Instruction manual

# ReproMed™ iPSC Medium

Cat. No. RCRM101
Version 1.3

## Overview

This instruction manual explains how to passage and maintain human iPSC which have been cultured on feeder cells to feeder-free culture using **ReproMed™ iPSC Medium** (after supplementing with bFGF).

Please use this product in a biosafety cabinet.

## About product

**Sale of this product to a third party or other commercial purposes is strictly prohibited without prior permission from ReproCELL.**

## Storage

This product is shipped frozen. Upon receipt store immediately at −20 °C. Thaw overnight at 2 °C to 8 °C before use. Aliquot and store at −20 °C. Avoid repeated freeze-thaw cycles. Before using, supplement with bFGF and use within 2 weeks.

## Characteristics

・Feeder-free culture.
・ Each lot has been tested on PChiPS771 human iPSC established by ReproCELL.
・Quality control testing of critical criteria, including osmolality, pH, sterility, and mycoplasma has been performed on each lot.
・ Does not contain any serum.

## Please take note of the following points when using this product.

・It is recommended to incubate cells in a **5% $O_2$, 5% $CO_2$ incubator.**
(It is possible to use a 5% $CO_2$ incubator, but the proliferation rate is about twice as fast in a 5% $O_2$, 5% $CO_2$ incubator.)
・ Supplement with bFGF (at a final concentration of 10 ng/mL) before using. Hereinafter, this product is referred to as **ReproMed™ iPSC Medium**.
・ Allow ReproMed™ iPSC Medium to sit for 30 min at room temperature to allow the medium to warm up to room temperature before using. Do not use a water bath to warm the medium.
・ While 10 ng/mL of bFGF is recommended, the amount of bFGF can be adjusted to suit different cells.
・ After the initial transition to feeder-free culture, there may be many floating cells observed in the medium. This is normal and will decrease with subsequent passages.
・ As listed below are the recommended dissociation solutions and coating reagent for feeder-free culture:
Dissociation solutions: 0.5mM EDTA/PBS+ ESGRO Complete™ Accutase™ & TrypLE™ Select Enzyme (1X), no phenol red
Coating reagent: iMatrix-511

## Table of contents

## Product

| Product Description | Cat. No. | Amount | Storage |
|---|---|---|---|
| **ReproMed™ iPSC Medium** | RCRM101 | 500 mL | -20°C |

## Required reagents & equipment

| Product Description | Cat. No. | Amount | Storage |
|---|---|---|---|
| **bFGF** | REPROCELL RCHEOT002 | 25 µg | -80°C |
| **iMatrix-511** | REPROCELL NP892-011 | 350 µg | 4°C |
| **Dissociation Solution for human ES/iPSC** | RCHETP002 | 30 mL | -20°C |
| **TrypLE™ Select Enzyme (1X), no phenol red** | GIBCO Cat. 12563011 | 100 mL | room temperature |
| **ESGRO Complete™ Accutase™** | Millipore SF006 | 100 mL | 4 °C |
| **UltraPure™ 0.5M EDTA, pH 8.0** | Invitrogen 15575020 | 100 mL | room temperature |
| **Y27632** | Wako 257-00511 | - | - |
| **PBS (-). $Ca^{2+}$- and $Mg^{2+}$-Free** | - | - | - |
| **60 mm Tissue Culture Dish** | - | - | - |
| **5% $CO_2$ incubator** | - | - | - |

## ReproMed™ iPSC Medium culture protocol

### 1. Coating

<For 4 x 60 mm dish (surface area = 22 $cm^2$)>

1. Place iMatrix-511 on ice.
2. Aliquot 8 mL PBS (-) to a 15 mL tube and place it on ice.
3. Aliquot 88 µL of iMatrix-511 to the PBS (-) and mix gently. (92-fold dilution of iMatrix-511)
4. Coat each dish with 2 mL of the prepared iMatrix-511 solution. (0.5 µg iMatrix-511/$cm^2$)
5. Incubate for 1 hour in a 37 ℃, 5% $CO_2$ incubator. Seal the coated dish with parafilm and store at 4 ℃ if not used immediately and use within a week.

### 2. Transition from feeder-dependent culture on 100 mm dish to feeder-free culture using ReproMed™ iPSC Medium on 60 mm dish

**Note 1:** The reagent volumes stated below are for transition to a 60 mm dish.

**Note 2:** Use a 100 mm dish that is about 30% confluent for the transition (Refer to Figure 1).

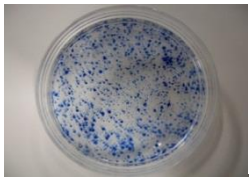


Figure 1
Alkaline Phosphatase (ALP) stained 30% confluent 100mm dish

1. Aspirate to remove the spent medium.
2. Wash dish with 4 mL of PBS (-).
3. Add 1.5 mL of dissociation solution for human ES/iPSC.
4. Incubate for 4 min in a 37 ℃, 5% $CO_2$ incubator.
5. Aspirate to remove the dissociation solution for human ES/iPS cells.
6. Wash dish with 2 mL of PBS (-).
7. Add 1.5 mL of 0.5X TrypLE Select (TrypLE™ Select Enzyme (1X) diluted with an equal volume of 0.5 mM EDTA).
8. Incubate for 7 min in a 37 ℃, 5% $CO_2$ incubator.
9. Flush the surface of the dish 10 times with a P1000 pipette to detach the cells from the dish.
10. Collect and transfer the cells to a 15 mL tube.
11. Collect the remaining cells with 2 mL/dish of ReproMed™ iPSC Medium containing Y27632 (at a final concentration of 10 µM).
    **Note 3:** Adding Y27632 increases cell viability and stability of the culture.
12. Centrifuge at 300×g for 5min.
13. Aspirate to remove the supernatant.
14. Resuspend the cells with 2 mL of ReproMed™ iPSC Medium with 10 µM Y27632 and count the

number of viable cells.

15. Aliquot an appropriate volume of cells into a new 15 mL tube and create a 4 mL cell suspension with ReproMed™ iPSC Medium with 10 μM Y27632. Transfer this cell suspension to an iMatrix-511 coated 60 mm dish.
    (※) Recommended seeding number:
    For the first 3 passages after the transition to feeder-free culture, seed the cells at $5\times10^5$ cells/dish.
    After 3 passages, optimize according to cell type.
    Option (1): For slow growing strains:
    $5\times10^5$ cells/dish
    Option (2): For fast growing strains with a high ability to remain undifferentiated:
    $2\times10^5$ cells/dish
16. Incubate in a 37 ℃, 5% $CO_2$ incubator.
17. Replace with 4 mL/dish of fresh ReproMed™ iPSC Medium (without Y27632) daily.
    **Note 4:** Option (1): Replace with 4 mL/dish of fresh ReproMed™ iPSC Medium (without Y27632) on Day 1, 24 hours after seeding.
    Option (2): Replace with 4 mL/dish of fresh ReproMed™ iPSC Medium with 10 μM Y27632 on Day 1, 24 hours after seeding. On Day 2, replace with 4 mL/dish of fresh ReproMed™ iPSC Medium (without Y27632), 48 hours after seeding. (Including Y27632 in the culture medium for 24 hours increases the stability of the cells cultured.)
    **Note 5:** Passage the cells once they have reached 80~90% confluent. (Cells seeded on Day 0 can be passaged on Day 7. For cells that are grown in a 5% $O_2$, 5% $CO_2$ incubator, cells can be passaged on Day 5.)
    **Note 6:** After the initial transition to feeder-free culture, there may be many floating cells observed in the medium. This is normal and will decrease with subsequent passages.

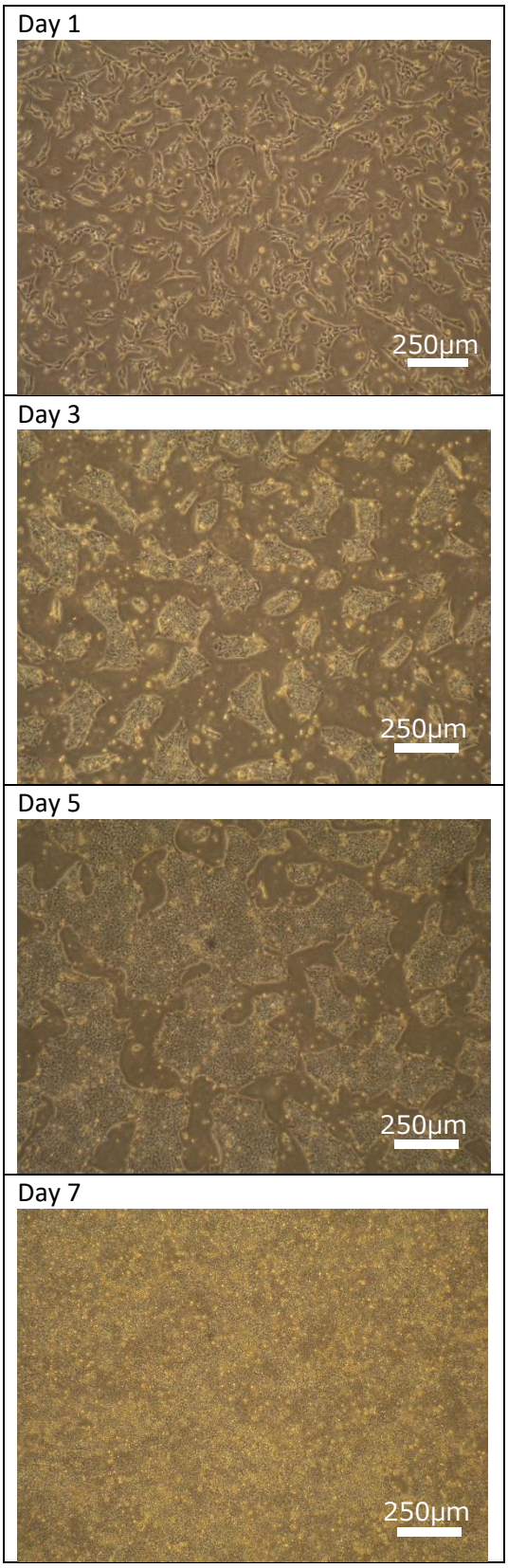
## 3. ReproMed™ iPSC Medium culture protocol

**Note 7:** The reagent volumes stated below are for cultures in a 60 mm dish.

**Note 8:** The protocol below is different from the transition of feeder-dependent to feeder-free culture protocol.

1. Aspirate to remove the spent ReproMed™ iPSC Medium.
2. Wash twice with 2 mL of PBS (-).
3. Add 1 mL/ dish of 0.5 mM EDTA/PBS and incubate for 2 min at room temperature on a rotator before removing the 0.5 mM EDTA/PBS.
4. Add 1 mL/dish of ESGRO Complete™ Accutase™.
5. Incubate for 7 min in a 37 ℃, 5% $CO_2$ incubator.
6. Flush the surface of the dish with a P1000 pipette to detach the cells from the dish.
7. Collect and transfer the cells to a 15 mL tube.
8. Collect the remaining cells with 1 mL/dish of ReproMed™ iPSC Medium with 10 μM Y27632.
   **Note 9:** Adding Y27632 increases cell viability and stability of the culture.
9. Centrifuge at 300×g for 5 min.
10. Aspirate to remove the supernatant.
11. Resuspend the cells with 2 mL of ReproMed™ iPSC Medium with 10 μM Y27632 and count the number of viable cells.
12. Aliquot an appropriate volume of cells into a new 15 mL tube and create a 4 mL cell suspension with ReproMed™ iPSC Medium with 10 μM Y27632. Transfer this cell suspension to an iMatrix-511 coated 60 mm dish.
    (※) Recommended seeding number:
    For the first 3 passages after the transition to feeder-free culture, seed the cells at $5\times10^5$ cells/dish.
    After 3 passages, optimize according to cell type.
    Option (1): For slow growing strains:
    $5\times10^5$ cells/dish
    Option (2): For fast growing strains with a high ability to remain undifferentiated:
    $2\times10^5$ cells/dish
    Incubate in a 37 ℃, 5% $CO_2$ incubator.
13. Replace with 4 mL/dish of fresh ReproMed™ iPSC Medium (without Y27632) daily.
    **Note 10:** Option (1): Replace with 4 mL/dish of fresh ReproMed™ iPSC Medium (without Y27632) on Day 1, 24 hours after seeding.
    Option (2): Replace with 4 mL/dish of fresh ReproMed™ iPSC Medium with 10 μM Y27632 on Day 1, 24 hours after seeding. On Day 2, replace with 4 mL/dish of fresh ReproMed™ iPSC Medium (without Y27632), 48 hours after seeding. (Including Y27632 in the culture medium for 24 hours increases the stability of the cells cultured.)
    **Note 11:** Passage the cells once they have reached 80~90% confluent. (Cells seeded on Day 0 can be passaged on Day 7. For cells that are grown in a 5% $O_2$, 5% $CO_2$ incubator, cells can be passaged on Day 5.)

4. Morphology of feeder-free human iPSC (253G1) grown in ReproMed™ iPSC Medium (seeded at $5x10^5$ cells/dish and cultured in a 5% $CO_2$ incubator)




## Frequently asked questions

**Q1. How do I switch to ReproMed™ iPSC Medium from another feeder-free medium?**

A1. Switch to ReproMed™ iPSC Medium when passaging cells. After dissociating cells, centrifuge cells and aspirate to remove the supernatant. Resuspend the cells in ReproMed™ iPSC Medium for counting and seeding.

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