KYOU-DXR0109B Human Induced Pluripotent Stem (IPS) Cells [201B7]

ACS-1023™

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.

Organism: *Homo sapiens*, human
Cell Type: iPSC
Tissue: Skin
Age: 36 years
Gender: Female
Disease: Normal
Cells per vial: ≥ 30 colonies after 5 days when seeded as directed

Storage Conditions

Product format: Frozen
Storage conditions: Vapor phase of liquid nitrogen

Intended Use

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

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BSL 2

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may 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 vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Handling Procedures

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Unpacking and 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.

Complete medium: ATCC iPSCs have been adapted to feeder- and serum-free culture conditions.

The base medium for this cell line is Pluripotent Stem Cell SFM XF/FF (ATCC® No. ACS-3002) which is a ready-to-use medium for serum-free and feeder-free iPSC culture.

Reagents for subculture:: Stem Cell Dissociation Reagent (ATCC ACS-3010)
D-PBS (ATCC 30-2200)
ROCK Inhibitor Y27632 (ATCC ACS-3030)
Pluripotent Stem Cell SFM XF/FF (ATCC ACS-3002)

Handling Procedure:

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

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 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 two 6-cm dishes.
12. Seed 0.5 mL of cell aggregates onto the two 6-cm 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.

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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 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 spilt ratio is 1:4. Volumes should be adjusted according to the size and number of the tissue culture vessels to be processed.

Reconstitution of Stem Cell Dissociation Reagent:

Lyophilized proteins tend to be hygroscopic. Bring the vial of Stem Cell Dissociation Reagent to room temperature before opening. The vial should not be cool to the touch. Once opened, the lyophilized material should be stored desiccated. The specific activity of the reagent is found on the certificate of analysis. Dissolve the appropriate amount of Stem Cell Dissociation Reagent in DMEM: F-12 Medium to prepare a 0.5 U/mL working solution.

1. Dissolve the appropriate amount of Stem Cell Dissociation Reagent in DMEM: F-12 Medium to prepare a 0.5 U/mL working solution. **Example:** To prepare 40 mL of a 0.5 U/mL working solution: Specific activity of Stem Cell Dissociation Reagent (on certificate of analysis) =1.46 U/mg (40 mL) x (0.5 U/mL)/(1.46 U/mg) = 13.7 mg Dissolve 13.7 mg Stem Cell Dissociation Reagent in 40 mL DMEM: F-12 Medium.
2. Filter sterilize through a 0.22 µm filter membrane.
3. Aliquot into working volumes according to routine usage.
4. Store aliquots at -20°C for up to three months. Avoid repeated freezing and thawing. Thawed aliquots may be kept at 2°C to 8°C for up to two weeks.

Note: Addition of ROCK inhibitor has been shown to increase the survival rate. 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 Stem Cell 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 DMEM:F12.
4. Add 2 mL of Stem Cell Dissociation Reagent working solution to the dish.
5. Incubate at 37°C for 2 to 5 minutes.
6. Aspirate the Stem Cell 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 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 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. 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 on CellMatrix Gel-coated dishes containing 5 mL Pluripotent Stem Cell XF/FF medium/6-cm dish.
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).

Culture 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.

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.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: KYOU-DXR0109B Human Induced Pluripotent Stem (IPS) Cells [201B7] (ATCC ACS-1023)

References

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

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Contact Information

ATCC

10801 University Boulevard

Manassas, VA 20110-2209

USA

US telephone: 800-638-6597

Worldwide telephone: +1-703-365-2700

Email: tech@atcc.org or contact your local distributor