Instruction Manual

Feeder Cells (MEF)

Cat.# RCHEFC003

Shipping and Storage

This product is shipped in the frozen state. The cells are sensitive to temperature shift, therefore, store the product under Liquid Nitrogen immediately upon receipt.

Features

・ Used as feeder cells for human and mouse ES/iPS cells.
・ Treated with Mitomycin C
・ The cells are culture tested with human iPS cells (Takahashi, K., et al., *Cell*, 131, 861-72, 2007).

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.

Procedure

The optimal culture conditions provided by MEF feeder cells for each ES/iPS cell line have to be determined empirically. For primate ES/iPS cell culture, 6 × 10^5 feeder cells every 60-mm dish is a good starting point. Adjust the density of the feeder cells according to your cell line and purpose of the experiment. To ensure good condition of the ES/iPS cells, please use feeder cells-coated dish 4 days of seeding.

Additional materials required

・Medium for MEF (Allow all reagents to equilibrate to room temperature before use.)
DMEM-high glucose/ 10% FBS(Penisillin-Streptomycin) (hereafter referred to as mdium)
・ReproCoat

Seeding

1) Prepare new culture dish in advance by incubating with the required amount (see Table 1) of ReproCoat for 30 min at 37℃ in a CO_2 incubator.
2) Prepare a 15-mL tube with 8 mL of medium at 4℃.
3) Thaw 1 vial of frozen MEF cells in a 37℃ water bath and transfer the contents to the 15-mL tube (from step 2). Add another 1 mL of medium to the vial in order to collect any remaining cells, and transfer the contents to the 15-mL tube.
4) Centrifuge the mixture at 170 x *g* (1,000 rpm) for 5 min, remove the supernatant, and re-suspend the cells in medium to make a cell suspension of the desired concentration (1.5 × 10^5 cells/mL).
5) Remove all of the ReproCoat solution from the dish. (prepared in step 1)
6) Add MEF cell suspension to the ReproCoat-treated dish. (See Table 2)
7) Incubate MEF cells overnight in a CO_2 incubator at 37℃
8) After overnight incubation, the MEF-coated dish can be used within 4 days.

Table 1. Dish size and required volume of ReproCoat

Dish size	Volume of ReproCoat
24-well	200～300 µL
12-well	500 µL
6-well/35 mm	1 mL
60 mm	2 mL
100 mm	4～5 mL

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

Dish size	Volume of cell suspension
24-well	0.4 mL
12-well	0.8 mL
6-well/35 mm	2 mL
60 mm	4 mL
100 mm	12 mL

Related products

RCHEMD001	Primate ES Cell Medium
RCHEMD003, 004	ReproFF
RCHEMD005	Repro Stem
RCHETP002	Primate ES Cell Dissociation Solution
RCHEFM001	Primate ES Cell Freezing Medium
RCHEOT001	ReproCoat
RCHEOT002, 003	bFGF
RCHEOT004	Laminin-5
RCHEFC001	Feeder Cells (SL10)

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