iPSC-derived Mesenchymal Stem Cells; BYS0112

ACS-7010™

Description

iPSC-derived Mesenchymal Stem Cells; BYS0112 are induced pluripotent stem cells that have been differentiated into multipotent stromal cells. iPSC-derived MSCs may be further differentiated into osteoblasts, chondrocytes, myocytes, and adipocytes. These cells can be used in bone cell lineage differentiation, regenerative medicine, cell therapy, exosome research, and cancer immunology research.

Organism: *Homo sapiens*, human
Gender: Male
Morphology: spindle-shaped, fibroblast-like
Growth properties: Adherent
Cells per vial: Approximately 2.0 to 3.0 x 10^6
Volume: 1.0 mL

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.

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.

All tissues used for isolation are obtained under informed consent and conform to HIPAA regulations to protect the privacy of the donor's Personally Identifiable Information. It is best to use caution when handling any human cells. We recommend that all human cells be accorded the same level of biosafety consideration as cells known to carry Human immunodeficiency virus (HIV) and other bloodborne pathogens. With infectious virus assays or viral antigen assays, even a negative test result may not exclude the possibility of the existence of a latent viral genome or infectious viral particles below the lower limit of detection of that assay.

ATCC recommends that appropriate safety procedures be used when handling all primary cells and cell lines, especially those derived from human or other primate material. Handle as a potentially biohazardous material using universal precautions. Cells derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens.

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.

Growth Conditions

Atmosphere: 95% Air, 5% CO_2

Handling Procedures

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: 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.

Handling Procedure: 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 cm^2.
2. Prepare the desired combination of flasks. Add 5 mL of complete growth media per 25 cm^2 of surface area. Place the flasks in a 37°C, 5% CO_2, 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% CO_2 atmosphere. Incubate for at least 24 hours before processing the cells further.

Subculturing procedure:

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 cm^2) 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 cm^2
15. Place freshly seeded flasks in a 37°C, 5% CO_2 incubator for at least 24 to 48 hours before processing the cells further. Refer to Maintenance for guidelines on feeding.

Reagents for cryopreservation: Stem Cell Freezing Medium (ATCC ACS-3020)

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: iPSC-derived Mesenchymal Stem Cells; BYS0112 (ATCC ACS-7010)

References

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

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