ANGIO-PROTEOMIE

DATA SHEET

Human Induced Pluripotent Stem (IPS) Cells

ORDER INFORMATION

Name of Cells: Human Induced Pluripotent Stem (IPS) Cells
Catalogue Number: **cAP-0500-001**
Product Format: Cells in Frozen Vials
Cell Number: Sufficient for seeding 1 x T25 flask

General Information

Description

cAP-0500-001 primary fibroblasts were obtained from a healthy donor. The fibroblasts were reprogrammed by the expression of OCT4, SOX2, KLF4 and MYC gene sequences using retroviral transduction. This cell line provides a unique model system for better understanding cell development and differentiation, as well as source material for the development of iPSC derived cells.

Cell Type: retroviral reprogrammed hiPSC
Reprogramming Method: Retroviral expression of OCT4, SOX2, KLF4, and MYC genes
Disease: normal
Gender: male
Age: 31 years
Isolation Date: 2018
Source: Primary human fibroblast

SAFETY PRECAUTION

We highly recommend that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If, upon arrival, continued storage of the frozen vial is necessary, it should be stored in liquid nitrogen vapor phase, not at -80C. Storage at –80C will result in loss of viability.

1. Preparation for Culture

1. Thaw iPSC Coating Solution (cAP-50) at room temperature. Aa soon the solution is thawed, then keep it at 2C to 8C (stable for 2 weeks).
2. One hour prior to thawing the iPSC – Prepare coated plates as described below.
3. 30 minutes prior to handling cells – Pre-warm iPSC SFM (Serum Free Medium, cAP-49) at 37C for at least 30 minutes before adding to cells. If using ROCK Inhibitor Y27632 (500x, cAP-52), prepare stem cell culture medium supplemented with final concentration of 10uM ROCK Inhibitor Y27632. Stem cell culture medium with ROCK inhibitor must be used immediately.

Note: Addition of ROCK inhibitor has been shown to increase the survival rate during sub cultivation and thawing of human iPSCs. The use of ROCK inhibitor may cause a transient spindle like morphology effect on the cells. However, the colony morphology will recover after subsequent media change without ROCK inhibitor.

2. Protocol for Coating Flasks

Add 4ml of iPSC Coating Solution into each T25 flask and leave the flask at 37C for one hour. The flask is now ready for use.

3. Initiation of Cultures

1. Rapidly thaw the cells by placing the cryovial in a 37C water bath, swirling gently. Remove the cryovial from the water bath when only a few ice crystals are remaining.
2. Sterilize the cryovial by rinsing with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
3. Using a 1 mL or 5 mL pipette, gently transfer the cell suspension to a 15 mL conical tube.
4. Slowly add 4 mL iPSC SFM dropwise, to the conical tube. Rinse the cryovial by adding and removing an additional 1 mL of medium and transfer the liquid to the 15 mL conical tube. Shake the conical tube gently to mix the cells while adding media. Do not break apart the aggregates into a single cell suspension, as it is crucial to maintain the cells in aggregates.
5. Centrifuge the cells at 200 x g for 5 minutes.
6. Aspirate the supernatant and discard. Gently tap on the bottom of the tube to loosen the cell pellet.
7. Add 4ml of stem cell culture medium with ROCK Inhibitor Y27632. Gently resuspend the pellet by pipetting up and down 2 to 3 times with a 1 mL tip. Do not over pipette, as it is crucial to maintain the cells in aggregates.
8. Aspirate the iPSC Coating Solution from the flask prepared as in the Protocol for Coating flask section.
9. Seed 4.0 mL of cell aggregates into the ONE flask prepared in step 8.
10. Incubate the culture at 37C in a suitable incubator. A 5% CO2 in air atmosphere is recommended.

Post thaw day 1, perform a 100% medium change and remove all cells that did not attach.

Perform a 100% medium change every day. Passage the cells every 4 to 5 days (80% confluent) at an appropriate split ratio. If the colonies are close to, or touching each other, the culture is overgrown. Overgrowth will result in differentiation.

ROCK Inhibitor Y27632 is not necessary each time the culture medium is changed. It is required when cells are recovering from thaw or immediately after cells being passaged on iPSC Coating Solution coated flasks with 5 mL iPSC SFM/T25 flask.

4. Sub culturing Procedure

This protocol is designed to passage stem cell colonies cultured in a T25 flask, using iPSC Non-Enzymatic Dissociation Reagent (cAP-51) to detach the cell colonies. The recommended spilt ratio is 1: 20 to 30. Volumes should be adjusted according to the size and number of the tissue culture vessels to be processed.

1. Add 4ml of iPSC Coating Solution into each T25 flask and leave the coated flask at 37C for one hour. The flasks are now ready for use.
2. Warm an aliquot of iPSC Non-Enzymatic Dissociation Solution to 37C
3. Aspirate and discard the stem cell culture medium.
4. Add 2ml of iPSC Non-Enzymatic Dissociation Solution to the flask.
5. Incubate at 37C for 2 to 5 minutes.
6. Add 3 mL of iPSC SFM to the flask, and detach the cells by pipetting up and down 2 to 3 times with a 5 mL pipette. **Take care not to over pipette the culture into a single cell suspension as single cells will not establish colonies after seeding**.
7. Transfer the cell aggregates to a 15 mL conical tube.
8. Add an additional 3 mL of stem cell culture medium to the flask to collect any remaining cells. Transfer this rinse to the 15mL conical tube containing the cell aggregates.
9. Centrifuge the cell aggregates at 200 x g for 5 minutes.
10. Aspirate the supernatant and discard.
11. Add 10-15 mL of iPSC SFM with 10um ROCK inhibitor. Gently resuspend the pellet by pipetting up and down **2 to 3 times** with a 10 mL pipette, maintaining the small cell aggregates. **Take care not to over pipette the culture into a single cell suspension as single cells will not establish colonies after seeding.**

12. Add 0.5ml of cells on iPSC Coating Solution coated flask containing 4.5 mL iPSC SFM (with 10um ROCK inhibitor)/T25 flask.
13. Incubate the culture at 37C in a humidified 5% CO2/95% air incubator. Perform a 100% medium change every day (with the Medium without ROCK inhibitor thereafter). Passage the cells when cells are close to 80% confluent.

Cryopreservation

For optimal results, cryopreserve stem cell colonies when the cell cultures are 80% confluent. This protocol is designed to cryopreserve stem cell colonies cultured in a T25 flask.
1. Detach stem cell colonies from the dish as described in the recommended subculturing protocol (steps 1-10). Gently tap the bottom of the tube to loosen the cell pellet.
2. Take the Stem Cell Freezing Media from storage and swirl to mix. Keep cold. Decontaminate by dipping in or spraying with 70% alcohol.
3. Add 2 mL of **cold** Stem Cell Freezing Media (cAP-53) to the tube. Gently resuspend the pellet by pipetting up and down **2 to 3** times with a 1 mL tip, maintaining the cell aggregates. And then add 18 ml cold Stem Cell Freezing Media and mix the cells by gently turn the tube upside down 2-3 times.
4. Immediately transfer 1 mL each of the cell suspension into 20 (1 to 20 split ratio) labeled cryovials.
5. Freeze the cells gradually at a rate of 1 C/min until the temperature reaches -70 C to 80 C.
6. The cells should not be left at 80 C for more than 24 to 48 hours. Once at 80 C, frozen cryovials should be transferred to the vapor phase of liquid nitrogen for long-term storage.

Use Restrictions

These cells are distributed for research purposes only.

Biosafety Level: 2
Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

Warranty

The viability of our products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. We list the media formulation that has been found to be effective for this strain. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the our recommended media may affect recovery, growth and/or function of this strain. If an alternative medium formulation is used, our warranty for viability is no longer valid.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans. While we use reasonable efforts to include accurate and up to date information on this product sheet, we make no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. We do not warrant that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. We are not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of strains on deposit, we are not liable for damages arising from the misidentification or misrepresentation of cultures.

Related products

iPSC SFM Culture Medium (Xeno-Free)	cAP-49	500ml	Angio-Proteomie
iPSC Coating Solution	cAP-50	50ml	Angio-Proteomie
iPSC Non-Enzymatic Dissociation Solution	cAP-51	50ml	Angio-Proteomie
ROCK Inhibitor (500 x) Solution	cAP-52	1 ml	Angio-Proteomie
iPSC Freezing Medium	cAP-53	50ml	Angio-Proteomie

Caution: Handling human tissue derived products is potentially bio-hazardous. Although each cell strain is tested negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate; therefore, proper precautions must be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination.