

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% CO₂. Ensure that proper gas exchange is achieved in culture vessels.

Prepare Complete Essential 8™ Medium (500 mL)

Refer to **Culturing Pluripotent Stem Cells (PSCs) in Essential 8™ Medium** (Pub. No. MAN0007035).

Recover Frozen iPSCs in Complete Essential 8™ Medium

- 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 thermofisher.com).
- Remove the vial of iPSCs from liquid nitrogen storage and transfer it on dry ice to the cell culture hood.
- 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.
- 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.

- Centrifuge the cells at 200 × g for 5 minutes, aspirate and discard the supernatant, and resuspend the cell pellet in 1 mL of complete Essential 8™ Medium by gently pipetting the cells up and down a few times.
- Add 1 mL of pre-warmed Essential 8™ Medium to one well of a VTN-N-coated plate or Geltrex™ matrix-coated plate.
- Slowly add 1 mL of the iPSC suspension into the pre-warmed, VTN-N- or Geltrex™ matrix-coated plate containing Essential 8™ Medium, for a total volume of 2 mL.
- (Optional):* For best results, add RevitaCell™ Supplement (Cat. no. A26445) at 1X final concentration to the cell culture (i.e., 20 µL per 2 mL of cell suspension) for the first 24 hours post-thaw to minimize apoptosis and necrosis. Using this supplement for the recovery of PSCs requires a lower seeding density; therefore, seed 2- to 3-fold lower cell seeding density than for recovery in Essential 8™ Medium alone.
- 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% CO₂ incubator, and incubate overnight.
- 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

- 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.**
- 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).
- 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.
- 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.
- Aspirate the Versene solution, and add pre-warmed complete Essential 8™ Medium to the vessel (refer to Table 1).
- 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.

- 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.
 - Mix the cell suspensions from step 6 by gentle inversion a few times and transfer the appropriate volume of cell suspension into each well containing pre-warmed complete Essential 8™ Medium.
 - 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% CO₂ incubator and incubate the cells overnight.
 - Feed the iPSCs the day after splitting. Replace the spent medium daily.
- Note:** It is normal to see cell debris and small colonies after passage.
- (Optional): To improve cell survival, you can add RevitaCell™ Supplement (Cat. no. A26445) to 1X final concentration (i.e., 20 µL per 2 mL of cell suspension) for the first 24 hours post-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.

Freeze iPSCs

Refer to **Culturing Pluripotent Stem Cells (PSCs) in Essential 8™ Medium** (Pub. No. MAN0007035).

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

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Essential 8 is a trademark of Cellular Dynamics International, Inc.

For support, visit thermofisher.com/support or email techsupport@lifetech.com.

Explanation of symbols and warnings

				
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.

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