Mouse iPS Cell Lines (Matched Sets)

Cat # SC201A-1

User Manual

Store kit at -80°C on receipt

(ver. 3 - 112113)

Contents

List of Components

Each iPS cell line set comes as one vial of MEF-derived mouse iPS cells and one vial of MEF at 2 x 10^5 cells each.

SC201A-1	Mouse iPS Cell Line Set [MEF & iPS cells]	2x10^5 cells each

The product is shipped on dry ice and should be immediately stored in the **gas phase** of liquid nitrogen.

In general, iPS cells are challenging to culture and should only be operated by researchers experienced in the intricacies of mouse embryonic stem (mES) cell culture. The methods for culture are nearly identical to mES cell culture, although more careful maintenance will be required.

The MEF cells provided in the iPSC kit are meant to be used as **source cells only** and not as feeder cells for culturing iPS cells. MEF cells for feeder cells can be obtained from Applied Stemcell, Inc (http://www.appliedstemcell.com).

I. Mouse iPS Cells

A. Description

Mouse induced pluripotent stem cells (iPSCs, Cat# SC201A-iPSC) were generated by transducing genetically unmodified mouse embryonic fibroblasts with viruses individually encoding the four murine transcription factors (Oct4, Sox2, Klf4, and c-Myc) that have been shown to induce the reprogramming of somatic cells to 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 SBI 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 AP activity.

Mouse iPS cells should be grown on a feeder cell layer. Appropriate feeder cells for mouse iPS cells are mouse embryonic fibroblasts (MEFs, available from Applied Stemcell, Inc (http://www.appliedstemcell.com/).

Mouse iPS cells from SBI are provided at passage 10 and can be passaged 50 times before differentiation.

B. Culture Conditions for MEF Feeder Cells

1. Required media and reagents

Reagent	Information
MEF Medium	DMEM containing 10% FBS, 2 mM glutamine, $1x10^{-4}$ M nonessential amino acids and 50 U and 50 µg/ml penicillin and streptomycin.
2x Cold Freezing Media	20% DMSO and 80% FBS
Mitomycin C solution	1 mg/ ml

2. Gelatin treatment of plates for MEF feeder cells

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

Approximate amounts: 10cm plate – 5 ml
6 well – 1.5 ml/ well
24 well – 0.5 ml/ well
96 well – 100 µl/ well

2) Incubate the gelatin-coated dishes for at least 15 min at 37°C.

3) Aspirate excess gelatin solution before use.

3. 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 10^4 cells/ cm^2.

1) Remove the vial from liquid nitrogen and thaw quickly in a 37°C water bath.

2) Remove the vial from the water bath as soon as the cells are half-thawed, and sterilize the tube 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 *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 10^4 cells/ cm^2.

5) Incubate at 37°C with 5% CO_2, until the cells reach 80-90% confluency.

6) Change medium twice a week or when pH decreases.

4. Passaging MEF cells

Cells should be split when they reach confluency. We recommend splitting the cells based on $0.5x10^4$ cells/ cm^2.

1) Discard the medium and wash the cells once with PBS.

2) Aspirate PBS, and add 2 ml per T75 flask of 0.25% 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 *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 $0.5x10^4$ cells/ cm^2 and incubate at 37°C with 5% CO_2.

5. Freezing MEF cells

1) Follow steps 1-4 from the Passaging MEF cells protocol (above).

2) Discard the supernatant, and resuspend the pellet in MEF medium.

3) Count the number of cells and adjust the cell suspension to 4×10^6 cells/ ml.

4) Add equal volume of cold 2X Freezing Media to the cell suspension.

5) Aliquot 1 ml of suspension into each cryovial (2×10^6 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.

6. Mitomycin C treatment of MEF

Mitomycin C acts to halt the division of MEF cells so that they can be used to condition the medium for human iPS cells. MEF cells should be at confluence when treated with mitomycin C.

1) Add 6 ml of fresh MEF medium contain 50 µl of mitomycin C solution (1 mg/ ml) to one T75 flask of confluent MEF cells, and swirl it briefly. The final concentration of mitomycin C is 8 µg/ ml.

2) Incubate at 37°C for at least 3 hrs.

3) Aspirate the mitomycin C-containing medium off the cells and wash the cells twice with 10 ml PBS.

4) Aspirate PBS and add 1 ml of 0.25% trypsin-EDTA, swirl to cover the entire surface, and incubate for 2 min at room temperature.

5) Add 5 ml MEF medium and break up the cells to a single-cell suspension by pipetting up and down. Count the number of cells.

6) Seed the cells on gelatin-coated dishes (3×10^6 cells per 100-mm dish, or 5×10^5 cells per well of a 6-well plate).

7) Cells should be ready to use by the next day.

C. Growth Conditions for mouse iPS cells

1. Required Media and Reagents

Reagent	Information
Mouse ES Medium	DMEM containing 15% FBS, 2 mM glutamine, 0.1M nonessential amino acids, 0.1 mM 2-mercaptoethanol, 10^3 U/ml LIF, 50 U and 50 µg/ ml Penicillin and Streptomycin
2x Cold Freezing Media	20% DMSO and 80% FBS
Trypsin-EDTA	GIBCO

1. 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 a 37°C water bath.

2) Remove the vial from the water bath as soon as the cells are half-thawed, and sterilize by spraying with 70% ethanol.

3) Transfer the cells with 10 ml of mouse ES medium to a 15 mL conical tube and pellet the cells by centrifugation at 200 *g* for 5 min.

4) While centrifuging, remove MEF medium from the feeder cell plates, and wash the wells twice with DMEM. Then add 1 ml of mouse ES Medium.

5) Discard the supernatant from the mouse iPS cells, and resuspend cells with 1 ml fresh mouse ES medium. Plate the cells on MEF feeder cells in a 6-well plate.

6) Incubate at 37°C with 5% CO_2 until the cells reach 80% confluency. The ES media must be changed every day.

2. Maintenance of mouse iPS cells

It is important not to keep mouse iPS cells in culture for long period of time without passaging, to maintain the pluripotency.

1) Aspirate the medium and wash the cells twice with 1 ml PBS.

2) Remove PBS completely and 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 of feeder cells (mitomycin C-treated MEFs). Aspirate the 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 2 ml of mouse ES medium, and suspend the cells by pipetting up and down to single cell suspension.

6) Transfer the cell suspension to a 15 ml conical tube and spin the cells at 200 *g* for 5 min.

7) Add 2 ml of ES medium to the plate and suspend the cells by pipetting up and down to single cell suspension.

8) 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 with 5% CO2 until the cells reach 80% confluency.

9) The ES media must be changed every day and mouse iPS cells subcultured ~1:10 every 2-3 days. Track the passage number of the iPS cells.

3. 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 0.25% trypsin-EDTA and incubated for 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 *g* for 5 min.

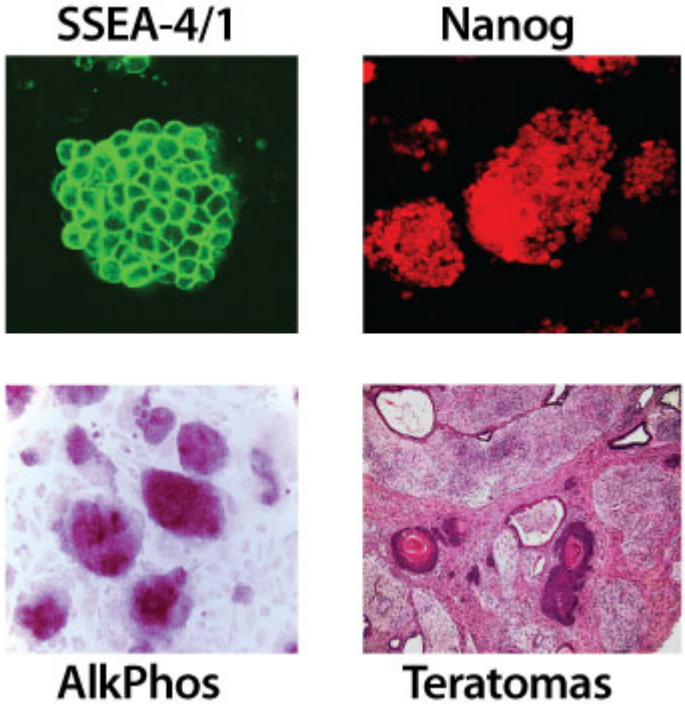
6) Discard the supernatant, and resuspend the cells with mouse ES medium to the concentration of $1x10^6$ cells per ml.

7) Add equal volume of 2x freezing medium and aliquot it at 0.5 ml per vial.

8) Put the vials in a cell-freezing container, and store them at -80°C overnight.

9) Transfer the vials to a liquid nitrogen tank for long-term storage.

D. Validation of mouse iPS cells



Stem cell markers for SSEA-1 and Nanog were determined by immunocytochemistry using primary antibodies for SSEA1 (Millipore) and Nanog (Abcam) followed by Alexa Fluor fluorescent-labeled secondary antibodies (Invitrogen). Detection of Alkaline Phosphatase activity was performed using the AP Detection Kit (Millipore). Teratoma assays were performed by subcutaneous injection of $1x10^6$ iPS cells into the dorsal flank of SCID mice. Teratomas were visualized 4 weeks later with hematoxylin and eosin staining.

II. References

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III. Technical Support

For more information about SBI products or to download manuals in PDF format, please visit our website:

http://www.systembio.com

For additional information or technical assistance, please call or email us at:

tech@systembio.com

650-968-2200

IV. Licensing and Warranty Statement

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