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-dependent culture conditions

Preparation of human ES medium
Knockout DMEM/F12 (Cat.no. 12660-012, Invitrogen) containing 20% knockout serum replacement (Cat.no. 10828-028, Invitrogen), 2mM glutaMAX (Cat.no. 35050-061, Invitrogen), 0.1 mM nonessential amino acids (Cat.no. 11140-050, Invitrogen), 0.1 mM 2-mercaptoethanol (Cat.no. 21985-023, Invitrogen), 10 ng/ml bFGF (Cat.no. 233-FB-025, R&D Systems), and 50 U and 50 µg/ ml penicillin and streptomycin (Cat.no. 15140-122, Invitrogen).

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Thawing cryopreserved human iPS cells

Warm medium to 37°C before use to insure the highest level of cell viability. Due to the low survival rate of cryopreserved human iPS cells, the recovery is expected to take at least one week.

1. Quickly thaw the human iPS cells in a 37°C waterbath by gently shaking the cryovial continuously till half thawed. Remove the cryovial from the waterbath and spray with 70% ethanol.
2. Transfer the contents of the cryovial to a 15 mL conical tube. Add 5 mL warm human ES medium dropwise to the tube, mixing gently while adding medium.
3. Centrifuge cells at 200 x g for 5 minutes at room temperature.
4. While centrifuging, remove MEF medium from the feeder cell plates, and wash the wells twice with Knockout DMEM/F12. Then add 1 ml of human ES Medium with 10 µM ROCK inhibitor (Y-27632, Cat.no. Y-05, StemRD) to one well of 6-well plate.
5. After centrifugation, aspirate the medium from 15 mL tube. Gently resuspend the cell pellet in 1 mL fresh human ES medium containing 10 µM ROCK inhibitor (Y-27632), taking care to maintain the cells as small cell clumps.
6. Transfer the medium containing the cell clumps to the well on the 6-well plate with MEF feeder cells.
7. Rock the plate gently to distribute the clumps evenly in the wells. 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-dependent conditions

1. Aspirate the medium and wash the cells twice with 1 ml of PBS.
2. Remove PBS completely and add 0.5 ml of Accutase (Cat.no. SCR005, Millipore, diluted 1:1 with DPBS before use) and incubate for 1-2 min at room temperature.
3. Tap the bottom of the plate to dislodge the cells from the bottom of the plate. Then aspirate the supernatant.

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4. Add 1 ml of DMEM/F12 to the plate and gently wash off the feeder cells. Remove the supernatant. Repeat.
5. Add 1 ml of human ES medium containing 10 µM ROCK inhibitor to the plate and suspend the cell colonies by pipetting up and down, or using a cell lifter (Cat.no. 3008, Corning) to harvest human iPS cells. It is important not to break up the colonies into single cells.
6. Remove a plate of MEF feeder cells from the incubator. Aspirate the MEF medium. Wash once with KO DMEM/F12 medium.
7. Distribute 0.2 – 0.3 ml of the human iPS cell suspension to each well of a 6-well plate. Add human ES medium with ROCK inhibitor to a final volume of 2 ml per well. Right after plating the iPS cells, gently rock the plate and incubate at 37°C.
8. After 24 hours, remove the media and replace with human ES media (without ROCK inhibitor).
9. The human ES media must be changed every day and human iPS cells subcultured every 5-7 days. Track the passage number of the cells.