iPSC-derived Mesenchymal Stem Cells; BYS0112 (ATCC® ACS-7010™)

Please read this FIRST

Storage Temp. liquid nitrogen vapor phase

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.

Complete Growth Medium

The base medium for these primary cells is Mesenchymal Stem Cell Basal Medium for Adipose, Umbilical and Bone Marrow-derived MSCs (ATCC PCS-500-030). To make the complete medium, add the contents of Mesenchymal Stem Cell Growth Kit for Bone Marrow-derived MSCs (ATCC PCS-500-041) as per the product sheet.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: iPSC-derived Mesenchymal Stem Cells; BYS0112 (ATCC® ACS-7010™)

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.

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

Refer to the batch specific information for the total number of viable cells recovered from this lot of ATCC® ACS-7010

1. Using the total number of viable cells, determine how much surface area can be inoculated to achieve an initial seeding density of between 10,000 and 20,000 cells per cm².
2. Prepare the desired combination of flasks. Add 5 mL of complete growth media per 25 cm² of surface area. Place the flasks in a 37°C, 5% CO₂ humidified incubator and allow the media to pre-equilibrate to temperature and pH for 30 minutes prior to adding cells.
3. While the culture flasks equilibrate, remove one vial of ATCC® ACS-7010 cells from storage and thaw the cells in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 1 to 2 minutes).
4. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All operations from this point onward should be carried out under strict aseptic conditions.
5. Transfer the vial content into 5mL of complete media taken in a 15 mL sterile conical tube – Centrifuge the tube at 270-300 xg for 5min. Aspirate supernatant and re-suspend the cell pellet in 5 mL of complete culture media. Take an aliquot for cell counting.
6. seed 10,000 to 20,000 cells/cm² to pre-equilibrated culture flasks prepared in steps 1 to 3 of Handling Procedure for Frozen Cells and Initiation of Culture. Cap and gently rock each flask to evenly distribute the cells.
7. Place the seeded culture flasks in the incubator at 37°C with a 5% CO₂ atmosphere. Incubate for at least 24 hours before processing the cells further.

Handling Procedure for Flask Cultures

1. Pre-warm complete growth media in a 37°C water bath. This will take between 10 to 30 minutes, depending on the volume. If using a small volume of medium (50 mL or less), warm only the volume needed in a sterile conical tube. Avoid warming complete growth media multiple times.
2. 24 to 36 hours after seeding, remove the cells from the incubator and view each flask under the microscope to determine percent cellular confluence.
3. and return the flasks to the incubator.
4. After 48 hours, view each flask under the microscope to determine percent cellular confluence and change media with 5mL for a T25 flask. When cultures have reached 80% to 90% confluence, and are actively proliferating, it is time to subculture.

Subculturing Procedure

Refer to the Certificate of Analysis for batch-specific test results.
1. Passage iPSC-derived MSC when culture has reached approximately 80% to 90% confluence, and are actively proliferating.
2. Warm both the Trypsin-EDTA for Primary Cells (ATCC PCS-999-003) and growth medium to 37°C prior to use with the cells.
3. For each flask, carefully aspirate the spent media without disturbing the monolayer.
4. Briefly rinse the cell layer with 3 to 5 mL DPBS (ATCC 30-2200) to remove residual traces of serum and then aspirate and discard the DPBS.
5. Add pre-warmed trypsin-EDTA solution (2 to 3 mL for every 25 cm²) to each flask.
6. Gently rock each flask to ensure complete coverage of the trypsin-EDTA solution over the cells.
7. Incubate at 37°C for 3 min. Observe the cells under the microscope. When the cells pull away from each other and round up (typically within 3 to 5 minutes), remove the flask from the microscope and gently tap it from several sides to promote detachment of the cells from the flask surface.
8. When the majority of cells are detached, quickly add an equal volume of complete growth medium to each flask. Gently pipette or swirl the culture to ensure all of the trypsin-EDTA solution has been neutralized.
9. Transfer the dissociated cells to a sterile centrifuge tube and set aside while processing any remaining cells in the flask.
10. Add 3 to 5 mL complete growth media to the flask to collect any remaining dissociated cells. Transfer remaining cells into the centrifuge tube.
11. Repeat steps 10 as needed until all cells have been collected from the flask.
12. Centrifuge the cells at 270-300 x g for 3 to 5 minutes.
13. Carefully aspirate the neutralized dissociation solution from the cell pellet and re-suspend the cells in 5 to 8 mL fresh, pre-warmed, complete growth medium.
14. Count the cells and seed in new flasks at a density of 10,000 to 20,000 cells per cm².
15. Place freshly seeded flasks in a 37°C, 5% CO₂ incubator for at least 24 to 48 hours before processing the cells further. Refer to Maintenance for guidelines on feeding.

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**Cryopreservation Medium**

Stem Cell Freezing Medium (ATCC ACS-3020)

**Comments**

Human iPSC-derived mesenchymal stem cells (MSC) enable researchers to carry out applications that are typically performed using primary MSCs. These cells can be used for bone cell lineage differentiation, regenerative medicine, cell therapy, exosome research, and cancer immunology.

A distinct advantage of incorporating iPSC-derived MSCs: While there will be lot-to-lot donor variability in primary MSC, there will be no donor variability in iPSC-derived MSC from multiple lots as they are all derived from the parental iPSC line (ATCC ACS-1026). In addition, there is an availability of large number vials of cells from a single donor.

**References**

References and other information relating to this product are available online at [www.atcc.org](http://www.atcc.org).

**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**

ATCC® products are warranted for 30 days from the date of shipment, and this warranty is valid only if the product is stored and handled according to the information included on this product information sheet. If the ATCC® product is a living cell or microorganism, ATCC lists the media formulation that has been found to be effective for this product. 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 product. 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.
Please read this FIRST

**Storage Temp.**
- liquid nitrogen
- vapor phase

**Biosafety Level**
- 2

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