Product Sheet
ATCC-DYR0530 Human Induced Pluripotent Stem (IPS) Cells (ATCC® ACS-1012™)

Please read this FIRST

IMPORTANT: ATCC strongly recommends that users download and read the ATCC® Stem Cell Culture Guide: Tips and Techniques for Culturing Stem Cells (http://www.atcc-guides.org/stemcell) before initiating their cultures.

ATCC-DYR0530 Human Induced Pluripotent Stem Cells (iPSCs) were derived from adult dermal fibroblasts obtained from a deceased 63 year-old donor who was diagnosed with Parkinson's disease (as well as asthma and depression; same donor as ACS-1013™, ACS-1014™). 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 the molecular pathology associated with Parkinson's disease. The availability of ACS-1012™, ACS-1013™ and ACS-1014™ provide a set of iPSCs that allow a side-by-side comparison of various reprogramming methods (episomal plasmid, retrovirus and Sendai virus) on iPSC performance.

Cell Type: retroviral reprogrammed hiPSC
Reprogramming Method: Retroviral expression of OCT4, SOX2, KLF4, and MYC genes
Disease: Parkinson's disease, asthma, depression

Gender: Male
Age: 63 years
Isolation Date: 2011
Source: Primary dermal fibroblasts
Number of Colonies per Vial: ≥ 30 colonies after 5 days when seeded as directed

Batch-Specific Information
Refer to the Certificate of Analysis for batch-specific test results.

SAFETY PRECAUTION
ATCC highly recommends 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.

Unpacking & Storage Instructions
1. Check all containers for leakage or breakage.
2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

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 culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -80°C. Storage at -80°C will result in loss of viability.

Preparation for Culture
1. Night before thawing iPSC cells – Thaw CellMatrix™ Basement Membrane Gel on ice in refrigerator or cold room (2°C to 8°C).
2. One Hour Prior to Thawing the iPSC Cells – Prepare coated plates as described.
3. 30 Minutes Prior to Thawing Cells – Pre-warm Pluripotent Stem Cell SFM XF/FF (stem cell culture medium) at 37°C for at least 30 minutes before adding to cells. If using ROCK Inhibitor Y27632, prepare stem cell culture medium supplemented with final concentration of 10 μM 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 subcultivation 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.

Protocol for Coating Plates
Important: CellMatrix™ Gel will solidify in 15 to 30 minutes above 15°C. Keep CellMatrix™ Gel and labware on ice at all times to prevent the matrix from gelling prematurely.

Calculate the appropriate CellMatrix volume per plate based on concentration and usage. The concentration of CellMatrix is found on the product label.

Example: 2 mL of Cell Matrix at 150 μg/mL is required to coat one 6 cm dish. To coat two 6 cm dishes, prepare as follows:
ATCC-DYR0530 Human Induced Pluripotent Stem (IPS) Cells (ATCC® ACS-1012™)

Please read this FIRST

Storage Temp.
Liquid Nitrogen
Vapor Phase (-130°C or colder)

Biosafety Level 2

Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: ATCC-DYR0530 Human Induced Pluripotent Stem (IPS) Cells (ATCC® ACS-1012™)

Initiation of Cultures

1. Rapidly thaw the cells by placing the cryovial in a 37°C 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 stem cell culture medium drop-wise, 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 1 mL of stem cell culture medium with ROCK Inhibitor Y27632. Gently resuspend the pellet by pipetting up and down 1 to 2 times with a 1 mL tip. Do not over pipette, as it is crucial to maintain the cells in aggregates.
8. Aspirate the coating solution from the plates prepared in step 4 of the Protocol for Coating Plates section. Add 5 mL of stem cell culture medium with ROCK Inhibitor Y27632 to each of two 6 cm dishes.
9. Seed 0.5 mL of cell aggregates onto the dishes prepared in step 8.
10. Incubate the culture at 37°C in a suitable incubator. A 5% CO₂ in air atmosphere is recommended if using the medium described on this product sheet.

Products for Feeder-Free Stem Cell Culture

- Pluripotent Stem Cell SFM XF/FF (ATCC® ACS-3002)
- CellMatrix Basement Membrane Gel (ATCC® ACS-3035)

Propagation

CellMatrix™ Basement Membrane Gel, ATCC ACS-3035
Pluripotent Stem Cell SFM XF/FF, ATCC ACS-3002

Subculturing Procedure

Cell culture dishes are coated with CellMatrix Basement Membrane Gel (ATCC® No. ACS-3035) to provide a surface for the attachment of iPSCs.

Coating Procedure:

1. Thaw CellMatrix Gel on ice and swirl gently to mix. Important: CellMatrix Gel will solidify in 15 to 30 minutes above 15°C. Keep CellMatrix Gel, vials and pipette tips on ice at all times to prevent CellMatrix Gel from solidifying. If air bubbles form, they may be eliminated by centrifuging CellMatrix Gel at 300 x g for 10 minutes at 2°C to 8°C.
2. Determine the appropriate volume per aliquot based on concentration and usage. Example: 2 mL of CellMatrix at 150 µg/mL is required to coat one 6-cm dish. To coat two 6-cm dishes, prepare as follows:
   
   \[
   \text{Dilute CellMatrix in DMEM:F12 to a working concentration of 150 µg/mL. For instance, if the protein concentration of CellMatrix (on certificate of analysis) is 14 mg/mL, then:} \ (4 \text{ mL}) \times (0.15 \text{ mg/mL})/(14 \text{ mg/mL}) = 0.043 \text{ mL. Therefore, add } 43 \text{ µL CellMatrix directly in } 4 \text{ mL cold DMEM: F-12 Medium} \\
   \text{3. Cell culture dishes coated with CellMatrix Basement Membrane Gel should be incubated at 37°C for one hour. Aspirate coating solution and immediately plate the cells. It is critical that the coating does not dry out.} \\
   \text{Volumes used in this protocol are for a 75 cm² flask.} \\
   
   \text{Post thaw day 1, perform a 100% medium change and remove all cells that did not attach. Perform} 

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Storage Temp. Liquid Nitrogen
Vapor Phase (~130°C or colder)

Biosafety Level
2

Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

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a 100% medium change every day. Passage the cells every 4 to 5 days (80% confluent) at an appropriate split ratio (a 1:4 split ratio is recommended). 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 on CellMatrix Gel-coated dishes containing 5 mL Pluripotent Stem Cell XF/FF medium/6-cm dish.

This protocol is designed to passage stem cell colonies cultured in a 6 cm dish, using Stem Cell Dissociation Reagent (ATCC ACS-3010) to detach the cell colonies. The recommended split ratio is 1:4. Volumes should be adjusted according to the size and number of the tissue culture vessels to be processed.

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 6 cm dish.

1. Detach stem cell colonies from the dish as described in the recommended subculturing protocol (steps 1-11). 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 to the tube. Gently resuspend the pellet by pipetting up and down 2 to 3 times with a 1 mL tip, 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.
4. Immediately transfer 1 mL each of the cell suspension into two labeled cryovials.
5. Freeze the cells gradually at a rate of -1°C/min until the temperature reaches -70°C to -80°C. A cryopreservation container (e.g., CoolCell® freezing container) may also be used.
6. The cells should not be left at -80°C for more than 24 to 48 hours. Once at -80°C, frozen cryovials are ready for storage in a cryopreservation container (e.g., CoolCell® freezing container) at -130°C or colder.)
should be transferred to the vapor phase of liquid nitrogen for long-term storage.

References

References and other information relating to this product are available online at www.atcc.org.

Use Restrictions

These cells are distributed for research purposes only. ATCC recommends that individuals contemplating commercial use of any cell line first contact the originating investigator to negotiate an agreement. Third party distribution of this cell line is discouraged, since this practice has resulted in the unintentional spreading of cell lines contaminated with inappropriate animal cells or microbes.

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.

ATCC Warranty

The viability of ATCC® 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. ATCC lists 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 ATCC recommended media may affect recovery, growth and/or function of this strain. If an alternative medium formulation is used, the ATCC 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 ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org.

Additional information on this culture is available on the ATCC web site at www.atcc.org.

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