nacalai tesque
The quality for certainty.

Contact us
Email : info.intl@nacalai.co.jp
TEL : +81-75-251-1730
FAX : +81-75-251-1763

Product No. 11325

Cell Reservoir One, Vitrify

Cell Reservoir One (Vitrify) is a novel serum-free cell culture freezing medium for vitrification method, which contains a water-soluble glycoprotein SERICIN isolated from the silkworm cocoon as a major constituent. It provides high survival rate of primate cells, such as Monkey ES cells and Human iPS cells.
* Cell Reservoir One (Vitrify) is produced in corporation with SEIREN CO.,LTD. (Patent pending)

Features

- High viability with a longer freezing protocol (up to 60 seconds)
- Serum and animal-derived component free
- Low toxicity to cells (DMSO and acetamide free)

Protocol

Preparation

In order to obtain high viability, it is important to freeze and thaw cells very rapidly. Prepare all necessary materials and equipments at hand before starting the freezing and thawing procedure.

Freezing protocol

1. Prepare tweezers and liquid nitrogen near a clean bench.
2. Detach primate ES/iPS cells with dissociation solution (0.25% trypsin/collagenase IV solution), and carefully collect the solution not to break down the cell colonies.
3. Aliquot the cell colonies into centrifuge tubes, and centrifuge them to remove as much supernatant as possible.
   * If Cell Reservoir One (Vitrify) is diluted by the remaining supernatant, the viability may decrease.
4. Add 200µl of Cell Reservoir One (Vitrify), and carefully mix by pipetting 4-5 times not to break down colonies. Transfer them quickly into a cryopreservation tube, and tighten a cap.
5. Immerse 2/3 height of the tube into liquid nitrogen for 10 seconds using tweezers, and then immerse it completely.
   * For the successful cryopreservation, [4.]–[5.] process should be performed within 60 seconds.
6. Transfer the tube into a liquid nitrogen storage tank.

Thawing protocol

1. Pre-warm 10 ml of cell culture medium in a centrifugation tube at 37°C .
2. Take out the cryopreservation tube containing frozen cells from the liquid nitrogen storage tank, and transfer it to a clean bench leaving it immersed in liquid nitrogen.
3. Take out the tube from liquid nitrogen. Open a cap and discard liquid nitrogen in the tube by turning it upside down.
4. Thaw the cell quickly by adding more than 800µl of the pre-warmed cell culture medium to the tube and pipetting a few times.
   * The larger volume of medium is used, the quicker the sample thaws. Add suitable volume of medium depending on the tube size.
5. Transfer the cell suspension [4.] to the centrifugation tube [1.] .
   * Operate [3.]-[5.] as quickly as possible.
6. Wash the cryopreservation tube with cell culture medium, and transfer the medium to the centrifugation tube [5.] .
7. Remove as much supernatant as possible after centrifugation, and seed cells in fresh medium.

(References)

Human iPS cells (201B7 cell line [1)]), Human iPS cells (253G1 cell line [2)]), Common marmoset ES cells (CMES40 cell line [3)])

1) Takahashi, K. et al. Cell, 2007 Nov 30;131(5):861-872
2) Nakagawa, M. et al. Nat Biotechnol, 2008 Jan;26(1):101-106
3) Sasaki, E. et al. Stem Cells, 2005 Oct;23(9):1304-1313

Attention

- Mix Cell Reservoir One (Vitrify) by pipetting before use.
- Freezing and thawing procedure should be performed tube by tube as quickly as possible.

Caution

- Cell Reservoir One(Vitrify) is designed for vitrification method. Do not use it for a slow freezing method.
- Nacalai Tesque takes no responsibility for any damages or losses arising from the use of this product.

Storage

Refrigerator (0-10°C)

Expiration

Expiration date is stated on the product label.

Packing

25 ml (Product No. 11325-62)

Manufactured by NACALAI TESQUE, INC.
498 Higashitamaya-cho, Nijo Karasuma,
Nakagyo-ku, Kyoto 604-0855, Japan