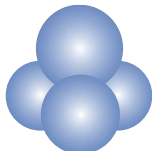


# Product Specification Sheet

<b>Product Name</b>	<b>Human Induced Pluripotent Stem Cells (mRNA)</b>
<b>Description</b>	Footprint-free human iPS (induced pluripotent stem) cell line was derived from human fibroblasts (female, Caucasian) by ectopic expression of OCT4, SOX2, KLF4, and c-MYC genes using mRNA. 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 human iPS cells is identical to that of human ES cells. The cells also express the pluripotency markers SSEA-4 and NANOG and demonstrate strong endogenous alkaline phosphatase activity.
<b>Catalog Number</b>	iPS209
<b>Size</b>	5 x 10 <sup>5</sup> cells/vial
<b>Shipping</b>	Dry ice
<b>Storage and Stability</b>	Store in gas phase of liquid nitrogen immediately upon receipt. This product is stable for 12 months when stored as directed.
<b>Quality Control</b>	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 SSEA-4 and NANOG, as well as the activity of alkaline phosphatase.
<b>Safety Precaution</b>	<b>ALSTEM highly recommends that protective gloves, a lab coat, and a full-face mask always be worn when handling frozen vials.</b> 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.
<b>Restricted Use</b>	For Research Use Only. Not for use in diagnostic or therapeutic procedures.



## **ALSTEM, INC**

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# 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 foreskin fibroblasts. 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 SSEA-4 and Nanog, and demonstrate a strong endogenous AP activity.

### PROCEDURE

#### 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. 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.

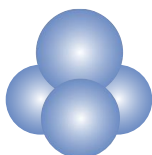
##### Coating plates with Matrigel

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 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 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.



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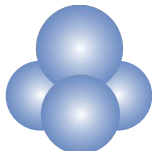
4. While centrifuging, remove the matrigel solution from a coated tissue culture 6-well plate. Add 1 mL of warm mTeSR1 containing 5  $\mu$ M ROCK inhibitor (Y-27632) to one well of 6-well plate.
5. After centrifugation, aspirate the medium from 15 mL tube. Gently resuspend the cell pellet in 1 mL mTeSR1 with 10  $\mu$ M 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<sub>2</sub> 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 (Cat.no. SCR005, Millipore, diluted 1:1 with DPBS before use). Let it stand at room temperature for 1 minute.
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 minutes at room temperature.
9. Aspirate the supernatant. Resuspend pellet in mTeSR1 containing 5  $\mu$ M 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). *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<sub>2</sub> 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 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.



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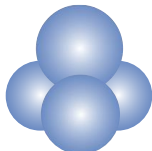
7. Transfer to a liquid nitrogen tank next day.

### **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.**



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