Instruction Manual

Dissociation Solution for human ES/iPS Cells

Cat.# RCHETP002

Storage

This product is shipped frozen. Store at −20°C soon after arrival. Thaw before use, and store at 2°C to 8°C after thawing. Use it up within about one week after thawing. Avoid repeated freezing and thawing

Characteristics

·Passage with high viability.
·Quick and easy operation.
·Each lot is culture-tested with human iPS cells (Takahashi K, et al., *Cell*, 131, 861–72, 2007).
·Inspected for sterilization and mycoplasma
·Ready-to-use.
·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

Described below are the procedures for passage of human ES/iPS cells with the use of Dissociation Solution for human ES/iPS Cells.

Procedures for passage (Allow all reagents to equilibrate to room temperature before use.)

A. Feeder-layer culture (60-mm dish)
Materials required
·Dissociation Solution for human ES/iPS Cells (hereafter referred to as Dissociation Solution).
·Primate ES Cell Medium supplemented with 5 ng/mL of bFGF (hereafter referred to as ES Medium).[Note 1)]
·Feeder-layer dish.
·PBS(-): Ca^{++},Mg^{++}-free PBS
·Other materials commonly used for culture procedures

A1. Prepare new feeder-layer dish in advance, remove feeder cell medium from the feeder-layer dish, and add 4mL of fresh ES Medium.
A2. Remove ES Medium from a dish containing human ES/iPS cells that are ready for passage, 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.[Note 2]
A6. Centrifuge at approximately 170 × g (1,000 rpm) for 5 minutes at room temperature and remove as much of the supernatant as possible.
A7. Add 1 mL of fresh ES Medium to precipitated cells. Allow the tip of a p-1000 Pipetman to come in contact with the bottom of the tube and make the size of colonies to about 100 to 200 µm by slowly pipetting cell clusters.[Note 3]
A8. Transfer about 1/3 to 1/4 of suspension onto fresh feeder-layer dish prepared in procedure A1, swirl the dish to spread cells uniformly, and culture overnight at 37°C in a CO_2 incubator. The dilution ratio for passage may differ depending on the growth rate of cell line used.[Note 4]
From the next day, change ES Medium once daily.

B. Feeder-free culture (Laminin-5-coated 35-mm dish)
Materials required
·Dissociation Solution for human ES/iPS Cells
·ReproFF supplemented with 5 ng/mL of bFGF (hereafter referred to as ReproFF)[Note 1)]
·Laminin-5-coated 35-mm cell culture dish.
·PBS(-):Ca^{++},Mg^{++}-free PBS

B1. Prepare new Laminin-5-coated dish in advance, wash a Laminin-5-coated dish twice with PBS (-), and add 2 mL of ReproFF (do not dry the dish).
B2. Remove ReproFF from a dish containing human ES/iPS cells that are ready for passage, and wash the cells with 1 mL of PBS (-).
B3. Add 0.5 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. (The heating time should be adjusted by observing the cells under a microscope.)
B4. Add 1 mL of fresh ReproFF, detach all ES/iPS cell colonies, and collect them with a p-1000 Pipetman in a 15-mL tube.
B5. Centrifuge at approximately 170 × g (1,000 rpm) for 5 minutes at room temperature and remove as much of the supernatant as possible.
B6. Add 1 mL of fresh ReproFF to precipitated cells. Allow the tip of a p-1000 Pipetman to come in contact with the bottom of the tube and make the size of colonies to about 200 to 300 µm by slowly pipetting cell clusters.[Note 5]
B7. Passage about half of the cell suspension. (Use a higher concentration of cells because the cell adhesion rate is slightly lower than that for feeder-layer culture.)
B8. Swirl the dish to spread cells uniformly, and culture overnight at 37°C in a 5% CO_2 incubator.
From the next day, change ReproFF once daily.

C. Feeder-free culture (Matrigel-coated 35-mm dish)
Materials required
·Dissociation Solution for human ES/iPS Cells.
·ReproFF supplemented with 5 ng/mL of bFGF (hereafter referred to as ReproFF).[Note 1]
·Matrigel-coated 35-mm cell culture dish.
·PBS(-):Ca^{++},Mg^{++}-free PBS.

C1. Prepare new Matrigel-coated dish in advance, remove excess liquid from a Matrigel-coated dish, and add 2 mL of ReproFF.
C2. Remove ReproFF from a dish containing human ES/iPS cells that are ready for passage, and wash the cells with 1 mL of PBS(-).
C3. Add 0.5 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.
C4. Add 1 mL of fresh ReproFF, scrape cells with a cell scraper, and collect them in a 15-mL tube.
C5. Centrifuge at approximately 170 × g (1,000 rpm) for 5 minutes at room temperature and remove as much of the supernatant as possible.
C6. Add 1 mL of fresh ReproFF to precipitated cells, allow the tip of a p-1000 Pipetman to come in contact with the bottom of the tube, and make the size of colonies to about 200 to 300 μm by slowly pipetting cell clusters.[Note 5]
C7. Passage about half of the cell suspension. (Use a higher concentration of cells because the cell adhesion rate is slightly lower than that for feeder-layer culture.)
C8. Swirl the dish to spread cells uniformly, and culture overnight at 37°C in a 5% CO_2 incubator.
From the next day, change ReproFF once daily.

Note:
1) Culture of human ES/iPS cells requires addition of bFGF. The concentration may differ depending on the cell line used.
2) In most cases, both ES/iPS cells and feeder cells are detached.
3) In rare cases, some ES/iPS cell colonies are included in feeder cell aggregates. In this case, remove the aggregates and use the remaining ES/iPS cell colonies for passage.
4) During passage, old feeder cells are also transferred. To avoid transfer of feeder cells, allow cells to stand for about 5 to 10 minutes after suspension. Colonies of ES/iPS cells precipitate first, and single feeder cells remain in the supernatant. The majority of single feeder cells may be removed by aspirating the supernatant.
5) Passage larger colonies of ES/iPS cells for feeder-free culture compared with feeder-layer culture. Excessive pipetting may be associated with too small colonies, low adhesion rates and growth efficiency, and poor cell conditions.

Related products

RCHEMD001	Primate ES Cell medium
RCHEMD003, 004	ReproFF
RCHEMD005	Repro Stem
RCHEFM001	Freezing Medium 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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