ReproCELL

Instruction Manual

ReproFF2

Cat. # RCHEMD006

Storage

This product is shipped frozen. Store at -20℃ upon arrival. After thawing, store it at 2 to 8℃, and use it within two weeks. Avoid repeated freezing and thawing.

Characteristics of the product

- Human ES/iPS cells can be maintained with culture maintenance only on Monday, Wednesday, and Friday.

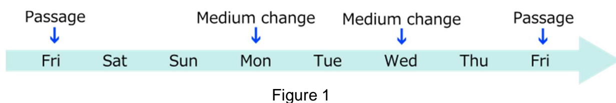

Figure 1

- Suitable for feeder-free culture of human ES/iPS cells.
- Each lot is culture-tested with human iPS cells as described in Takahashi K, et al., *Cell*, 131, 861–72, 2007.
- Lot-to-lot control of other critical criteria including osmolarity, pH, sterility, and mycoplasma.
- Serum-free.
- Includes 2-Mercaptoethanol.

Conditions of Use

This product is for research use only and 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.

Characteristics of feeder-free culture

The human ES/iPS cells cultured in a feeder-free environment have a slightly different morphology from the ones cultured on the feeder cells. Under standard feeder-free culture with ReproFF2, it takes around 7 days for the human iPS cells to become confluent, whereas it takes 3 or 4 days in feeder-dependent culture. These are the normal and the typical characteristics of feeder-free culture and are not specific to ReproFF2.

The condition of the cells can get unstable during the transition from feeder-dependent to feeder-free culture, but it usually gets back to normal after 3 to 4 passages. During this period, the growth rate of the cells is still as high as that of the cells in feeder-dependent culture, so passaging is necessary every 3 to 4 days instead of every 7 days in established feeder-free culture. Changing the medium every two days is usually acceptable during this transition period (See B10 below), but if the cells become very unstable, then we recommend to change the medium everyday until the cells get back to a stable state.

Instructions for Use

Described below is the procedure for passaging of human ES/iPS cells by using ReproFF2.

Materials required

Except for the coating materials (Laminin-5 or Matrigel), allow all reagents to equilibrate to room temperature before use.

- ReproFF2 supplemented with 5 ng/mL of bFGF (RCHEOT002, 003). (hereafter referred to as ReproFF2) [Note 1].
- Dissociation Solution for human ES/iPS Cells (RCHETP002)
- Laminin-5 (RCHEOT004) or other coating material, e.g., Matrigel (BD). When Matrigel is used, the culture medium DMEM and a cell scraper are also required.
- PBS (-): Ca^{++} and Mg^{++}-free PBS
- Standard equipments for cell culture

A. Surface Coating

A1. Coating with Laminin-5

Coat a 35-mm dish with 1 to 2 µg of Laminin-5. For better results, using 2 µg/dish is generally recommended, but the best concentration depends on each cell line.

A1-1. Thaw Laminin-5 at 4℃ or on ice. (Do not thaw at room temperature or by hand)

A1-2. Add 1mL of PBS (-) into a 35 mm dish. (Do not add PBS (-) into the vial containing Laminin-5 to dilute it.)

A1-3. Add Laminin-5 to the dish. Immediately and gently rock the dish to cover the surface equally. (Do not use the pipet to mix, or part of Laminin-5 will stick to it and be lost)

A1-4. Seal with Parafilm and Incubate at 4℃ overnight or 37℃ for 2 h.

A1-5. If the dish is not used immediately, seal it with Parafilm and store at 4℃. The coated dish can be stored for up to 1 week at 4℃.

A2. Coating with Matrigel

Making aliquots

A2-1. Thaw Matrigel at 4℃ overnight. Re-suspend Matrigel by rocking gently. Avoid warming up of Matrigel as it turns into a gel when warmed.

A2-2. Put 15 mL tubes and pipette tips on ice to cool them down.

A2-3. Make 300 µL aliquots of Matrigel quickly on ice.

A2-4. Seal the tubes with Parafilm, and store them at -80℃.

Coating

A2-5.Thaw the aliquoted Matrigel overnight at 4℃. Undiluted Matrigel can be viscous but should not have gelated.

A2-6. Add 8.7 mL serum-free DMEM to 300 µL of Matrigel, and mix by pipetting. Avoid making bubbles.

A2-7. Add 0.75 mL of diluted Matrigel to a 35 mm dish, and spread by swirling gently. Seal with Parafilm.

A2-8. Incubate at room temperature for 3 h. If not used immediately, store at 4℃.

B. Transition from feeder-dependent to feeder-free culture

B1. Prepare Laminin-5 or Matrigel coated dishes in advance as described in A. For Laminin-5-coated dishes, discard the coating solution and wash the dishes twice with PBS (-), and add 1.5 mL/dish of ReproFF2 (do not dry the coated surface). For Matrigel-coated dishes, remove the coating solution, and add 1.5 mL/dish of ReproFF2.

B2. Remove the medium from the dish containing feeder-dependently cultured human ES/iPS cells that are ready for passaging. Wash the cells with 1 mL of PBS (-).

B3. Add 1 mL of Dissociation Solution to the dish, allow the solution to cover the whole surface of cells, and then incubate in a CO_2 incubator at 37℃ for about 5 min. The incubation time should be adjusted appropriately (see B4).

B4. Periodically observe the cells under a microscope until more than half of the colonies are about to be detached from the dish Note 2).

B5. Add 1 mL of fresh ReproFF2, detach all ES/iPS cells and feeder cells from the dish by pipetting, and collect them in a 15 mL tube Note 3).

B6. Centrifuge at 170 × *g* (1,000 rpm) for 5 min at room temperature and remove as much of the supernatant as possible.

B7. Add 1 mL of fresh ReproFF2 to the pelleted cells. By pipetting gently, break down the pellet to 200 to 300-μm clusters Note 4).

B8. Transfer about the half of the cell suspension into a Laminin-5-coated or Matrigel-coated dish prepared in B1 Note 5). The actual amount of the cell suspension has to be determined depending on the concentration of Laminin-5 used for coating and the growth rate of the cell line used.

B9. Swirl the dish to spread the cells uniformly, and incubate at 37℃ in a CO_2 incubator.

B10. A suggested schedule during the transition

- 1st passage (transition on Friday): Passaging in three days on Monday.
- 2nd passage: Change medium in two days on Wednesday, and passaging in two days on Friday.
- From 3rd passage: Change medium on every Monday and Wednesday. Passaging on every Friday.

C. Passaging the established feeder-free culture

C-1. Passaging feeder-free culture with Laminin-5

C1-1. Prepare new Laminin-5-coated dishes in advance as described in A1. Wash the Laminin-5-coated dishes twice with PBS (-), and add 1.5 mL of ReproFF2 (do not dry the coated surface).

C1-2. Remove medium from the dish containing the cells that are ready for passaging, and wash the cells with 1 mL of PBS (-).

C1-3. Add 1 mL of Dissociation Solution to the cells, allow the solution to cover the whole surface of cells, and then incubate in a CO_2 incubator at 37℃ for about 5 min. The incubation time should be adjusted appropriately (see C1-4).

C1-4. Observe the cells under a microscope to confirm that more than half of the colonies are about to be detached from the dish Note 2). Add 1 mL of fresh ReproFF2, detach all ES/iPS-cell colonies by pipetting, and collect them into a 15 mL tube Note 3).

C1-5. Centrifuge at 170 × g (1,000 rpm) for 5 min at room temperature and remove as much of the supernatant as possible.

C1-6. Add 1 mL of fresh ReproFF2 to the pelleted cells. Break down the pellet to 200 to 300-μm clusters by pipetting gently Note 4).

C1-7. Transfer about the half of the cell suspension into the dish prepared in C1-1 Note 5).

C1-8. Swirl the dish to spread cells uniformly, and start incubation at 37℃ in a 5% CO_2 incubator. Change medium every two days. Passage the cells every 7 days as shown in Figure 1.

C-2. Passaging feeder-free culture with Matrigel

C2-1. Prepare new Matrigel-coated dish in advance as described in A2. Remove excess liquid from the Matrigel-coated dish, and add 1.5 mL of ReproFF2.

C2-2. Remove the medium from the dish containing the cells ready for passaging, and wash the cells with 1 mL of PBS (-).

C2-3. 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℃ for about 5 min.

C2-4. Add 1 mL of fresh ReproFF2, detach the cells with a cell scraper, and collect them in a 15 mL tube.

C2-5. Centrifuge at 170 × *g* (1,000 rpm) for 5 min at room temperature, and remove as much of the supernatant as possible.

C2-6. Add 1 mL of fresh ReproFF2 to the pelleted cells. Break down the pellet to 200 to 300-μm clusters by pipetting gently Note 4).

C2-7. Passage about the half of the cell suspension Note 5).

C2-8. Swirl the dish to spread cells uniformly, and culture at 37℃ in a 5% CO_2 incubator. Change ReproFF2 every two days. Passage the cells every 7 days as shown in Figure 1.

Notes:

Note 1) Addition of bFGF may improve the culture of human ES/iPS cells. The concentration differs depending on the cell line used.

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

Note 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 passaging.

Note 4) Passage larger colonies in feeder-free culture than those you would passage in feeder-dependent culture. Excessive pipetting results in formation of too small colonies, with low adhesion rates and poor cell conditions.

Note 5) During transition, some of the the feeder cells are also transferred. To minimize this, allow cells to stand for about 5 to 10 min after suspension. Colonies of ES/iPS cells precipitate first, and single feeder cells remain in the supernatant. The majority of single feeder cells can be removed with the supernatant.

Optional procedure for induction of differentiation:

The procedure for inducing differentiation can be different in feeder-free environment from that in feeder-dependent environment. In cases where the procedure for feeder-dependent culture does not work in feeder-free culture with ReproFF2, the following might help to improve the differentiation efficiency.

1) Upon passaging, switch the medium from ReproFF2 to Primate ES Cell Medium (RCHEMD001) supplemented with 5 ng/mL FGF-2 (RCHEOT002).
2) Continue culturing the cells using Primate ES Cell Medium with FGF-2 for two weeks. Except for the medium you do not have to change any of other materials or procedures.
3) Start induction.

Related products

RCHEMD001	Primate ES Cell medium
RCHEMD003	ReproFF
RCHEMD005	Repro Stem
RCHETP002	Dissociation Solution for human ES/iPS Cells
RCHEFM001	Freezing Medium for human ES/iPS Cells
RCHEOT002, 003	bFGF
RCHEOT004	Laminin-5

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