gibco
by life technologies

Gibco® Episomal hiPSC Line

Description

The Gibco® Episomal hiPSC line is a zero-footprint, viral-integration-free human induced pluripotent stem cell (iPSC) line generated using cord blood-derived CD34+ progenitors with seven episomally expressed factors (Oct4, Sox2, Klf4, Myc, Nanog, Lin28, and SV40 T). The iPSC line was cultured on mouse feeder cells and subsequently adapted to feeder-free, serum-free culture conditions. Whole genome expression and epigenetic profiling analyses demonstrated that the Gibco® Episomal hiPSC line is molecularly indistinguishable from human embryonic stem cell lines. In directed differentiation and teratoma analyses, the hiPSCs retained their differentiation potential for the ectodermal, endodermal, and mesodermal lineages. In addition, viral-free vascular, hematopoietic, neural, and cardiac lineages can be derived with robust efficiencies (Burridge *et al*., 2011). Grown in Essential 8™ medium under feeder-free conditions, the Gibco® Episomal hiPSC line can be used as a positive control in reprogramming procedures for iPSC generation.

Product	Catalog No.	Amount	Storage
Gibco® Episomal hiPSC Line	A18945	1×10^6 viable cells/vial	Liquid nitrogen (vapor phase)

Product Use

For Research Use Only. Not for use in diagnostic procedures.

Important Information

Gibco® Episomal hiPSCs cultured in Essential 8™ Medium on vitronectin- or Geltrex® matrix-coated culture vessels should be passaged using Versene solution (Cat. no. 15040), which is 0.48 mM EDTA in PBS. Alternatively, cell passaging can be performed using 0.5 mM EDTA prepared in Dulbecco's Phosphate-Buffered Saline (DPBS) without calcium or magnesium (Cat. no. 14190). The use of enzymes, such as collagenase and dispase, for passaging these cells results in compromised viability and attachment.

Safety Information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Culture Conditions

Media: Complete Essential 8™ medium (Cat. no. A1517001)

Culture Type: Adherent, feeder-free, serum-free

Recommended Substrate: Vitronectin (VTN-N) (Cat. no. A14700) or Geltrex® hESC-qualified Reduced Growth Factor Basement Membrane Matrix (Cat. no. A1413301)

Temperature Range: 36°C to 38°C

Incubator Atmosphere: Humidified atmosphere of 5% CO_2. Ensure that proper gas exchange is achieved in culture vessels.

Prepare Complete Essential 8™ Medium (500 mL)

1. Thaw the frozen Essential 8™ Supplement at 2°C to 8°C overnight. **Do not thaw the frozen supplement at 37°C.**
2. Mix the thawed supplement by gently inverting the vial a couple of times, remove 10 mL from the bottle of Essential 8™ Basal Medium, and then aseptically transfer the entire contents of the Essential 8™ Supplement to the bottle of Essential 8™ Basal Medium. Swirl the bottle to mix and to obtain 500 mL of homogenous complete medium.
3. Complete Essential 8™ Medium can be stored at 2°C to 8°C for up to 2 weeks. Before use, warm complete medium required for that day at room temperature until it is no longer cool to the touch. **Do not warm the medium at 37°C.**

Recover Frozen iPSCs in Complete Essential 8™ Medium

1. Pre-warm complete Essential 8™ Medium and VTN-N- or Geltrex® matrix-coated 6-well plates to room temperature.

   Note: Refer to the Vitronectin (VTN-N) or Geltrex® matrix product information sheet for the coating procedure (available at **www.lifetechnologies.com**).
2. Remove the vial of iPSCs from liquid nitrogen storage and transfer it on dry ice to the cell culture hood.
3. Immerse the vial in a 37°C water bath without submerging the cap. Swirl the vial gently. When only an ice crystal remains, remove the vial from the water bath, spray the outside of it with 70% ethanol, and place it in the hood.
4. Transfer the thawed cells to a 15-mL conical tube and slowly add 10 mL of complete Essential 8™ Medium drop-wise to cells. This reduces osmotic shock to the cells. While adding the medium, gently move the tube back and forth to mix the iPSCs. Rinse the vial with 1 mL of complete Essential 8™ Medium and add to the 15-mL tube with cells.
5. Centrifuge the cells at 200 × *g* for 5 minutes, aspirate and discard the supernatant, and resuspend the cell pellet in 3–6 mL of complete Essential 8™ Medium by gently pipetting the cells up and down a few times. Recommended resuspension volume is 3 mL when using VTN-N-coated plates and 6 mL when using Geltrex® matrix-coated plates.
6. Add 1 mL of pre-warmed Essential 8™ Medium to 3 wells of a VTN-N-coated plate or 6 wells of a Geltrex® matrix-coated plate.
7. Slowly add the iPSC suspension into pre-warmed, VTN-N- or Geltrex® matrix-coated 6-well plate containing Essential 8™ Medium, plating 1 mL of cell suspension per well for a total volume of 2 mL per well.
8. Move the plate in several quick figure eight motions to disperse cells across the surface of the wells, place it gently into the 37°C, 5% CO_2 incubator, and incubate overnight.
9. The next day, replace the spent medium with fresh complete Essential 8™ Medium. Replace the medium daily thereafter until the cells are approximately 85% confluent.

Passage iPSCs in Essential 8™ Medium using Versene

1. Pre-warm complete Essential 8™ Medium and VTN-N- or Geltrex® matrix-coated culture vessels to room temperature. **Do not warm the medium at 37°C.**
2. Aspirate the spent medium from the vessel containing iPSCs and rinse the vessel twice with DPBS without Calcium and Magnesium (refer to Table 1 for the recommended volume).
3. Add 1X Versene solution to the vessel containing iPSCs (refer to Table 1). Swirl the vessel to coat the entire cell surface.

   Note: 0.5 mM EDTA in DPBS may be substituted for Versene solution throughout. Prepare 0.5 mM EDTA by combining 50 µL of UltraPure™ 0.5 M EDTA, pH 8.0 with 50 mL of DPBS without Calcium and Magnesium. Filter sterilize and store at room temperature.
4. Incubate the vessel at room temperature for 5 to 8 minutes or at 37°C for 4 to 5 minutes. When the cells start to separate and round up, and the colonies appear to have holes in them when viewed under a microscope, they are ready to be removed from the vessel.

5. Aspirate the Versene solution, and add pre-warmed complete Essential 8™ Medium to the vessel (refer to Table 1).
6. Remove the cells from the well(s) by gently squirting medium and pipetting the colonies up. Avoid creating bubbles. Collect the cells in a 15-mL conical tube. There may be obvious patches of cells that were not dislodged and left behind. **Do not scrape the cells from the dish in an attempt to recover them. Do not pipet vigorously or the colonies will break apart.**

   Note: Depending upon the cell line, work with no more than 1 to 3 wells at a time, and work quickly to remove cells after adding Essential 8™ Medium to the well(s), which quickly neutralizes the initial effect of the Versene solution. Some lines re-adhere very rapidly after medium addition, and must be removed 1 well at a time. Others are slower to re-attach, and may be removed 3 wells at a time.

   Note: Gibco® iPSCs grown in Essential 8™ medium can typically be passaged at a ratio of 1:4 to 1:10 on day 4 or 5 post-thaw based on the culture vessel surface area. Once cells have been established for a couple of passages post-thaw, the passaging ratio can be slightly increased and cells can continue to be passaged every 4 to 5 days. In general, cells should be passaged before the colonies begin to touch and/or the vessel is 70–80% confluent.
7. Add an appropriate volume of pre-warmed complete Essential 8™ Medium to each well of a VTN-N- or Geltrex® matrix-coated 6-well plate so that each well contains 2 mL of medium after the cell suspension has been added. Refer to Table 1 for the recommended volumes for other culture vessels.
8. Move the vessel in several quick figure eight motions to disperse the cells across the surface of the vessels. Place the vessel gently into the 37°C, 5% CO_2 incubator and incubate the cells overnight.
9. Feed the iPSCs starting on the second day after splitting. Replace the spent medium daily.

   Note: It is normal to see cell debris and small colonies after passage.

Table 1 Reagent Volumes (in mL per well or per dish)

Culture vessel (approx. surface area)	DPBS	1X Versene solution	Complete medium
6-well (10 cm^2/well)	2 mL	1 mL	2 mL
12-well (4 cm^2/well)	1 mL	0.4 mL	1 mL
24-well (2 cm^2/well)	0.5 mL	0.2 mL	0.5 mL
35-mm (10 cm^2)	2 mL	1 mL	2 mL
60-mm (20 cm^2)	4 mL	2 mL	4 mL
100-mm (60 cm^2)	12 mL	6 mL	12 mL
T-25 (25 cm^2)	4–5 mL	2–3 mL	4–5 mL
T-75 (75 cm^2)	12–15 mL	5–8 mL	12–15 mL

*0.5 mM EDTA in DPBS may be substituted for Versene solution.

Freezing iPSCs

1. Pre-warm the required volume of complete Essential 8™ Medium at room temperature until it is no longer cool to the touch. **Do not warm the medium at 37°C.**
2. Prepare Essential 8™ Freezing Medium. For every 1 mL of freezing medium needed, aseptically combine 0.9 mL of complete Essential 8™ Medium and 0.1 mL of DMSO in a sterile 15-mL tube.
3. Place the tube with Essential 8™ Freezing Medium on ice until use. Discard any remaining freezing medium after use.
4. Aspirate the spent medium from the dish using a Pasteur pipette, and rinse the cells twice with DPBS without Calcium and Magnesium (refer to Table 1).
5. Add 1X Versene solution to the dish. Adjust the volume of Versene solution for various dish sizes (refer to Table 1). Swirl the dish to coat the entire cell surface.
6. Incubate the vessel at room temperature for 5 to 8 minutes or at 37°C for 4 to 5 minutes. When the cells start to separate and round up, and the colonies appear to have holes in them when viewed under a microscope, aspirate the Versene solution and add 1 mL of ice-cold Essential 8™ Freezing Medium to each well of a 6-well plate.
7. Remove the cells from the well(s) by gently squirting medium and pipetting the colonies up. Avoid creating bubbles. Collect the cells in a 15-mL conical tube and place on ice.
8. Resuspend the cells gently. Aliquot 1 mL of the cell suspension into each cryovial.
9. Quickly place the cryovials in a cryofreezing container (e.g., Mr. Frosty®) and freeze the cells by decreasing the temperature by 1°C per minute. Once frozen, transfer the cells to –80°C overnight.
10. After overnight storage at –80°C, transfer the cells to a liquid nitrogen tank vapor phase for long-term storage.

Related Products

Product	Cat. no.
Essential 8™ Medium	A1517001
Dulbecco's PBS (DPBS) without Calcium and Magnesium	14190
Vitronectin, truncated human recombinant (VTN-N)	A14700
Geltrex® LDEV-Free hESC-qualified Reduced Growth Factor Basement Membrane Matrix	A1413301
UltraPure™ 0.5 M EDTA, pH 8.0	15575
Versene Solution	15040

Explanation of Symbols and Warnings

The symbols present on the product label are explained below:

				
Temperature limitation	Use by	Batch code	Catalog number	Manufacturer

Reference

Burridge, P. W., Thompson, S., Millrod, M. A., *et al*. (2011) A universal system for highly efficient cardiac differentiation of human induced pluripotent stem cells that eliminates interline variability. PLoS One *6*, e18293.

Limited Product Warranty

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