Instruction Manual

Primate ES Cell Medium

Cat.# RCHEMD001

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 two weeks after thawing. Avoid repeated freezing and thawing

Characteristics

· Each lot functionally tested using human iPS cells. (Takahashi K, et al., *Cell*, 131, 861–72, 2007)
·Lot-to-lot control of other critical criteria including osmolarity, pH, sterility and mycoplasma..
·Ready-to-use formulation – no mixing required.
·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 Primate ES Cell Medium and Dissociation Solution for human ES/iPS Cells.

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

Materials required

·Primate ES Cell Medium supplemented with 5 ng/mL of bFGF (hereafter referred to as ES Medium). Culture of human ES/iPS cells requires addition of bFGF. The concentration may differ depending on the cell line used.
·Dissociation Solution for human ES/iPS Cells (hereafter referred to as Dissociation Solution).
·Feeder-layer dish.
·PBS(-): Ca^{++},Mg^{++}-free PBS
·Other materials commonly used for culture procedures

1. Prepare new feeder-layer dish in advance, remove feeder cell medium from the feeder-layer dish, and add 4mL of fresh ES Medium.
2. 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(-).
3. 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.
4. 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)
5. 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 1]
6. Centrifuge at approximately 170 × g (1,000 rpm) for 5 minutes at room temperature and remove as much of the supernatant as possible.
7. 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 2]
8. Transfer about 1/3 to 1/4 of suspension onto fresh feeder-layer dish prepared in procedure 1, 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 3]
From the next day, change ES Medium once daily.

Note:

1) In most cases, both ES/iPS cells and feeder cells are detached.
2) 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.
3) 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.

Related products

RCHEMD003, 004	ReproFF
RCHEMD005	Repro Stem
RCHETP002	Dissociation Solution for human ES/iPS Cells
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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