Product Specification Sheet

Product Name	**PLN Knockout Human Induced Pluripotent Stem Cells (Episomal, HFF)**
Description	Footprint-free human iPS (induced pluripotent stem) cell line with phospholamban (PLN) deficiency was derived using genome editing method to delete whole coding sequence of PLN gene. Sequencing results confirmed that this isogenic iPSC line has a stable homozygous 189 bp deletion that contains 159 bp of coding sequence of PLN. When cultured under standard human ES cell culture conditions, the morphology of human iPS cells is 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.
Catalog Number	iPS41
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 are always 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 gas 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.

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Overview

Phospholamban (PLN) is a membrane protein that is regulated by Ca2+-ATPase 2a (SERCA2a), an ATP-driven pump that drives calcium into the cardiac muscle cells. Previous studies show that inhibition of PLN expression and reduction of SERCA2a protein expression results in impaired Ca^{2+} uptake, which leads to degeneration of cardiomyocytes in heart failure disease state. Additionally, heart failure murine models demonstrate decreased mortality rate with ablation of PLN protein.

ALSTEM's footprint-free human iPS (induced pluripotent stem) cell line (cat# iPS41) carries PLN KO mutation in both alleles. Sequencing results confirmed that this isogenic iPSC line has a stable homozygous 189 bp deletion including 159 bp of coding sequence of PLN gene. The morphology of this human iPS cell line is identical to that of human ES cells. The cells express the pluripotency markers TRA-1-60, SSEA-3 and OCT4. They also demonstrate a strong endogenous alkaline phosphatase (AP) activity.

ALSTEM's human iPS is good model for studying mechanisms of heart failure associated with PLN, as well as for drug discovery.

Data Analysis

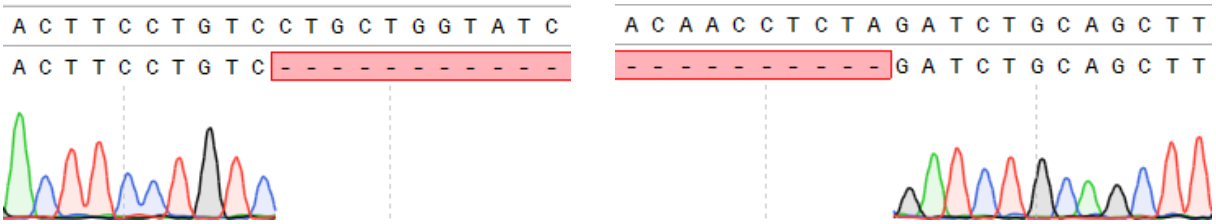

Figure 1. The sequencing results showed this iPSC line (cat# iPS41) had a 189-bp deletion including 159 bp of coding sequence of PLN.

Protocol

Human iPS Cell Culture

OVERVIEW

This protocol can be used for culturing human iPS cells. Footprint-free human iPS cells were generated by transient introduction of episomal plasmids encoding the human transcription factors into human peripheral blood mononuclear cells. The cells were derived using morphological selection criteria without the use of fluorescent markers or drug selection. When cultured under standard human ES cell culture conditions, the morphology of footprint-free human iPS cells is identical to that of human ES cells. The cells express the pluripotency markers TRA-1-60 and Oct4, and they demonstrate a strong endogenous AP activity.

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PROCEDURE

I. Feeder free culture conditions

Preparation of feeder-free medium

1. Thaw mTeSR1 5X Supplement (Cat.no. 05850, STEMCELL Technologies) 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 and filter through a 0.2 µm, low-protein binding filter, if desired.
3. Aliquot into appropriate amount according to usage and store the aliquots at 4°C.

Coating plates with Matrigel

Matrigel (Cat.no. 354277, BD) 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 1 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 h 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 minutes before removing the matrigel solution.

Thawing cryopreserved human iPS cells

1. Quickly thaw the human iPS cells in a 37°C water bath by gently shaking the cryovial continuously until half thawed. Remove the cryovial from the water bath 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. After centrifugation, aspirate the medium from 15 mL tube. Gently resuspend the cell pellet in 2 mL mTeSR1 with 5 µM ROCK inhibitor, taking care to maintain the cells as small cell clumps.
5. Remove the matrigel solution from a coated tissue culture 6-well plate. Transfer the medium containing the cell clumps to the matrigel coated 6-well plate.
6. 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.
7. 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).

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4. Add 1 mL per well of EZStem Enzyme-Free Stem Cell Dissociation Solution (cat. no. M100, ALSTEM) and incubate at 37°C for 2-3 minutes.
Note: Addition of 0.5 mL per well of accutase (Cat.no. SCR005, Millipore, diluted 1:1 with DPBS before use) and incubate at 37°C for 1 minute can also be used to detach the cells from the plate.
5. Remove EZStem Enzyme-Free Stem Cell Dissociation Solution or accutase, gently rinse each well 2 - 3 times with 2 mL of DMEM/F-12 per well and transfer the detached cell aggregates to a 15 mL conical tube.
6. Add 2 mL/well mTeSR1 and scrape colonies off with a cell lifter. Transfer the detached cell aggregates to a 15 mL conical tube.
7. Rinse the well with an additional 2 mL mTeSR1 to collect any remaining aggregates. Add the cell suspension rinse to the 15 mL tube.
8. Centrifuge the 15 mL tube containing the aggregates at 200 x *g* for 5 minutes at room temperature.
9. Aspirate the supernatant. Resuspend pellet in mTeSR1 containing 5 µM ROCK inhibitor by gently pipetting to ensure that the cells are maintained as aggregates.
10. Plate the human iPS cell aggregates with mTeSR1 in a new plate coated with matrigel. (Remove matrigel solution before plating). *If the colonies are at an optimal density, the cells can be split every 5 - 7 days using 1:3 to 1:6 ratios.*
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 cell culture medium daily.

Cryopreserving human iPS cells

1. Prepare EZStem freezing medium (Cat.no. M050, ALSTEM) 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 that would normally be done for passaging.
5. Transfer 1 mL of cells 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.

IMPORTANT NOTICE

Store the vials at vapor phase of liquid nitrogen immediately upon receipt.

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 the use of protective gloves, clothing, and a full face mask to be always be worn when handling frozen vials.

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