NIPPON Genetics EUROPE

Customers product feedback

Product name: Bambanker hRM (BBH01)

Serum-free cryopreservation solution for regenerative medicine research

Application: Comparison of the cryopreservation efficiency for human iPS cells

Data kindly provided by US corporation Hasumi International Research Foundation, Tokyo Research Center, Japan.

Methods

The cryopreservation efficiency of Bambanker hRM was compared to the vitrification method and a 10 % DMSO-containing self-made freezing medium for human iPS cells.

Vitrification:

Vitrification is an instant solidification of a solution achieved by an extrem increase of viscosity during cooling without ice crystal formation. It is used for the fast and gentle freezing of cells.

The used cells for comparison of the three media were human iPSC 201B7 (RIKEN BRC Bio Resource Center).

1.) The human iPSC 201B7 line was cultured together with feeder cells (SNL cell line which was treated with Mitomycin C (MMC)) in a 10 cm dish.

Feeder cells:

Feeder cells support the iPS cells growth in culture. They provide auxillary substances including attachment substrates, nutrients or other factors that are needed for growth in culture.

Mitomycin C:

Mitomycin C (MMC) intercalates between DNA strands. The resulting covalent bound leads to a dissociation incapability of the DNA strands and thus replication is not possible and cell proliferation of feeder cells is inhibited.

2.) Cultured human iPS cells were isolated by a dissociation solution. In order to reduce size of the colonies 1 ml of culture was pipetted up and down.

3.) The recovered cells were divided into three equal parts resuspended with:

- Bambanker hRM,
- 10 % DMSO + human ES/iPS cell culture medium and
- vitrification freezing preservation solution.

Cell preservation method	Preservation solution
Vitrification freezing method	Vitrification freezing preservation solution
Slow method	Bambanker hRM
Slow method	10 % DMSO containing medium

Slow method:

Freezing and storage of the samples at -80 °C.

4.) Cells resuspended in Bambanker hRM and 10 % DMSO + human ES/iPS cell culture medium were stored at -80 °C. Cells resuspended in vitrification freezing preservation solution were directly frozen in liquid nitrogen. All three cell cultures were stored for one week.

5.) The thawed cells were seeded in a 6 cm dish together with SNL feeder cells which were treated with MMC.

6.) Cells were cultured for 8 days. In the first 24 h rock inhibitor was added.

Rock inhibitor:

Rock is a serine/threonine kinase that acts as an effector of the GPTase Rho. Rho is a regulator for the actin-myosin contraction. Addition of rock inhibitor (Y-27632) to the culture medium can inhibit the human ES/iPS cell death.

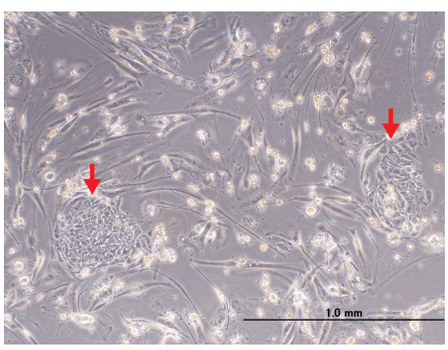
7.) After growing period the number of colonies were counted.

Result

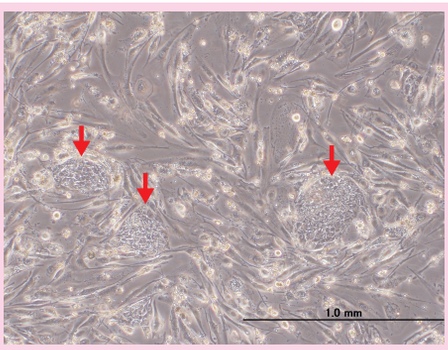
The abovementioned comparison test was repeated three times and the number of colonies was compared. The number of colonies in case of the vitrification freezing method was determined as 100 % reference.

	test 1	test 2	test 3
Vitrification freezing method preservation solution	100 %	100 %	100 %
Bambanker hRM	317 %	310 %	422 %
10% DMSO containing medium	122 %	56 %	56 %

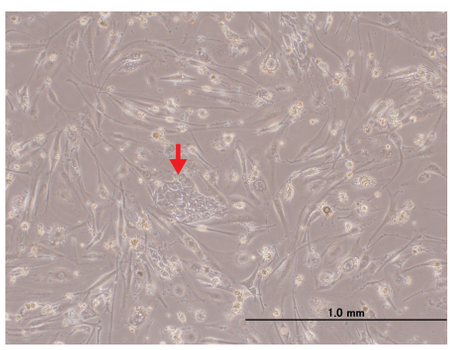
In all three trials the most efficient cell harvest was achieved with Bambanker hRM. Storage efficiency with Bambanker was much higher than with 10 % DMSO containing medium. Additionally, the freezing procedure with Bambanker hRM is much easier and less prone to error compared with the vitrification freezing method. The following photos show the state of cells on the fourth day after thawing. In case of 10 % DMSO containing medium only small colonies could be recovered what suggests a small survival rate while with Bambanker hRM it was possible to recover large colonies with almost the same size as achieved with vitrification freezing preservation solution. These results indicate that the same storage efficiency can be achieved with Bambanker hRM as with the vitrification preservation solution with the additional advantage of an easier handling.



Cells cryopreserved with vitrification freezing preservation solution 4 days after thawing



Cells cryopreserved with Bambanker hRM 4 days after thawing



Cells cryopreserved with 10 % DMSO containing medium 4 days after thawing

Customers comment

For the cryopreservation of human iPS cells we used until now the vitrification freezing method. This method has the disadvantage to require a lot of skills and the simultaneous processing of many cryocultures is not possible. Because of the test results we believe that Bambanker hRM can improve the working efficiency and storage stability and thus leading to a better efficiency of storage and maintenance of human iPS cells.