

Protocol of STEM-CELLBANKER®

A case of cryopreservation of human ES and iPS cells

Freezing

1. Examine and make sure the cell culture free of contamination, in healthy situation and proper confluency, etc.
 2. Transfer cells into cryotube.
 3. Remove culture medium by pipetting without centrifugation (*1).
 4. Directly add STEM-CELLBANKER® (*2) cooled at 4°C to cells in the cryotube at 500 uL.
 5. Place the cryotube into a container (*2) for programmable freezing cooled at 4°C.
 6. Place the container (*3) into -80°C freezer.
 7. Next day, remove the cryotube from the container and transfer it to -196°C liquid nitrogen storage tank.
- Perform 5. and 6. quickly.

*1. The procedure is also available to collect cells into centrifugation tube and remove culture medium by centrifugation at 300rpm (20G).

*2. Prior to the procedure, Rho-associated kinase (ROCK) inhibitor, Y-27632 (Merck) can be added at 10 uM (final conc.) to improve the viability in some cases.

*3. As programmable freezing container, Mr.Frosty (5100-0001) (Nalgene Nunc) and BICELL DEWAR FLASK (Nihon Freezer) can be used.

Thawing

1. Remove the frozen cell from storage and quickly thaw in a 37°C shaking water bath.
2. Immediately dilute and gently mix cells with 8~10ml of cell wash and recovery solution CELLOTION™ (*1) or culture medium.
3. Centrifuge the cryotube (*2) and remove the supernatant by aspirator.
4. Gently suspend the cells with appropriate volume of complete cell culture medium.
5. Continue the further culture procedures according to standard protocols.

*1. CELLOTION™ helps to increase the yield of cells after washing cells.

<http://www.zenoag.jp/cellbanker/ja/cellotion.html>

*2. 5~10 minutes at 300G recommended.

Storage and Stability:

Store at 2 - 8°C. The product will be stable for 3 years after the date of manufacture at this storage temperature. If storing for 3 months or longer after the bottle opened, separate into aliquot and keep at -20°C. To prevent from deterioration of the product, frequent freeze-thaw method (more than 3 times) should be avoided.