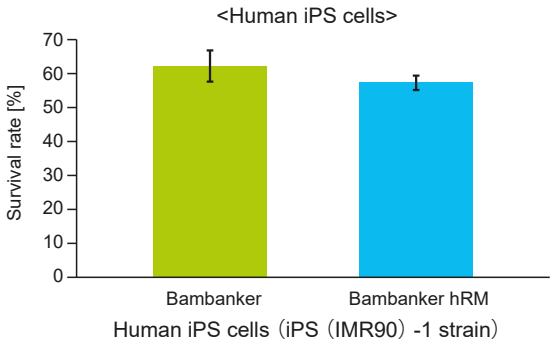
[Freezing] Human ES cells (KhES-1 strain) (subcultured on MEF feeder cells using a medium for human pluripotent stem cells) were subjected to single cell dispersion by using EDTA/DPBS and TrypLE. Cells were collected at 1 x 10^6 cells/ tube, and the cell suspension was dispensed and centrifuged (300 x g, 3 minutes). The cells were suspended in a cryopreservation solution (0.5 ml), transferred to a freezing tube, placed in Mr. Frosty, cooled overnigth at -80 ° C, transferred to a -150°C deep freezer and stored.

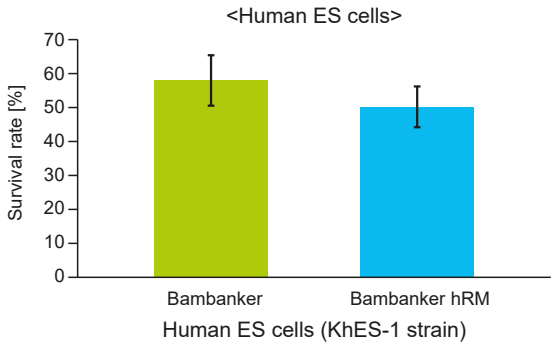
[Thawing and Survival Rate Measurement] After one month, frozen cells were thawed in a 37° C warm bath, centrifuged (500 x g, 5 minutes) by adding 10 times the amount of medium. Cells were suspended in an appropriate amount of medium, stained with PI and the number of surviving hSC fractions was counted using flow cytometry. The average value of 3 thawed samples was evaluated as survival rate.

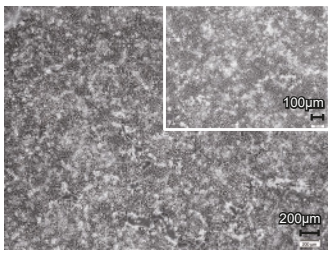
Result

The above test was carried out and the cell viability was compared immediately after thawing.

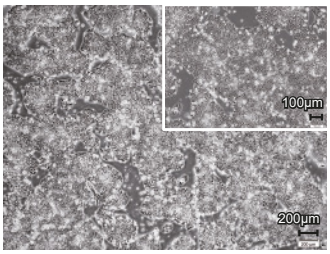
● Feeder-free culture



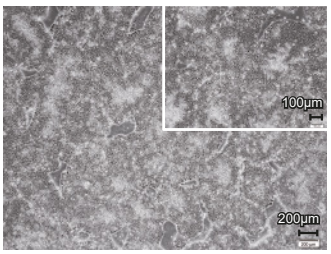




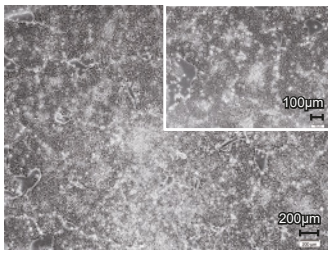
Four days after seeding
Human iPS cells (iPS (IMR 90)-1 strain)
Bambanker



Four days after seeding
Human iPS cells
（iPS（IMR90）-1 strain）
Bambanker hRM



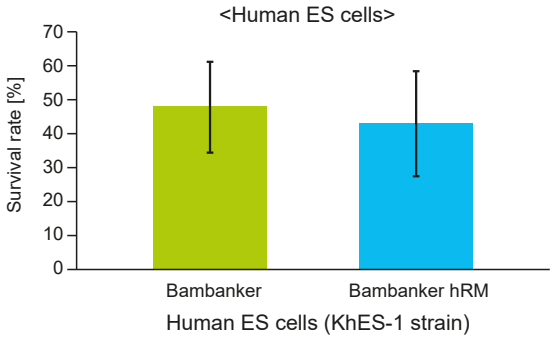
Three days after seeding
Human ES cells（KhES-1 strain）
Bambanker



Three days after seeding
Human ES cells（KhES-1 strain）
Bambanker hRM

High survival rates were observed in any of the cryopreservation solutions and good cell adhesion and proliferation could be confirmed after reseeding.

● On feeder culture



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Customer's comment

Human pluripotent stem cells can be handled in the same way as other general purpose animal cell lines by combining improved slow freezing method with ready-to-use Bambanker and the hurdle of cryopreservation has dramatically decreased.
Both of these experiments are carried out without using ROCK inhibitors, but survival rates are maintained very high immediately after thawing and after re-seeding, so I think both series are efficient reagents.

●NGC comment

Bambanker hRM is serum-free and Xeno-free and has a survival rate comparable to that of Bambanker.
In future, it is expected to be a product expected for regenerative medicine research using human cells.

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