Instruction Manual

Feeder Cells (SL10)

Cat.RCHEFC001
Ver.1.0

Overview

This document is the thawing and plating protocol for SL10 feeder cells.

Use of this product

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.

Shipping and Storage

This product is shipped in the frozen state. The cells are highly sensitive to temperature shift, therefore, store the product in liquid nitrogen immediately upon receipt.

Features

・Used as feeder cells for human and mouse ES/iPS cells.
・These SL10 cells are treated with mitomycin C, and suitable for plating immediately after thawing.
・The cells are culture tested with human iPS cells (Takahashi, K., et al., *Cell*, 131, 861-72, 2007).

General note

- Do not thaw more than two vials at once for best result.
- Precisely follow the recommended centrifugation conditions of 170×g for 5 minutes at room temperature.
- Optimal SL10 cell density is dependent on the iPS cell line.
- Feeder cell density requirements may be different when cultivating with ReproNaïve™ media. It is necessary to consult the manual of ReproNaïve™.
- The cell density is optimized for 201B7 iPS cell line. The required cell density for other iPS cell lines may need to be optimized for best performance.
- Do not use feeder cells after 5 days. Older cells will not stabilize the undifferentiated state of iPSCs.
- Cell culture time may be different when using ReproNaïve™ media. It is necessary to consult the manual of ReproNaïve™.

Contents

Feeder cells (SL10)

Component	Cat. No.	Volume	Storage
Feeder cells SL10	RCHEFC001	3x10^{6}cell per vial (5 vials)	Liquid nitrogen

Required reagents and equipment

Product	Cat. No.	Volume	Storage
DMEM-high glucose	Sigma, Cat.D5796	500 mL	4℃
Fetal bovine serum (FBS)	GIBCO, Cat.10437	500 mL	4℃-
Sodium Pyruvate	Sigma, Cat.S8636	100 mL	4℃-
ReproCoat	ReproCELL, Cat.RCHEOT001	500 mL	4℃-
Standard cell culture equipment			

Feeder cells SL10 protocol

Coating dish/plate (Day –0)

1. Prepare new culture dish in advance by incubating with the required amount (see Table 1) of ReproCoat for 30 min at 37^{0}C in a CO_2 incubator.

Table 1. Dish size and required volume of ReproCoat

Dish size	Volume of ReproCoat
24-well	0.3 mL
12-well	0.5 mL
6-well/35 mm	1 mL
60 mm	3 mL
100 mm	5 mL

Preparation of medium

1. Add FBS and sodium pyruvate to DMEM-high glucose at final concentration of 10% and 1 mM, respectively (SL10 medium).

Thawing and seeding

1. Aliquot 9 mL of SL10 medium to 50 mL tube and warm to 37^{0}C in water bath (=Tube A). Move it to a cell culture safety cabinet.
2. Aliquot 40 mL of SL10 medium to 50 mL tube and warm to 37^{0}C in water bath (=Tube B). Move it to a cell culture safety cabinet.
3. Prepare an ice box full of dry ice.
4. Remove a cryogenic vial from the liquid nitrogen tank and place it into the dry ice box. Bring the ice box near to a water bath.
   Note 1. Do not thaw more than two vials at once for best results.
5. Transfer a cryogenic vial into a 37 ^{o}C water bath and incubate. Swirl the vial while warming in the water bath.
6. Dry the cryogenic vial, removing all traces of water. Transfer all cell suspension into Tube A by decantation as quickly as possible.
7. Add 1 mL of SL10 medium to the cryogenic vial to rinse the contents and add it to Tube A.
8. Centrifuge in 170×g for 5 minutes at room temperature.
   Note 2. The centrifugation conditions described above are critical.
9. Remove the supernatant.
10. Add 10 mL of SL10 medium from Tube B to

the pelleted SL10 cells and suspend.

11. Count the number of cells and adjust to 1.5 × 10^5 cells/mL using SL10 medium from Tube B. Save the remaining SL10 medium in Tube B for later use.
12. Remove the coating solution from the dish/ plate. And add cell suspension to desired culture dish/plate (Refer to table 2).
    Note 3. Optimal cell density is dependent on the cell line.
    Note 4. Required cell density is different in the cultivation when using ReproNaïve™ media. It is necessary to consult the manual of ReproNaïve™.
13. Swirl the dish to spread the cells evenly and incubate at 37°C in a 5% CO_2 incubator.
14. The cells are ready to be used as a feeder cell layer within 4 days.
    Note 5. Do not use feeder cells after 5 days. Older cells will not stabilize the undifferentiated state of iPSCs.

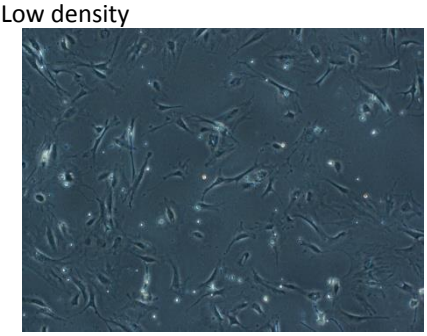
・ **Note 6.** Required cell culture time may be different when using ReproNaïve™ media. It is necessary to consult the manual of ReproNaïve™.

Table 2. Dish size and required volume of SL10 suspension
(for a seeding concentration of 1.5 x 10^5 cells/mL)

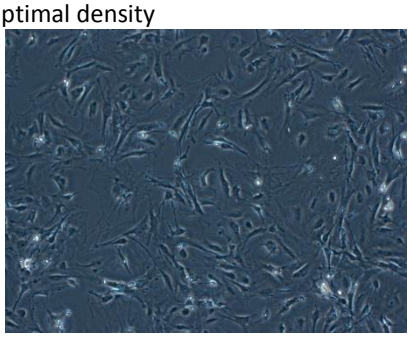
Dish size	Volume of cell suspension
24-well	0.3 mL
12-well	0.6 mL
6-well/35 mm	1.6 mL
60 mm	4 mL
100 mm	11 mL

Cell density at Day 1

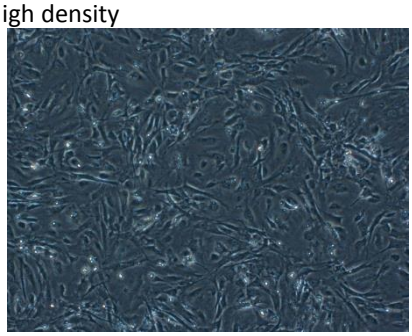
Low density



Optimal density



High density



Note 7. The cell density is optimized for 201B7 iPS cell line. The optimal cell density for other iPS cell lines may need to be optimized for best performance.

Related products

RCHEMD001	Primate ES Cell Medium
RCHEMD003, 004	ReproFF
RCHEMD005	Repro Stem
RCHEMD008	ReproNaïve
RCHETP002	Primate ES Cell Dissociation Solution
RCHEFM001	Primate ES Cell Freezing Medium
RCHEOT001	ReproCoat
RCHEOT002, 003	bFGF
RCHEOT004	Laminin-5
RCHEFC003	Feeder Cells (MEF)

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