ATCC

Product Sheet

KYOU-DXR0109B Human Induced Pluripotent Stem (IPS) Cells [201B7] (ATCC® ACS-1023™)

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.
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Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: KYOU-DXR0109B Human Induced Pluripotent Stem (IPS) Cells [201B7] (ATCC® ACS-1023™)

Shipping Information

Frozen

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800-638-6597 (US, Canada, Puerto Rico)
Tel: 703-365-2700
Fax: 703-365-2750
Tech@atcc.org

Description

KYOU-DXR0109B Human Induced Pluripotent Stem Cells (iPSCs) were derived at Kyoto University from dermal fibroblasts obtained from a healthy donor. Fibroblasts were reprogrammed by the expression of *OCT4, SOX2, KLF4* and *MYC* using retroviral transduction. This cell line is one of the pioneer lines from the Yamanaka laboratory and serves as a "normal" control for designed experiments.
Cell Type: Yamanaka retrovirus reprogrammed hiPSC
Reprogramming Method: Retroviral expression of *OCT4, SOX2*, *KLF4*, and *MYC* genes
Disease: Normal
Gender: Female
Age: 36 years
Isolation Date: 2007
Source: KYOU-DXR0109B Human Induced Pluripotent Stem Cells (iPSCs) were derived at Kyoto University from dermal fibroblasts obtained from a healthy donor.
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 Flask Cultures

Perform a 100% medium change every day. Passage the cells every 4 to 5 days (80% confluent) at a split ratio of 1:4. If the colonies are close to, or touching each other, the culture is overgrown. Overgrowth will result in differentiation. ROCK Inhibitor Y27632 is not required each time the culture medium is changed. It is required when the cells are recovering from thaw and recommended when cells are passaged.

Products for Feeder-Free Stem Cell Culture

Pluripotent Stem Cell SFM XF/FF: ATCC ACS-3002
CellMatrix™ Basement Membrane Gel: ATCC ACS-3035
Stem Cell Dissociation Reagent: ATCC ACS-3010
D-PBS: ATCC 30-2200
ROCK Inhibitor Y27632: ATCC ACS-3030
Stem Cell Freezing Media: ATCC ACS-3020

Propagation

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.
Important: CellMatrix™ will gel in 15 to 30 minutes above 15°C. Keep CellMatrix 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.
Preparation of Culture

1. Thaw CellMatrix™ in the refrigerator (2°C to 8°C), in ice, overnight.
2. Dilute the thawed CellMatrix™ to 150 µg/mL in cold DMEM: F-12 (ATCC 30-2006) on ice and mix well. Immediately coat each 6 cm dish with 2 mL diluted CellMatrix™.
3. Swirl dish gently to ensure that the entire dish is evenly covered.
4. Leave the coated dishes at 37°C for one hour.
5. Aspirate the coating solution and immediately plate the cells. It is critical that the coating does not dry

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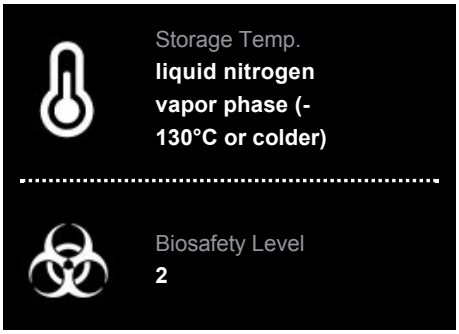

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out.

Protocol for Coating Plates

1. One Hour Prior to Thawing the iPS Cells - Prepare coated plates as described.
2. 30 Minutes Prior to Handling Cells - Pre-warm Pluripotent Stem Cell SFM XF/FF 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 spindlelike morphology effect on the cells. However, the colony morphology will recover after subsequent media change without ROCK inhibitor.
3. 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.
4. Sterilize the cryovial by rinsing with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
5. Using a 1-mL or 5-mL pipette, gently transfer the cell suspension to a 15-mL conical tube.
6. Slowly add 4 mL stem cell culture medium drop-wise, to the conical tube. Use an additional 1 mL of media to rinse the cryovial and transfer the liquid to the 15-mL conical tube. Shake the conical tube gently to mix the cells while adding media.
7. Gently pipette the cells up and down several times to mix thoroughly. Avoid breaking apart the aggregates into a single-cell suspension.
8. Centrifuge the cells at 200 x g for 5 minutes.
9. Aspirate the supernatant and discard. Gently tap on the bottom of the tube to loosen the cell pellet.
10. Add 1 mL of stem cell culture medium that has been supplemented with ROCK Inhibitor Y27632 to a final concentration of 10 µM to the tube. Gently resuspend the pellet by pipetting up and down 5-6 times with a 1-mL tip, maintaining the cell aggregates.
11. Add 4 mL of pre-warmed Pluripotent Stem Cell SFM SF/FF, also with ROCK Inhibitor Y27632 at 10 µM, to each of four 6-cm dishes.
12. Seed 0.5 mL of cell aggregates onto the dishes prepared in step 11.
13. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

Maintenance

Perform a 100% medium change every day. Passage the cells every 4 to 5 days (80% confluent) at a split ratio of 1:4. If the colonies are close to, or touching each other, the culture is overgrown. Overgrowth will result in differentiation. ROCK Inhibitor Y27632 is not required each time the culture medium is changed. It is required when the cells are recovering from thaw and recommended when cells are passaged.

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:

   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 mL) x (0.15 mg/mL)/(14 mg/mL) = 0.043 mL. Therefore, add 43 µL CellMatrix directly in 4 mL cold DMEM: F-12 Medium
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.

Volumes used in this protocol are for a 75 cm^2 flask.

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 (a 1:4 split ratio is recommended). If the colonies are close to, or touching each other, the culture is

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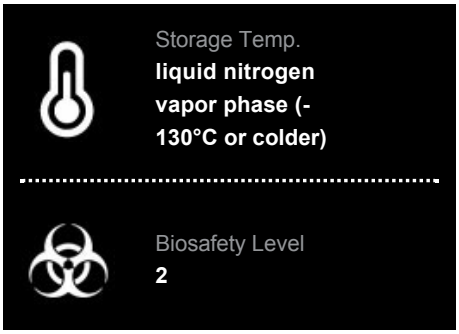

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ROCK Inhibitor Y27632 is not necessary each time the culture medium is changed. It is required when cells are recovering from thaw; it is recommended when the cells are being passaged.

This protocol is designed to passage stem cell colonies cultured in a 6 cm dish, using EDTA Dissociation Reagent to detach the cell colonies. The recommended spilt ratio is 1:4. Volumes should be adjusted according to the size and number of the tissue culture vessels to be processed.

EDTA Dissociation Reagent:
500ul 0.5M EDTA
0.9g NaCl
in 500ml Calcium/Magnesium free PBS
Sterile filter and store at 4°C

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.

1. Warm an aliquot of EDTA Dissociation Reagent working solution to room temperature.
2. Aspirate and discard the stem cell culture medium.
3. Rinse the cells twice by adding and discarding 4 mL of D-PBS.
4. Add 2 mL of EDTA Dissociation Reagent working solution to the dish.
5. Incubate at 37°C for 2 to 5 minutes.
6. Aspirate the EDTA Dissociation Reagent and gently rinse the colonies with 4 mL of DMEM: F-12 Medium, taking care not to dislodge the cells during manipulation. Aspirate the DMEM: F12 rinse and discard.
7. Add 2 mL of stem cell culture medium with ROCK Inhibitor Y27632 to the dish, and detach the cells by pipetting up and down 2 to 3 times with a 1 mL tip. **Take care not to over-pipette the culture into a single-cell suspension as single cells will not establish colonies after seeding**.
8. Transfer the cell aggregates to a 15 mL conical tube.
9. Add an additional 3 mL of stem cell culture medium with ROCK Inhibitor Y27632 to the dish to collect any remaining cells. Transfer this rinse to the 15 mL conical tube containing the cell aggregates.
10. Centrifuge the cell aggregates at 200 x g for 5 minutes.
11. Aspirate the supernatant and discard.
12. Add 1 mL 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, 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.**
13. Plate the cells as desired on feeder or feeder-free cultures.
14. Incubate the culture at 37°C in a humidified 5% CO2/95% air incubator. Perform a 100% medium change every day. Passage the cells every 4 to 5 days (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 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 5-6 times with a 1-mL tip, maintaining the cell aggregates.
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. An isopropanol freezing container also may 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 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.

Quality Control Information

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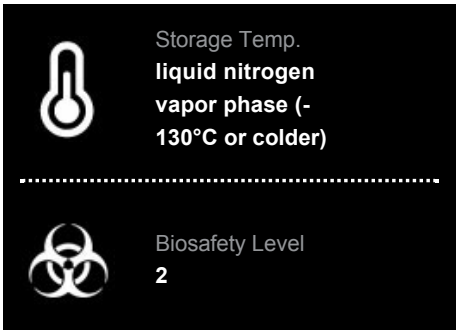

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Cells are tested for post-freeze viability and growth, sterility (including mycoplasma), identity by STR analysis and karyotype by G-banding. Each lot is tested for pluripotency using flow cytometry for the expression of the pluripotent markers. A Certificate of Analysis is available upon request.

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.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is 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, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.

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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