Product Specification Sheet

Product Name	**Mouse Induced Pluripotent Stem Cells**
Description	The mouse iPS (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.
Catalog Number	iPS02m
Size	2 x 10^5 cells/vial
Shipping	Dry Ice
Storage and Stability	Store in gas phase of liquid nitrogen immediately upon receipt. This product is stable for 6 months when stored as directed.
Quality Control	Mouse iPS cells were grown in mouse ES medium supplemented with 103 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	**ALSTEM highly recommends that protective gloves, a lab coat, and a full-face mask always be worn when handling frozen vials.** It is important to note that some liquid nitrogen can leak into the vials when submersed in liquid nitrogen. Upon thawing, the liquid nitrogen returns to the gas phase, resulting in excessive pressure within the vial that can cause the vial to explode or expel the cap with dangerous force.
Restricted Use	For Research Use Only. Not for use in diagnostic or therapeutic procedures.

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Protocol (iPS02m)

Mouse iPS Cell Culture

Overview

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.

Procedure

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, 103 U/ml LIF, and 50 U and 50 mg /ml penicillin and streptomycin.

2X Freezing medium 20% DMSO plus 80% FBS.

CULTURE CONDITION FOR MOUSE FIBROBLASTS

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 µl
48-well	300 µl
24-well	0.5 ml
12-well	1 ml
6-well	1.5-2 ml
30 mm	1.5-2 ml
60 mm	3 ml
100 mm	4-5 ml

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

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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 ml conical tube and pellet the cells by centrifugation at 200 x *g* for 5 min.
4. Discard the supernatant and resuspend 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.

Passage of MEF cells

Cells should be split when they reach confluency. A split based on seed density of 0.5 x 10^4 cells /cm^2 is recommended.

1. Discard the medium and wash the cells twice with PBS.
2. Aspirate PBS, and add 1 ml per T75 flask of 0.05% trypsin-EDTA, and incubate for 2 min.
3. Add 5 ml of MEF medium, and break up the cell clumps by gently pipetting up and down several times.
4. Transfer cells into a conical tube and centrifuge at 200 x *g* for 5 min.
5. Discard the supernatant, and resuspend the cell pellet in 10 ml MEF medium.
6. Count the number of cells, plate cells at 0.5 x 10^4 cells/cm^2, and incubate at 37° C with 5% CO_2.

Freezing MEF cells

1. Follow steps 1-4 from "Passage of MEF cells."
2. Discard the supernatant, and resuspend the pellet in MEF medium. Add approximately 1 ml for each T75 flask.
3. Count the number of cells and dilute the cell suspension to 1 x 10^7 cells/ml.
4. Add an equal volume of cold 2X Freezing Media (containing 20% DMSO and 80% FBS) to the cell suspension.
5. Aliquot 1 ml of suspension into each cryovial (5 x 10^6 cells/vial).
6. Place the vials in a cell-freezing container and keep it at -80° C overnight.
7. Transfer the vials to a liquid nitrogen tank for long-term storage.

Mitomycin C treatment of MEF

At confluence, MEF cells are treated with mitomycin C to halt the division of the cells when they are still able to condition the medium as the feeder layers for iPS or ES cells.

1. Add 6 ml of fresh MEF medium containing 50 µl of mitomycin C solution (1 mg/ml) to one T75 flask of confluent MEF cells, and swirl it briefly.

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2. Incubate at 37° C for at least 3 hours.
3. After incubation, aspirate the mitomycin C-containing medium off the cells, and wash the cells twice with 10 ml of PBS.
4. Aspirate off PBS, add 1 ml of 0.05% trypsin-EDTA, swirl to cover the entire surface, and incubate for 2 min at room temperature.
5. Add 5 ml of MEF medium, and break up the cells to a single cell suspension by pipetting up and down. Count the number of cells. Seed the cells on gelatin-coated dishes (1×10^6 cells per 100-mm dish, or 1.5×10^5 cells per well of 6-well plate).
6. Cells should be ready to use by the next day.

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 200 x *g* for 5 min.
4. Discard the supernatant, resuspend 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 everyday 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 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 days. 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 200 x *g* for 5 min.
6. Discard the supernatant, and resuspend 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.

WARNING

Do not use cryogenic vials for storage in the liquid phase of liquid nitrogen. Such use may cause entrapment of liquid nitrogen inside the vial and lead to pressure buildup resulting in possible explosion or biohazard release. Use appropriate safety procedures which are outlined by the ATCC when handling and disposing of vials. ALSTEM highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials.

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