Instruction Manual

Freezing Medium for human ES/iPS Cells

Cat.# RCHEFM001

Storage

This product is shipped frozen. Store at -80 °C soon after arrival. Thaw before use, and store at 2 °C to 8 °C after thawing. Use it up within about two weeks after thawing. Avoid repeated freezing and thawing

Characteristics

·High viability of human ES/iPS cells after long-term cryopreservation.
·Enable the sub-culturing at day3 to 5 after thawing.
·Pre-tested with human iPS cells.
(Takahashi K, et al., *Cell*, 131, 861–72, 2007)
·Inspected for pH, sterilization and mycoplasma.
·Serum free.

Conditions of Use

This product is for research use only, not for therapeutic or diagnostic purposes. It is not allowed to sell this product to a third party or use it for commercial purposes without permission from ReproCELL.

Instructions for Use

Please read the following instruction carefully before you use Freezing Medium for human ES/iPS Cells.

The same protocol of freezing/thawing method is also applicable for feeder-free culture system, if ReproFF is used instead of Primate ES Cell Medium.

A. Freezing Operation

Before freezing operation

In order to obtain the high viability of human ES/iPS cells, it is important to freeze the suspension including human ES/iPS cells and Freezing Medium for human ES/iPS Cells very rapidly in liquid nitrogen. If it takes longer than 15 seconds between making the cell suspension and freezing it, the viability of human ES/iPS cells should become extremely low. Please confirm that the preparation is all set before you start your freezing operation.

Materials required (The following reagent volume is for a 60mm dish.)

·Freezing Medium for human ES/iPS Cells (hereafter referred to as Freezing Medium).
·Dissociation Solution for human ES/iPS Cells (hereafter referred to as Dissociation Solution).
·Human ES/iPS Cell Medium supplemented with 5 ng/mL of bFGF (hereafter referred to as ES Medium) or ReproFF with 5ng/mL of bFGF.
·Human ES/iPS cells in the state of confluent. (Human ES/iPS cells in a 35mm to 100mm dish can be cryopreserved in a cryovial.)
·PBS(-): Ca^{++},Mg^{++}-free PBS
·15 mL tube
·Liquid nitrogen
·Ice
·Tweezers
·P-1000 Pipettman adjusted to 200µl and sterilized tip
·1ml Cryovial
·Cryovial rack
·Other materials commonly used for culture procedures

A1. Prepare the materials and equipments

Cryovial should be cooled on ice beforehand. You should write down necessary information (ex. the name of cell, the number of sub-culturing, date, etc.) on the label of the tube.

Put the liquid nitrogen near/in the lamina-flow cabinet. The viability of human ES/iPS cells is strongly affected by the interval between making the suspension of human ES/iPS cells and freezing it in liquid nitrogen. The interval must be less than 15 seconds.

Put a 15ml tube, cryovial rack, tweezers, p-1000 Pipettman and sterilized tip in the lamina-flow cabinet. Set the volume of P-1000 Pipettman to 200µl beforehand.

A2. Remove ES Medium from a dish containing human ES/iPS cells in the state of confluent, and wash the cells with 2 mL of PBS(-).

A3. Add 1 mL of Dissociation Solution to the dish, allow the solution to cover the whole surface of cells, and then warm in a CO_2 incubator at 37 °C for about 5 minutes.

A4. Observe cell conditions under a microscope to confirm that more than half the colonies are about to be detached from the dish. (The heating time should be adjusted)

A5. Add 2 to 3 mL of fresh ES Medium, detach all ES/iPS cells and feeder cells from the dish by pipetting, and collect them in a 15 mL tube. (Do not try to break down into cell clumps). Note 1)

A6. Centrifuge at approximately 170 × g (1,000 rpm) for 5 minutes at room temperature.

Please carry out the following operations using only one sample at a time.

A7. Remove the supernatant as much as possible. Note 2) If several samples are prepared simultaneously, they should be kept cool in the ice after remove the supernatant.

A8. Take 200µl of Freezing Medium with a P-1000 Pipettman.Note 3)

A9. Make a suspension by adding Freezing Medium to human ES/iPS cells in the 15 mL tube and then transfer the suspension to the cryovial which was cooled down on ice beforehand. Close a tab quickly and soak the cryovial in liquid nitrogen by using tweezers so that 2/3 height of the cryovial from the bottom should be in liquid nitrogen.

For the successful cryopreservation, it is very important to carry out this process very rapidly (within 15 seconds) [Note 4)]

A10. Freeze the human ES/iPS cells completely in liquid nitrogen and then transfer it to the liquid nitrogen tank or -135 °C freezer.

B. Thawing Operation

Before thawing operation

Thaw human ES/iPS cells very rapidly by adding the pre-warmed ES Medium to obtain the suspension. If it takes longer to do this operation, it causes extremely low viability of the human ES/iPS cells. Please carry out the operation one by one for each cryovial. Do not thaw it in a water bath because it causes extremely low viability.

Additional materials and equipments

·Frozen human ES/iPS cells.
·ES Medium or ReproFF.
·15 ml tube.
·Feeder-layer dish. (Prepare a dish of the same size as used in the freezing process.)
·Dry ice. (Liquid nitrogen)
·Common equipments for cell culture.

B1. Prepare 15 mL tube containing 10 ml of ES Medium and incubate in 37 °C waterbath.
B2. Take out frozen human ES/iPS cells in a cryovial from liquid nitrogen or freezer and then carry it to the lamina-flow cabinet while cooling it in dry ice.
B3. Put the tubes in procedure B1, in the lamina-flow cabinet .
B5. Thaw the frozen human ES/iPS cells quickly by adding 800µl of the pre-warm ES Medium in procedure B1 to the frozen human ES/iPS cells, and immediately make pipetting a few times for quick thawing. (This operation is very important to obtain high viability)
B6. Transfer the cell suspension to pre-warm ES Medium in procedure B1.
B7. Centrifuge at 4 °C for 5 minutes at about 170×g (1,000rpm).
B8 Remove the supernatant and then add 4mL of fresh ES Medium to obtain a suspension. (Do not try to break down into cell clumps)
B9. Transfer the cell suspension onto fresh feeder-layer dish removed the medium, swirl the dish to spread cells uniformly, and culture overnight at 37°C in a CO_2 incubator. From the day after next, change ES Medium once daily.

Note:
1) You should not break down the colonies into cell clumps in this process because the colonies will be broken down without any operation after thawing.
2) If too much supernatant is left, it causes low viability of ES cells after thawing because the freezing medium is diluted.
3) 200µl of the freezing medium is enough for one cryovial, regardless of the amount of human ES/iPS cells.
4) It is enough to pipette 2-3 times when you suspend the human ES/iPS cells in the freezing medium. If you pipette more than a few times, it causes low viability of human ES/iPS cells.

Related products

RCHEMD001	Primate ES Cell medium
RCHEMD003, 004	ReproFF
RCHEMD005	Repro Stem
RCHETP002	Dissociation Solution for human ES/iPS Cells
RCHEOT001	ReproCoat
RCHEOT002, 003	bFGF
RCHEOT004	Laminin-5
RCHEFC001	Feeder Cells (SL10)
RCHEFC003	Feeder Cells (MEF)

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