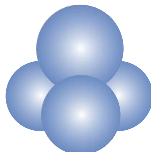
Product Specification Sheet

Product Name	**Human Induced Pluripotent Stem Cells (Episomal, $CD34^{+}$)**
Description	Footprint-free human iPS (induced pluripotent stem) cell line with ApoE2 isoform was derived from human iPSC (cat# iPS16) with ApoE4 isoform by changing Arg112 to Cys112 and Arg158 to Cys158 with genome editing technology. Sequencing results confirmed that this iPSC line has a stable homozygous conversion with Cys112 (TGC) from Arg112 (CGC) and Cys158 (TGC) from Arg158 (CGC) in the APOE gene. When cultured under standard human ES cell culture conditions, the morphology of this human iPS cell line 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	iPS46
Size	> $5x10^{5}$ cells/vial
Shipping	Dry ice
Storage and Stability	Store in gas 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

Apolipoprotein (apo) E, a polymorphic protein with 299 amino acids, has important and diverse functions in neurobiology. ApoE distributes lipids among cells in the central nervous system for normal lipid homeostasis and participates in neuronal repair and remodeling. However, the three major human isoforms (apoE2, apoE3, and apoE4) differ in their ability to accomplish these tasks. Human ApoE2 and ApoE4 differ from each another only at amino acid residues at positions 112 and 158. ApoE2, the common isoform, has Cys112 and Cys158, whereas ApoE4 has Arg112 and Arg158. ApoE4, the major known genetic risk factor for Alzheimer's disease (AD), is associated with an earlier onset of AD in a gene dose-dependent manner. It may also contribute to age-related shrinking of the hippocampus and memory deficits in humans. Of note, the lifetime risk estimate of developing AD by age 85 is ~65% in people with two copies of the APOE-ε4 allele, which encodes apoE4, but only ~10% in people with two copies of the APOE-ε3 allele, which encodes ApoE3. This notable difference highlights the importance of ApoE4 in the pathogenesis of AD. Recent studies suggest that apoE4 inhibits hippocampal neurogenesis by impairing neuronal maturation mediated by GABA signaling, ApoE4 increased Aβ production in human neurons leading to GABAergic interneuron degeneration, which could be dramatically ameliorated by treatment with a small-molecule ApoE4-structure corrector. The common APOE2 gene variant is neuroprotective against Alzheimer's disease (AD) and reduces risk by nearly 50%. Converting ApoE4 to ApoE2 by gene editing rescued these phenotypes, indicating the specific effects of ApoE4. Neurons that lacked APOE behaved similarly to those expressing ApoE3, and the introduction of ApoE4 expression recapitulated the pathological phenotypes, suggesting a gain of toxic effects from ApoE4. Treatment of ApoE4-expressing neurons with a small-molecule structure corrector ameliorated the detrimental effects, thus showing that correcting the pathogenic conformation of ApoE4 is a viable therapeutic approach for ApoE4-related AD.

ALSTEM's footprint-free human iPSC (induced pluripotent stem cell) line (cat# iPS16) carries APOE-ε4 in both alleles, and was used for generating an isogenic iPSC line (cat# iPS46) with APOE-ε2 in both alleles by changing Arg112 to Cys112 and Arg158 to Cys158. Sequencing results confirmed that this iPSC line has a stable homozygous conversion with Cys112 (TGC) from Arg112 (CGC) and Cys158 (TGC) from Arg158 (CGC) in the APOE gene. The morphology of this human iPSC line 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.

ALSTEM's human iPS (induced pluripotent stem) cell lines (cat# iPS16, iPS26, iPS36 and iPS46) are isogenic. Lines iPS26 (ApoE3), iPS36 (ApoE-KO) and iPS46 (ApoE2) are derived from line iPS16 (ApoE4). These lines have same genetic background and are good candidates for studying AD-related pathologies in neurons induced specifically by ApoE4, as well as for drug discovery.

Data Analysis

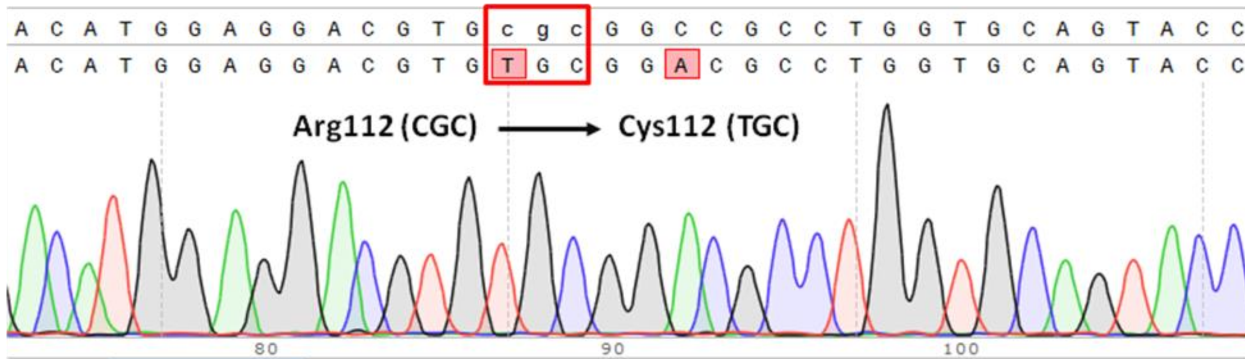


Figure 1. The sequencing results showed this iPSC line (cat# iPS46) had converted Arg112 (CGC) to Cys112 (TGC) with a silence mutation (C to A) in both alleles.

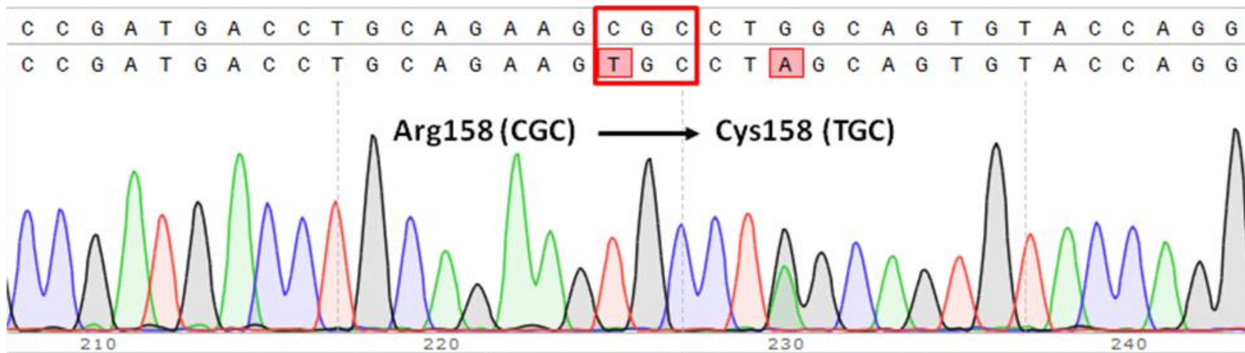


Figure 2. The sequencing results showed this iPSC line (cat# iPS46) had converted Arg158 (CGC) to Cys158 (TGC) with a silence mutation (G to A) in one allele.

Protocol

Human iPS Cell Culture

OVERVIEW

This protocol can be used for culturing human iPS cells. Footprint-free human iPS cells were generated by transiently introducing episomal plasmids encoding the human transcription factors into human peripheral blood mononuclear cells. The cells were derived using morphological selection criteria and 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 also express the pluripotency markers TRA-1-60 and Oct4, and demonstrate a strong endogenous AP activity.

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 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. 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).

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. Or add 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.
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 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 and ensure that 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 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 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.

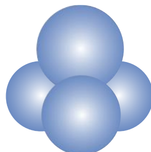
II. Feeder-dependent culture conditions

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 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.



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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 minutes 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 (Y-27632, Stemgent) 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 (Y-27632), 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

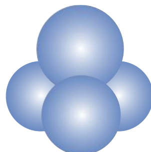
1. Aspirate the medium and wash the cells twice with 1 ml of PBS.
2. Remove PBS completely, add 0.5 ml of Accutase (Cat.no. SCR005, Millipore, diluted 1:1 with DPBS before use) and incubate for 1-2 min at 37°C.
3. Tap the bottom of the plate to dislodge the cells from the bottom of the plate. Then aspirate 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 gently 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.
8. Add 1 mL human ES medium to the original well and scrape colonies off with a cell lifter.
9. Distribute 0.2 – 0.3 ml of the human iPS cell suspension to each well of a 6-well plate.
10. 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.

Note: *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. After 24 hours, remove the media and replace with human ES media (without ROCK inhibitor).
12. 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.no. M050, ALSTEM) on ice.
2. Perform steps 1-6 from **Passaging human iPS cells grown under feeder-dependent conditions.**
3. Transfer the detached cell aggregates to a 15 mL conical tube.
4. Add 1 mL human ES medium and scrape colonies off with a cell lifter.



5. Transfer the cell suspension to the 15 mL conical tube.
6. Centrifuge the 15 mL tube containing the aggregates at 200 x *g* for 5 minutes at room temperature.
7. Gently aspirate the supernatant and loosen the cell pellet by tapping the bottom of the tube.
8. Gently resuspend the pellet in freezing medium, taking care to leave the clumps larger than that would normally be done for passaging.
9. Transfer 1 mL of clumps in freezing medium into each labeled cryogenic vial.
10. Place vials into an isopropanol freezing container and place the container at -80°C overnight.
11. Transfer to a liquid nitrogen tank next day.

IMPORTANT NOTICE

Store the vials at gas 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 that protective gloves and clothing always be used and a full-face mask always be worn when handling frozen vials.