It is important to note that some vials leak when submersed in liquid nitrogen: CellMatrix Gel will solidify in 15 to 30 minutes above 15°C. Keep CellMatrix Gel and labware on 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-HYR0103 Human Induced Pluripotent Stem (IPS) Cells (ATCC® ACS-1007™)

**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:

Dilute CellMatrix in DMEM:F12 at a working concentration of 150 μg/mL:

Protein concentration of CellMatrix (on product label): 14 mg/mL.

**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 Handling 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.

**Handling Procedure for Frozen Cells**

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 submerged 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.

**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 Handling 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.
Biologicals for Feeder-Free Stem Cell Culture

Subculturing 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:

\[
\text{Dilute CellMatrix in DMEM:F-12 to a working concentration of 150 µg/mL. For instance, if the protein concentration of CellMatrix (on certificate of analysis) is 14 mg/mL, then: (4 mL) x (0.15 mg/mL)/(14 mg/mL) = 0.043 mL. Therefore, add 43 µL CellMatrix directly in 4 mL cold DMEM: F-12 Medium.}
\]

3. Cell culture dishes coated with CellMatrix Basement Membrane Gel should be incubated at 37°C for one hour. Aspirate the coating solution from the plates prepared in step 4 of the Protocol for Coating Plates section. Add 4 mL of stem cell culture medium with ROCK Inhibitor Y27632 to each of two 6 cm dishes.

4. Seed 0.5 mL of cell aggregates onto the dishes prepared in step 8.

5. 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.

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 split 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.

- Warm an aliquot of Stem Cell Dissociation Reagent working solution to room temperature.
- Aspirate and discard the stem cell culture medium.
- Rinse the cells twice by adding and discarding 4 mL of DMEM:F12.
- Add 2 mL of Stem Cell Dissociation Reagent working solution to the dish.
- Incubate at 37°C for 2 to 5 minutes.
- 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.
- 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.
- Transfer the cell aggregates to a 15 mL conical tube.
- Add 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.
- Centrifuge the cell aggregates at 200 x g for 5 minutes.
- Aspirate the supernatant and discard.
- 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.
- Plate the cells on CellMatrix Gel-coated dishes containing 5 mL Pluripotent Stem Cell XF/FF medium/6-cm dish.
- Incubate 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 2 to 3 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. A cryopreservation container (e.g., CoolCell® freezing container) may also be used.
6. The cells should not be kept 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.
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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