Gibco™ episomal hiPSC line

Catalog Number A18945

Pub. No. MAN0009562 Rev. 4.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

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. Global gene expression 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 under feeder-free conditions, the Gibco™ Episomal hiPSC line can be used as a positive control in reprogramming procedures and many other stem cell applications.

Product	Catalog no.	Amount	Storage
Gibco™ Episomal hiPSC Line	A18945	1 × 10^6 viable cells/vial	Liquid nitrogen, vapor-phase

Important guidelines for thawing and storing cells

- Upon receipt, immediately thaw cells or place into vapor-phase liquid nitrogen storage until ready to use. **Do not store the cells at –80°C**.
- Avoid short-term extreme temperature changes. When storing cells in liquid nitrogen after shipping on dry ice, allow the cells to remain in liquid nitrogen for 3-4 days before thawing.

Important information

- Gibco™ Episomal hiPSCs can be cultured in Essential 8™ Medium or StemFlex™ Medium on Vitronectin-, Geltrex™, or rhLaminin 521 matrix-coated culture vessels.
- When using the above mentioned media-matrix combinations, cells should be passaged using Versene solution (Cat. no. 15040), which is 0.48 mM EDTA in PBS. The use of collagenase or dispase for passaging these cells results in compromised viability and attachment.
- The best post-thaw recovery can be achieved with StemFlex™ Medium (described below). For more detail on culturing cells with StemFlex™ Medium or a guide on using Essential 8™ Medium, refer to **StemFlex™ Medium Kit User Guide** (Pub No. MAN0016431) or **Culturing Pluripotent Stem Cells (PSCs) in Essential 8™ Medium** (Pub. No. MAN0007035).

Culture conditions

Media: Complete StemFlex™ Medium (Cat no. A3349401)

Culture type: Adherent

Recommended substrate: Geltrex™ hESC-qualified Reduced Growth Factor Basement Membrane Matrix™ (Cat. no. A1413301)

Temperature range: 36℃ to 38℃

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

Prepare complete StemFlex™ medium (500mL)

Refer to **StemFlex™ Medium Kit User Guide** (Pub No. MAN0016431).

Recover frozen iPSCs in complete StemFlex™ medium

1. Pre-coat plates with Geltrex™ matrix. Refer to Geltrex™ matrix product manual for the coating procedure.

   Note: To improve post-thaw cell survival, Geltrex™ matrix may be replaced with rhLaminin 521 (Cat no. A29248), Please refer to product manual for the coating procedure.

2. Remove a vial of PSCs from liquid nitrogen storage and transfer it on dry ice to the tissue culture room.

3. Immerse the vial in a 37℃ 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 or 50-mL conical tube and add 3 mL of complete StemFlex™ Medium drop-wise to the cells to reduce osmotic shock to the cells. While adding the medium, gently move the tube back and forth to mix the PSCs.

5. Rinse the vial with 1 mL of complete StemFlex™ Medium and add to the conical tube containing the cells.

6. Centrifuge the cells at 200 x g for 4 minutes, aspirate and discard the supernatant, and resuspend the cell pellet in 1 mL of complete StemFlex™ Medium by gently pipeting the cells up and down a few times.

7. Immediately prior to plating of cells and following coating of culture vessel for >1 hour at 37℃, 5% C02, aspirate Geltrex™ matrix from the wells and discard. Be certain to not allow the culture surface to dry out.

8. Slowly add the PSC suspension into the Geltrex™ matrix-coated plate, plating -100,000 viable cells per cm^2 of plate for conditions seeded in the absence of ROCK inhibitor. See Table 1 for recommended volume of complete medium.

   (Optional): To improve efficiency of cell survival, RevitaCell™ Supplement (Cat. No. A2644501) may be used at IX final concentration (i.e., 10 pL per 1 mL of cell suspension) for the first 24 hours post-thaw. When using this supplement for recovery of your PSCs, lower cell seeding densities are required; plating at a viable cell density of -20,000-40,000 viable cells/cm^2 will allow for recovery in three to four days post-thaw. Do not include additional ROCK inhibitors such as Y-27632 or thiazovivin when using RevitaCell™ Supplement.

9. Move the plate in several quick side-to-side motions to disperse the cells across the surface of the wells and place the plate gently into the 37℃, 5% C02 incubator.

10. Feed the PSCs the day after seeding followed by every-other-day thereafter. If the cells are to be left without feeding for two days (for example, over a weekend), then double the feed volume (i.e., 4 mL added per well of 6-well plate). Refer to StemFlex™ Medium Kit User Guide (Pub No. MAN0016431) for flexible feeding schedule.

    Note: Cells should be passaged once reaching -85% confluency to maintain optimum cell health of cultures.

Passage iPSCs in StemFlex™ medium using versene

If using pre-coated plates stored at 2℃ to 8℃, pre-warm Geltrex™ -coated plates to room temperature. Pre-warm StemFlex™ Medium and Versene Solution or 500 uM EDTA solution to room temperature.

1. Pre-coat plates with Geltrex™ matrix. Refer to Geltrex™ matrix product information sheet for the coating procedure.

2. Aspirate the spent medium from the vessel containing PSCs and rinse the vessel once with DPBS, no calcium, no magnesium. See Table 1 for recommended volumes.

3. Add Versene Solution or 500 uM EDTA to the side of the vessel containing PSCs (refer to Table 1), then swirl the vessel to coat the entire well surface.

4. Incubate the vessel at room temperature for 5 to 8 minutes or at 37℃ 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. Note: Cells should not be incubated to the extent that the colonies float off the surface of the culture vessel.

5. Aspirate the Versene Solution and remove the cells from the well(s) by gently flushing pre-warmed complete StemFlex™ Medium over the surface of the well(s) a few times. See Table 1 for recommended volumes.

6. Collect cells in a 15-mL or 50-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.

   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 StemFlex™ Medium to the well(s), which quickly neutralizes the initial effect of the Versene Solution. Some lines readhere very rapidly after medium addition, and must be removed 1 well at a time. Others are slower to reattach and may be removed 3 wells at a time.

7. Following coating of culture vessel for >1 hour at 37 ℃, 5% C02, aspirate Geltrex™ matrix from the culture vessel and discard. Be certain to not allow the culture surface to dry out.

8. Immediately add an appropriate volume of pre-warmed complete StemFlex™ Medium to each well of a Geltrex™ matrix-coated plate so that each well contains the recommended volume of complete medium after the cell suspension has been added. See Table 1 for recommended volumes.

   Note: Step 5 on page 2 can be completed immediately prior to passaging the cells and cells can be directly passaged onto the new plate rather than transferring to a conical tube.

   Note: The split ratio can vary, though it is generally between 1:2 and 1:4 for newly derived PSCs and between 1:8 and 1:16 for established cultures. Occasionally, cells may recover at a different rate and the split ratio will need to be adjusted.

9. Move the vessel in several quick side-to-side motions to disperse the cells across the surface of the vessels.

10. Place the vessel gently into the 37℃, 5% CO_2 incubator and incubate the cells overnight.

11. Feed the PSCs the day after passaging followed by every-other-day thereafter until the cells are approximately 85% confluent. If the cells are to be left without feeding for longer than 48 hours (for example, during a weekend), double the feed volume (i.e., 4 mL added per well of 6-well plate).

    Note: It is normal to see cell debris and small colonies after passage. Cells should be passaged once reaching ~85% confluency to maintain optimum cell health of cultures.

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.

Related products

Product	Cat. no.
StemFlex™ Medium	A3349401
Essential 8™ Medium	A1517001
Vitronectin, truncated human recombinant (VTN-N)	A14700
Geltrex™ LDEV-Free hESC-qualified Reduced Growth Factor Basement Membrane Matrix™	A1413301
rhLaminin 521	A29248
Versene Solution	15040
RevitaCell™ Supplement	A2644501

Reference

Burridge, P. W., Thompson, S., Millrod, M. A., *et at*. (2011) A universal system for highly efficient cardiac differentiation of human induced pluripotent stem cells that eliminates interline variability. PLoS One *6*, el8293.

Limited product warranty

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