Applied StemCell, Inc.
(866) 497-4180
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Datasheet

Mouse Induced Pluripotent Stem Cells (Retroviral)

Order information

Catalog Number	Quantity
ASE-9107	2-5 x 10^5 cells/ vial

Product Information

Description

Applied StemCell, Inc. provides mouse Induced Pluripotent Stem (iPS) cells. The mouse iPS cell line (induced pluripotent stem cell line) was derived from mouse embryonic fibroblasts (MEFs) by retroviral expression of Oct3/4, Sox2, Klf4 and c-Myc genes. The cells were derived using morphological selection criteria and without the use of fluorescent marker or drug selection. When cultured under standard mouse ES cell culture conditions, the morphology of mouse iPSCs are identical to that of mouse ES cells. The cells also express the pluripotency markers SSEA-1 and Nanog, and demonstrate strong endogenous alkaline phosphatase activity. Mouse iPS cells are grown on a feeder layer of mouse embryonic fibroblasts (MEFs) and require the pretreatment of the plate with Gelatin.

Mouse Strain

C57BL/6J

Shipping

Dry ice

Storage and Stability

Store in liquid nitrogen freezer immediately upon receipt. This product is stable for at least 6 months from the date of receiving when stored as directed.

Quality Control

Mouse iPS cells were grown in mouse ES medium supplemented with 10^3 U/ml LIF. Each lot of mouse iPS cells is tested for growth and viability following recovery from cryopreservation. In addition, each lot is tested for expression of SSEA-1 and Nanog, as well as the activity of alkaline phosphatase.

Safety Precaution

PLEASE READ BEFORE HANDLING ANY FROZEN VIALS. Please wear the appropriate PPE (**P**ersonal **P**rotection **E**quipment: lab coat, thermal gloves, safety goggles and a face shield). Handle the frozen vials with due caution. Please be aware that the following scenario can occur: Liquid nitrogen can leak into the vials when the vials are submerged in liquid nitrogen. Upon thawing, the liquid nitrogen returns to the gas phase, resulting in a dangerous build-up of pressure within the vial. This can result in the vial exploding and expelling not only the vial contents but also the vial cap and plastic fragments of the vial.

Restricted Use

This product is for research use only and not intended for human or animal diagnostic or therapeutic uses.

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Protocol

Mouse iPS Cell Culture

This protocol can be used for culturing mouse iPS cells. Mouse iPS cells were generated by transducing source cells with retroviruses individually encoding the four mouse transcription factors (Oct4, Sox2, Klf4, and c-Myc) that have been shown to induce the reprogramming of mouse embryonic fibroblasts into a pluripotent state. The cells were derived using morphological selection criteria and without the use of fluorescent markers or drug selection. When cultured under standard mouse ES cell culture conditions, the morphology of mouse iPS cells is identical to that of mouse ES cells. The cells also express the pluripotency markers SSEA-1 and Nanog, and demonstrate a strong endogenous AP activity.

Preparation of culture medium

- **MEF medium** DMEM containing 10% FBS, 2 mM glutamine, 1x 10^{-4} M nonessential amino acids, and 50 U and 50 mg /ml penicillin and streptomycin.
- **Mouse ES medium** KO-DMEM containing 15% ES-FBS, 2 mM glutamine, 1 x 10^{-4} M nonessential amino acids, 1 x 10^{-4} M 2-mercaptoethanol, 10^3 U/ml LIF, and 50 U and 50 mg /ml penicillin and streptomycin.
- **2X Freezing medium** 20% DMSO plus 80% FBS

Mouse Embryonic Fibroblasts (MEF) may be purchased from Applied StemCell, Inc. (untreated, Mitomycin C treated, or irradiated) in a variety of sizes and drug resistance options including DR4, CF-1, Neo-Resistant, or SNL 76/7. For ordering and information please see: **http://www.appliedstemcell.com/products/cell-culture/mef-feeder-cells/**

Culture Conditions for MEF

Gelatin treatment of plates

1. Add enough sterile/autoclaved 0.1% gelatin to cover the bottom of the wells. Approximate amounts:

Plate/Dish	Amount/Well
96 well	100 mL
48 well	300 mL
24 well	0.5 mL
12 well	1.0 mL
6 well	1.5-2.0 mL
30 mm	1.5-2.0 mL
60 mm	3.0 mL
100 mm	4.0-5.0 mL

2. Incubate the gelatin-coated dishes for at least 15 min at 37 °C.
3. Aspirate excess gelatin solution before using.

Thawing MEF cells

To insure the highest level of viability, be sure to warm medium to 37 °C before using it on the cells. Cells should be plated at a minimum cell density of 1 x 10^4 cells/cm^2.

1. Remove the vial from liquid nitrogen and thaw quickly in 37 °C water bath.
2. Remove the vial from the water bath as soon as the cells are half way thawed, and sterilize by spraying with 70% ethanol.
3. Transfer the cells with 10 ml of MEF medium to a 15-cm conical tube and pellet the cells by centrifugation at 200x g for 5 min.
4. Discard the supernatant and re-suspend the cells with 10 ml fresh MEF medium and plate the cells at seed density of 1 x 10^4 cells/cm^2.

5. Incubate at 37 °C with 5% CO_2 in air atmosphere, until the cells reach 80-90% confluency.
6. Change media twice a week or when pH decreases.

Culture Condition for Mouse iPS Cells

Thawing mouse iPS cells

To insure the highest level of viability, be sure to warm medium to 37 °C before using it on the cells.

1. Remove the vial from liquid nitrogen and thaw quickly in 37 °C water bath.
2. Remove the vial from the water bath as soon as the cells are half way thawed, and sterilize by spraying with 70% ethanol.
3. Transfer the cells with 10 ml of mouse ES medium to a 15-cm conical tube and pellet the cells by centrifugation at 200X g for 5 min.
4. Discard the supernatant, re-suspend the cells with fresh mouse ES medium, and *plate the cells in the wells of 6-well plate with MEF feeder cells.*
5. Incubate at 37 °C with 5% CO_2 in air atmosphere, until the cells reach 80% confluency.
6. Change the medium every day or when pH decreases.

Maintenance of mouse iPS cells

It is important to note that do NOT keep mouse iPS cells in culture for long periods in order to maintain the pluripotency.

1. Aspirate the medium, and wash the cells twice with 1 ml of PBS.
2. Remove PBS completely, add 0.5 ml of 0.25% trypsin-EDTA solution, and incubate at 37 °C for 2 min.
3. While incubating, remove a 6-well plate with mitomycin C treated (or irradiated) MEFs from the incubator. Aspirate MEF medium and add 2 ml of mouse ES medium to each well.
4. Remove the plate containing mouse iPS cells from the incubator and swirl to dislodge the cells from the bottom of the plate.
5. Add 1 ml of ES medium to the plate and suspend the cells by pipetting up and down to single cell suspension.
6. Distribute 0.2 ml of the mouse iPS cell suspension to each well of the 6-well plate. Right after plating iPS cells, gently swirl the plate back-and-forth and side-to-side and incubate at 37°C. The ES media must be changed every day and mouse iPS cells sub-cultured every 2-3 d. ***Track passage number of iPS cells***.

Freezing mouse iPS cells

1. Grow cells to the exponential phase in a 6-well plate.
2. Aspirate the medium, and wash the cells twice with 2 ml of PBS.
3. Add 0.5 ml of 0.25% trypsin-EDTA and incubate 2 min at 37 °C.
4. Add 2 ml of mouse ES medium, and suspend the cells by pipetting up and down to single cell suspension.
5. Transfer the cell suspension to a 15-ml conical tube, count the number of cells and spin the cells at 200x g for 5 min.
6. Discard the supernatant, and re-suspend the cells with mouse ES medium to the concentration at 1 x 10^6 cells per ml.
7. Add equal volume of 2X freezing medium (20% DMSO and 80% FBS), and aliquot it at 1 ml per vial.
8. Put the vials in a cell-freezing container, and store the vials at –80°C overnight.
9. Transfer the vials to liquid nitrogen for long-term storage.