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# 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 <sup>1)</sup>), Human iPS cells (253G1 cell line <sup>2)</sup>), Common marmoset ES cells (CMES40 cell line <sup>3)</sup>)

- 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)

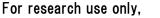
# Expiration

18 months from manufacturing. Expiration date is stated on the product label (Exp. yy/mm)

# Packina

25 ml (Product No. 11325-62)

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not intended for diagnostic or clinical use.

