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.

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.

Prepare the desired combination of flasks. Add 5 mL of complete growth media per 25 cm² surface area. 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.

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™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org
800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org
Or contact your local distributor
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.

**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.

**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

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™)

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

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