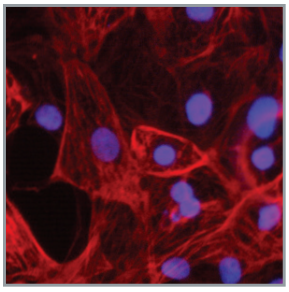

Stem Cell Application Protocol

Transfer of Human iPS Cells from Mouse Embryonic Fibroblast (MEF) Feeder Cells

To the Cellartis® DEF-CS™ 500 Culture System

I. Introduction

Undifferentiated human iPS cells maintained on MEF feeder cells can be readily transferred to the feeder-free DEF-CS Culture System. Cryopreserved, MEF-cultured human iPS cells can be thawed directly using the DEF-CS Culture System. Fresh cultures should be transferred on days when they would normally be passaged.

Materials Required

- Cellartis DEF-CS 500 Culture System (includes COAT-1, Basal Medium, GF-1, GF-2, and GF-3)
- TrypLE Select Enzyme (1X), w/o phenol red
- PBS Dulbecco's with Ca^{2+} & Mg^{2+} (D-PBS +/+)
- PBS Dulbecco's w/o Ca^{2+} & Mg^{2+} (D-PBS –/–)
- Cell culture vessels, tissue culture-treated polystyrene surface

II. Protocol

A. Transferring Fresh or Frozen Cultures to the DEF-CS System

Coating of Cell Culture Vessels

1. Dilute the required volume of DEF-CS COAT-1 in D-PBS +/+ before use. Make a 1:10 dilution.
2. Mix the diluted DEF-CS COAT-1 solution gently and thoroughly by pipetting up and down.
3. Add an appropriate volume of diluted DEF-CS COAT-1 solution to a cell culture vessel (use 0.1 ml/cm^2), making sure that the entire surface is covered.
4. Incubate the cell culture vessel at 37°C ± 1°C, 5% CO_2, and >90% humidity for a minimum of 20 min, or at room temperature (15–25°C) for 0.5–3 hr.
5. Aspirate the DEF-CS COAT-1 solution from the cell culture vessel immediately before use.

Preparing Supplemented DEF-CS Medium

1. Prepare the appropriate volume of supplemented DEF-CS medium by adding DEF-CS GF-1 (dilute 1:333), GF-2 (dilute 1:1,000), and GF-3 (dilute 1:1,000) to DEF-CS Basal Medium.
2. Prepare fresh medium on the day of intended use and warm it to 37°C ± 1°C immediately before use. Discard any leftover warm medium.
3. Warm all other necessary reagents to room temperature (15–25°C) before use.

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Transferring hiPS Cells from a Fresh MEF Feeder Culture

NOTE: Fresh cultures should be transferred on days when they would normally be passaged, using a consistent culture area (e.g. cells cultured on MEF feeder cells in a 35-mm dish should be transferred to a 35-mm dish for culturing with DEF-CS).

1. Check MEF-cultured colonies under a microscope; photo document as necessary.
2. Aspirate the medium from the cell culture vessel and wash the cell layer once with D-PBS –/–.
3. Add 20 µl/cm^2 of TrypLE Select Enzyme (1X) to the cell culture vessel and incubate for 5 min, or until the cell layer has detached. Detachment can be aided by swirling the cell culture vessel or by tapping the side of the cell culture vessel firmly but gently. **Any *inactivated* MEF-cells that are detached and passaged to the DEF-CS Culture System can be neglected, as they will NOT affect the result.**
4. Resuspend the cells in pre-warmed, supplemented DEF-CS medium (40 µl/cm^2) and pipet up and down several times to ensure a single-cell suspension. (The cells will aggregate if left too long in TrypLE Select Enzyme).
5. Centrifuge the cells at 200*g* for 2–5 min.
6. Discard the supernatant carefully and resuspend the cell pellet in the appropriate volume of pre-warmed, supplemented DEF-CS medium. Add the cell suspension to the newly coated cell culture vessel.
7. Tilt the vessel backwards and forwards gently to ensure that the cell suspension is dispersed evenly over the surface, then place in an incubator at 37°C ± 1°C, 5% CO_2, and >90% humidity.

Transferring Cryopreserved, MEF-Cultured hiPS Cells

NOTE: Thaw the cells onto the same culture area you would use for thawing the cells onto an MEF feeder layer.

1. Thaw the cells according to your preferred protocol.
2. Transfer the cells to a newly coated cell culture vessel with pre-warmed, supplemented DEF-CS medium.
3. Tilt the vessel backwards and forwards gently to ensure that the cell suspension is dispersed evenly over the surface, then gently place in an incubator at 37°C ± 1°C, 5% CO_2, and >90% humidity.

B. Scaling Up

NOTE: The single-cell passaging method employed by the DEF-CS Culture System causes iPS cells to initially assume a distinct morphology and sparser distribution relative to cells cultured using colony-based passaging methods. However, as the cells proliferate and form denser populations, morphologies commonly associated with undifferentiated stem cells (e.g., high nucleus-to-cytoplasm ratio, clearly defined borders, and prominent nucleoli) emerge.

- It may take 2–5 passages to adapt a cell line to the DEF-CS Culture System. Newly transferred cells might initially grow at a slightly slower rate. A suitable passage interval might therefore be between 3 and 7 days for the first few passages.
- Use a 1:10 dilution of DEF-CS COAT-1:D-PBS +/+ for the first few passages to provide extra support during the adaptation process.
- To prevent cell loss during scale-up, we recommend not counting cells at passage when the total number of cells is quite low.
- If the hiPS cells were sparsely seeded or thawed in aggregates, they will grow as colonies on COAT-1. As a general rule, when passaging hiPS cells that are growing as colonies, the area covered by the cells at passage should not be less than 20% of the area of the destination vessel.
- For passages involving cells growing in a homogeneous monolayer (normal DEF-CS Culture System characteristics), cells are ready for passage when they have acquired the morphology displayed in Figures 3 and 4 in the Cellartis DEF-CS 500 Culture System User Manual. However, if cells remain sparsely distributed after seven days in culture, a passage is still recommended. The area of the destination vessel should be 3–6 times the area of the current vessel.
- Once cells have been scaled up to a T-25 flask, they should be cultured according to the Cellartis DEF-CS 500 Culture System User Manual.