

## StemCell Keep

StemCell keep is a suitable ready-to-use vitrification solution for human ES/iPS cell cryopreservation. Human ES/iPS cells are very sensitive to freezing and thawing damage and their survival rate after cryopreservation by slow cooling is quite low. Vitrification is recommended for preservation of these cell lines. Vitrification means direct transition to the glassy state without the crystallization that is said to be the main reason for freezing damage. *StemCell Keep* has been developed as highly efficient, low toxic and easy to handle vitrification solution without any protein and DMSO that might affect differentiation.

### [Characteristics]

- ▶ Complete chemically defined: No Serum, No protein, No DMSO
- ▶ High efficiency: ES/iPS cells can be well preserved in the colony
- ▶ State by vitrification
- ▶ ES/iPS cells can be stored and fully retain their pluripotency
- ▶ Long shelf life (4°C 2 years)
- ▶ Ready-to-use medium



### [Operating Procedure]

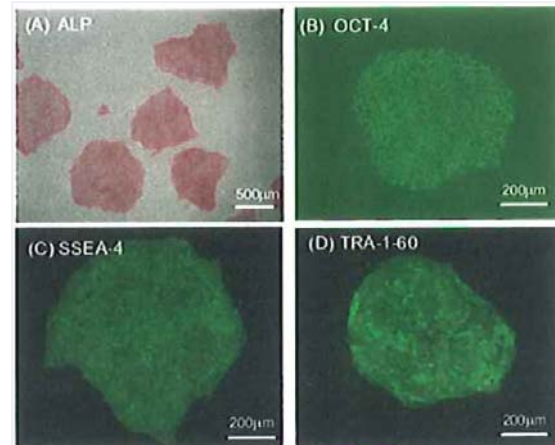
**Note:** It is necessary to immerse the vial into liquid nitrogen as soon as possible after making cell suspension with StemCell Keep if you want to obtain high viability. Also, when thawing, addition of pre-warmed medium to the vial should be recommended.

#### Freezing of ES/iPS cell colonies suspensions

- 1) Prepare liquid N<sub>2</sub> in the clean bench
- 2) Centrifuge suspended media of ES/iPS cell colonies and pellet them by elimination of supernatant at 4—10°C
- 3) Resuspend the cell pellet with 200μL of StemCell Keep.
- 4) Pipette the cell suspension into a cryotube and immediately put it to liquid N<sub>2</sub> directly. (If the successful vitrification would be achieved, the solution should be kept transparent after freezing.)
- 5) Transfer the cryotube into a liquid nitrogen chamber (-196°C)

#### Thawing from preserved state

- 1) Keep the vials to be thawed in the liquid N<sub>2</sub> in the Dewar vessel
- 2) Add the pre-warmed (37°C) media (1mL) directly to the vial immediately after taking out from the liquid N<sub>2</sub>
- 3) Thaw the solution by gentle pipetting and add into the excess amount of medium. Centrifuge and plated the colonies on the feeder cell layer in a culture dish and culture in an incubator.



[Characteristics of cyropreserved human iPS cells]

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