Human iPS Cell Line (Episomal, PBMC)

Product Description

Footprint-free human iPS (induced pluripotent stem) cell line (Cat# iPS15) was derived from human peripheral blood mononuclear cells (PBMCs) by ectopic expression of OCT4, SOX2, KLF4, and L-MYC genes using episomal plasmids. The cells were derived using morphological selection criteria and without the use of fluorescent marker or drug selection. When cultured under standard human ES cell culture conditions, the morphology of human iPS cells are identical to that of human ES cells. The cells also express the pluripotency markers TRA-1-60, SSEA-3 and OCT4, and demonstrate strong endogenous alkaline phosphatase activity.

Characterization

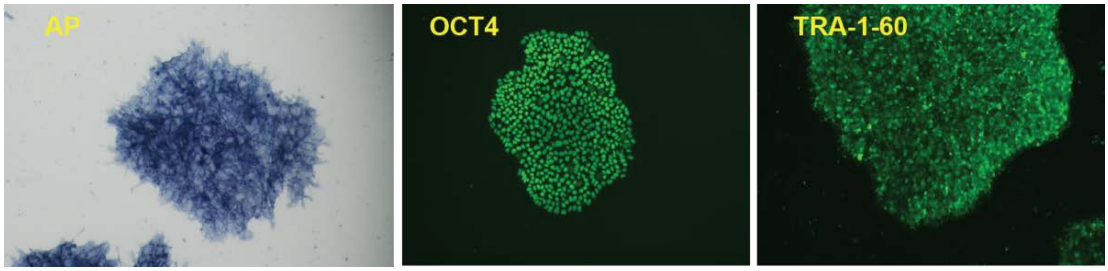

Figure 1. Characterization of iPSCs derived from human peripheral blood mononuclear cells (PBMCs) using ALSTEM episomal vectors. Alkaline phosphatase staining of iPSC clone, immunostaining of colonies expressing ESC specific markers OCT4 and TRA-1-60.

Highlights

Footprint-free human iPS15 cell lines generated by episomal plasmids are ideally suited for various research purpose including 1) differentiating various somatic cells or organoid models for phenotypic and target-based compound screening, 2) establishing genetically modified disease model through CRISPR/Cas9 editing, and 3) generating functional cells/tissues as regenerative biology initiatives. iPS15 has several features such as:

- Low passage and long-term viability
- Off the shelf - simple thaw the cells and plate them onto serum-free, feeder-free culture
- Transgene- and Virus-free (episomal)
- Homogeneity– Originated from a single iPSC clone

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Product Specifications

Product Name	Human iPS Cell Line (Episomal, PBMC)
Catalog #	iPS15
Cell Source	human peripheral blood mononuclear cells
Reprogramming method	Episomal plasmid transfection
Size	> $5x10^5$ cells/vial
Shipping	Dry Ice
Storage and Stability	Store in vapor phase of liquid nitrogen immediately upon receipt. This product is stable for 6 months when stored as directed.
Quality Control	Human iPS cells were grown in feeder free conditions with mTeSR1 medium. Each lot of human iPS cells is tested for growth and viability following recovery from cryopreservation. In addition, each lot is tested for expression of TRA-1-60 and Oct4, as well as the activity of alkaline phosphatase.
Safety Precaution	**ALSTEM highly recommends 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 vapor phase, resulting in excessive pressure within the vial that can cause the vial to explode or expel the cap with dangerous force.
Restricted Use	For Research Use Only. Not for use in diagnostic or therapeutic procedures.

Related Products

1. EZStem™ Enzyme-free Stem Cell Dissociation Solution: Cat # M100
2. Episomal iPSC Reprogramming Kit: Cat # RF202
3. Human iPS Cell Line (Episomal, HFF): Cat # iPS11
4. Human iPS Cell Line (Episomal, MSC): Cat # iPS12

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Protocols

I. FEEDER-FREE CULTURE CONDITIONS

Preparation of feeder-free medium

1. Thaw mTeSR1 5X Supplement (STEMCELL Technologies, Cat. # 05850) at room temperature or overnight at 4° C.
2. Add the 100 ml of thawed 5X Supplement to 400 ml Basal Medium for a total volume of 500 ml aseptically. Mix well. Filter through a 0.2 µm, low-protein binding filter, if desired.
3. Aliquot into appropriate amounts according to usage and store the aliquots at 4° C.

Coating plates with Matrigel

Matrigel (BD, Cat. # 354277) should be aliquoted and stored at -80° C for long-term use.

1. Thaw Matrigel on ice until liquid. Dilute Matrigel 1:30 to 1:50 with pre-chilled KO DMEM/F12.
2. Immediately use the diluted Matrigel solution to coat tissue culture-treated plates. For a 6-well plate, use 0.8 ml of diluted Matrigel solution per well, and swirl the plate to spread the Matrigel solution evenly across the surface.
3. Let the coated plate stand for 1 hour at 37° C or overnight at 4° C. If plate has been stored at 4° C,allow the plate to incubate at 37° C for at least 30 min before removing the Matrigel solution.

Thawing cryopreserved human iPS cells

1. Quickly thaw the human iPS cells in a 37° C waterbath by gently shaking the cryovial continuously until half thawed. Remove the cryovial from the waterbath and spray with 70% ethanol to sterilize.
2. Transfer the contents of the cryovial to a 15 ml conical tube. Add 5 ml warm mTeSR1 dropwise to the tube, gently mixing as the medium is added.
3. Centrifuge cells at 200 x *g* for 5 minutes at room temperature.
4. While centrifuging, remove the Matrigel solution from a coated tissue culture 6-well plate. Add 1 ml of warm mTeSR1 containing 5 µM ROCK inhibitor (StemRD, Y-27632) 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 mTeSR1 with 10 µM ROCK inhibitor, taking care to maintain the cells as small cell clumps.
6. Transfer the medium containing the cell clumps to the Matrigel coated 6-well plate.
7. Place the plate into the 37° C incubator and move the plate in quick side-to-side, forward-to-back motions to evenly distribute the clumps within the wells. Culture the cells at 37° C, with 5% CO_2 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 grown under feeder-free conditions

1. Use a microscope to identify regions of differentiation. Mark the differentiated colonies using lens marker on the bottom of the plate.

2. Remove regions of differentiation by scraping with a pipette tip or by aspiration.
3. Aspirate medium from the human iPS cell culture and rinse with DPBS (2 ml/well).
4. Add 0.5 ml per well of accutase (Millipore, Cat. # SCR005, diluted 1:1 with DPBS before use). Let it stand at room temperature for 1 min.
5. Remove accutase, and gently rinse each well 2-3 times with 2 ml of DMEM/F-12 per well to wash away remaining enzymes.
6. Add 2 ml/well mTeSR1 and scrape colonies off with a cell scraper.
7. Transfer the detached cell aggregates to a 15 ml conical tube and rinse the well with an additional 2 ml mTeSR1 to collect any remaining aggregates. Add the rinse to the 15 ml tube.
8. Centrifuge the 15 ml tube containing the aggregates at 200 x *g* for 5 min at room temperature.
9. Aspirate the supernatant. Resuspend pellet in mTeSR1 containing 5 µM ROCK inhibitor by gently pipetting and ensure that cells are maintained as aggregates.
10. Plate the human iPS cell aggregates with mTeSR1 onto a new plate coated with Matrigel (remove Matrigel solution before plating).
    Note: If the colonies are at an optimal density, the cells can be split every 5-7 days using 1:3 to 1:6 ratio.
11. Place the plate into the 37° C incubator and move the plate in quick side-to-side, forward-to-back motions to evenly distribute the clumps within the wells. Culture the cells at 37° C with 5% CO_2 and 95% humidity.
12. Change medium daily.

Cryopreserving human iPS cells

1. Prepare EZStem™ Freezing Medium (Cat. # M050) on ice.
2. Perform steps 1-8 from "Passaging human iPS cells grown under feeder-free conditions."
3. Gently aspirate the supernatant and loosen the cell pellet by tapping the bottom of the tube.
4. Gently resuspend the pellet in freezing medium, taking care to leave the clumps larger than would normally be done for passaging.
5. Transfer 1 ml of clumps in freezing medium into each labeled cryogenic vial.
6. Place vials into a freezing container and place the container at -80° C overnight.
7. Transfer to a liquid nitrogen tank next day.

II. FEEDER-DEPENDENT CULTURE CONDITIONS

Preparation of human ES medium

Knockout DMEM/F12 containing 20% knockout serum replacement, 2mM glutamine, 0.1 mM nonessential amino acids, 0.1 mM 2-mercaptoethanol, 10 ng/ml bFGF, and 50 U and 50 µg/ ml penicillin and streptomycin.

Thawing cryopreserved human iPS cells

To insure the highest level of viability, be sure to warm medium to 37° C before using it on the cells. 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 until half thawed. Remove the cryovial from the waterbath and spray with 70% ethanol to sterilize.
2. Transfer the contents of the cryovial to a 15 ml conical tube. Add 5 ml warm human ES medium dropwise to the tube, gently mixing as the medium is added.
3. Centrifuge cells at 200 x *g* for 5 min 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 5 µM ROCK inhibitor (StemRD, Y-27632) 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 5 µM ROCK inhibitor, taking care to maintain the cells as small cell clumps.
6. Transfer the medium containing the cell clumps to one well of 6-well plate with MEF feeder cells.
7. Place the plate into the 37° C incubator and move the plate in quick side-to-side, forward-to-back motions to evenly distribute the clumps within the wells. Culture the cells at 37° C with 5% CO_2 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 grown 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 (Millipore, Cat. # SCR005, 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 accutase.
4. Add 1 ml of DMEM/F12 to the plate and carefully wash the feeder cells, and aspirate the medium. Repeat.
5. Add 1 ml of human ES medium containing 5 µM ROCK inhibitor to the plate and suspend the cell colonies by pipetting up and down. 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 swirl the plate back-and-forth and side-to-side 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.

Cryopreserving human iPS cells

1. Prepare EZStem™ Freezing Medium (Cat. # M050) on ice.
2. Perform steps 1-6 from "Passaging human iPS cells grown under feeder-dependent conditions."

3. Gently aspirate the supernatant and loosen the cell pellet by tapping the bottom of the tube.
4. Gently resuspend the pellet in freezing medium, taking care to leave the clumps larger than would normally be done for passaging.
5. Transfer 1 ml of clumps in freezing medium into each labeled cryogenic vial.
6. Place vials into an isopropanol freezing container and place the container at -80° C overnight.
7. Transfer to a liquid nitrogen tank next day.

WARNING

Do not use cryogenic vials for storage in the liquid phase of liquid nitrogen. Such use may cause entrapment of liquid nitrogen inside the vial and lead to pressure buildup resulting in possible explosion or biohazard release. Use appropriate safety procedures which are outlined by the ATCC when handling and disposing of vials. ALSTEM highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials.

Publications

1. **Single-cell transcriptomics reveals multiple neuronal cell types in human midbrain-specific organoids**

   bioRxiv (2019)

2. **Clinical Applications of Induced Pluripotent Stem Cells – Stato Attuale**

   Cell Biology and Translational Medicine (2018)

3. **Pseudotyping exosomes for enhanced protein delivery in mammalian cells**

   International Journal of Nanomedicine (2017)

4. **(Patent) Engineered Exosomes for the Delivery of Bioactive Cargo Using Transmembrane VSV-G**

   United States Patent Application 20190015333A1 (2019)

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