ProMab Biotechnologies, Inc.

Protocol

Human Retroviral iPS Cell Culture Feeder Free Culture Conditions

OVERVIEW

This protocol can be used for culturing human iPS cells. Footprint-free human iPS cells were generated by transiently introducing episomal plasmids encoding the human transcription factors into human foreskin fibroblasts. The cells were derived using morphological selection criteria and without the use of fluorescent markers or drug selection. When cultured under standard human ES cell culture conditions, the morphology of footprint-free human iPS cells is identical to that of human ES cells. The cells also express the pluripotency markers SSEA-3 and Nanog, and demonstrate a strong endogenous AP activity.

PROCEDURE

It is highly recommended 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.

Feeder free culture conditions

Preparation of feeder-free medium

1. Thaw mTeSR1 5X Supplement (Cat.no. 05850, STEMCELL Technologies) at room temperature or overnight at 4°C.
2. Add 100 mL of the thawed 5X Supplement to 400 mL Basal Medium for a total volume of 500 mL aseptically. Mix well. Filter through a 0.2 µm, low-protein binding filter, if desired.
3. Aliquot into appropriate amount according to usage and store the aliquots at 4°C.

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Coating plates with Matrigel

Matrigel (Cat.no. 354277, BD) should be aliquoted and stored at -80°C for long-term use.

1. Thaw Matrigel on ice completely. Dilute Matrigel 1:30 to 1:50 with pre-chilled KO DMEM/F12 (Cat.no. 12660-012, Invitrogen).
2. Immediately coat tissue culture-treated plates with the diluted Matrigel solution. For a 6-well plate, use 0.8 mL of diluted Matrigel solution per well, and swirl the plate to spread the Matrigel solution evenly across the surface.
3. Let the coated plate stand for 1 h at 37°C or overnight at 4° C. If plate has been stored at 4°C, incubate the plate at 37°C for at least 30 minutes before removing the Matrigel solution.

Thawing cryopreserved human iPS cells

1. One hour before thawing human iPS cells, coat one well of a 6-well plate with 0.8 mL of diluted Matrigel solution per well, swirl the plate to spread the Matrigel solution evenly across the surface, and incubate 1 h at 37°C.
2. Quickly thaw the human iPS cells in a 37°C water bath by gently shaking the cryovial continuously until half thawed. Remove the cryovial from the water bath and spray with 70% ethanol.
3. Transfer the contents of the cryovial to a 15 mL conical tube. Add 5 mL warm mTeSR1 dropwise to the tube, mixing gently.
4. Centrifuge cells at 200 x g for 5 minutes at room temperature.
5. After centrifugation, aspirate the medium from 15 mL tube. Gently resuspend the cell pellet in 2 mL of mTeSR1 containing 10 µM ROCK inhibitor (Y-27632, Cat.no. Y-05, StemRD), taking extra care to maintain the cells as small cell clumps.
6. Remove the Matrigel solution from a coated 6---well tissue culture plate. Transfer the medium containing the cell clumps to the Matrigel coated 6-well plate immediately.

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7. Place the plate in a 37°C incubator and rock the plate in quick side to side, forward to back motions to evenly distribute the clumps in the well. Culture the cells at 37°C, with 5% CO2 and 95% humidity.
8. Change medium daily. Check for undifferentiated colonies that are ready to passage when colonies are big enough (approximately 7-10 days after thawing).

Passaging human iPS cells under feeder-free conditions

1. Use a microscope to identify regions of differentiation. Mark the differentiated colonies using lens marker on the bottom of the plate.
2. Remove regions of differentiation by scraping with a pipette tip or by aspiration.
3. Aspirate medium from the human iPS cell culture and rinse with DPBS (2 mL/well, Cat.no. 14190 ,Invitrogen).
4. Add 0.5 mL per well of accutase (Cat.no. SCR005, Millipore, diluted 1:1 with DPBS before use). Let it stand at room temperature for 1 minute.
5. Remove accutase, and gently rinse each well 2 - 3 times with 2 mL of DMEM/F-12 per well to remove remaining enzymes.
6. Add 2 mL/well mTeSR1 and scrape colonies off with a cell scraper.
7. Transfer the detached cell aggregates to a 15 mL conical tube and rinse the well with an additional 2 mL mTeSR1 to collect any remaining aggregates. Add the rinse to the 15 mL tube.
8. Centrifuge the aggregates at 200 x g for 5 minutes at room temperature.
9. Aspirate the supernatant. Resuspend pellet in mTeSR1 containing 10 µM ROCK inhibitor by gently pipetting and ensure that cells are maintained as aggregates.
10. Plate the human iPS cell aggregates with mTeSR1 onto a new Matrigel-coated plate. (Remove Matrigel solution before plating). If the colonies are at an optimal density, the cells can be split every 5 - 7 days using 1:3 to 1:6 ratio.
11. Rock the plate in quick side to side, forward to back motions to distribute the clumps evenly in the wells. Place the plate into the 37°C incubator with 5% CO2 and 95% humidity.
12. Change medium daily.