ATCC® Normal Human Umbilical Cord-Derived Mesenchymal Stem Cells, when grown in Mesenchymal Stem Cell Basal Media supplemented with Mesenchymal Stem Cell Growth Kit for Adipose and Umbilical-derived MSCs - Low serum components, provide an ideal cell system to propagate mesenchymal stem cells in low serum (2% FBS) conditions. When maintained under optimal growth conditions, ATCC Normal Human Umbilical Cord-Derived Mesenchymal Stem Cells have been shown to be multipotent, capable of differentiating down the adipogenic, osteogenic, and chondrogenic lineages. The cells are cryopreserved at the second passage to ensure the highest viability and plating efficiency. ATCC® Primary Cell Solutions™ cells, media, supplements and reagents are quality tested together to guarantee optimum performance and reliability.

Components: One vial of Umbilical Cord-Derived Mesenchymal Stem Cells; Normal, Human (ATCC® PCS-500-010™) containing a minimum of 5 x 10^6 viable cells (provided).

Also Required:
A. One bottle of Mesenchymal Stem Cell Basal Medium (ATCC PCS-500-030) plus one Mesenchymal Stem Cell Growth Kit for Adipose and Umbilical-derived MSCs - Low serum (ATCC PCS-500-040) that contains the following growth supplements: MSC Supplement (composed of FBS, rh FGF basic, rh FGF acidic, and rh EGF) and L-alanyl-L-glutamine.
B. Optional media supplements
   1. Gentamicin-Amphotericin B Solution (ATCC PCS-999-025)
   2. Penicillin-Streptomycin-Amphotericin B Solution (ATCC PCS-999-002)
   3. Phenol Red (ATCC PCS-999-001)
C. Reagents for subculture
   1. D-PBS (ATCC 30-2200)
   2. Trypsin-EDTA for Primary Cells (ATCC PCS-999-003) containing 0.05% Trypsin and 0.02% EDTA. Note: Do not use other Trypsin-EDTA concentrations with ATCC PCS-500-010.
   3. Trypsin Neutralizing Solution (ATCC PCS-999-004)

Cell Characteristics
Tissue: Umbilical

Morphology: spindle-shaped, fibroblast-like

Growth Properties: adherent

Batch-Specific Information
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 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 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.
3. Obtain one bottle of Mesenchymal Stem Cell Basal Medium (485 mL) from cold storage.
4. Decontaminate the external surfaces of all growth kit component vials and the basal medium bottle by
Handling Procedure for Frozen Cells and Initiation of Culture

1. **Refer to the batch specific information for the total number of viable cells recovered from this lot of ATCC® PCS-500-010.**

2. Using the total number of viable cells, determine how much surface area can be inoculated to achieve an initial seeding density of 5,000 cells per cm².

3. Prepare the desired combination of flasks. Add 5 mL of complete growth medium 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.

4. While the culture flasks equilibrate, remove one vial of ATCC® PCS-500-010 from storage and thaw the cells by gentle agitation 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).

5. 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.

6. Add the appropriate volume of complete growth medium [volume = (1 mL x number of flasks to be seeded)] mL] into a sterile conical tube. Using a sterile pipette, transfer the cells from the cryovial to the conical tube. Gently pipette the cells to homogenize the suspension. Do not centrifuge.

7. Transfer 1.0 mL of the cell suspension to each of the pre-equilibrated culture flasks prepared in steps 1 to 3 of Handling Procedure for Frozen Cells and Initiation of Culture. Pipette several times, then cap and gently rock each flask to evenly distribute the cells.

8. Place the seeded culture flasks in the incubator at 37°C, 5% CO₂ atmosphere. Incubate for at least 24 hours before processing the cells further.

**Maintenance**

1. Before beginning, pre-warm complete growth media in a 37°C water bath. This will take between 10 and 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 hours after seeding, remove the cells from the incubator and view each flask under the
microscope to determine percent cellular confluence.
3. Carefully remove the spent media without disturbing the monolayer.
4. Add 5 mL of fresh, pre-warmed complete growth medium per 25 cm² of surface area and return the
flasks to the incubator.
5. After 24 to 48 hours, view each flask under the microscope to determine percent cellular confluence.

Note: Umbilical cord-derived stem cells are contact inhibited. It is essential that the cells be
subcultured BEFORE reaching confluence as post-confluent cells exhibit changes in morphology,
slower proliferation, and reduced differentiation capacity after passing.

Subculturing

1. Passage normal umbilical cord-derived stem cells when the culture has reached approximately 70% to
80% confluence.
2. Warm both the Trypsin-EDTA for Primary Cells (ATCC PCS-999-003) and the Trypsin Neutralizing
Solution (ATCC PCS-999-004) to room temperature prior to dissociation. Warm the complete 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. Add pre-warmed trypsin-EDTA solution (1 to 2 mL for every 25 cm²) to each flask.
5. Centrifuge the cells at 150 x g for 3 to 5 minutes.
6. Repeat steps 10 and 11 as needed until all cells have been collected from the flask.
7. Centrifuge the cells at 150 x g for 3 to 5 minutes.
8. Add 3 to 5 mL D-PBS (ATCC 30-2200) to the tissue culture flask to collect any additional cells that
might have been left behind.
9. Add 5 mL of fresh, pre-warmed complete growth medium to 37°C, 5% CO₂ incubator for at least 24 to 48 hours before processing
the cells further. Refer to Maintenance for guidelines on feeding.

Quality Control Specifications

Growth
Each lot of ATCC PCS-500-010 is tested to ensure the cells can be passaged at least three times (i.e., ≥ 10
population doublings) after thaw in complete growth media (Mesenchymal Stem Cell Basal Medium plus one
Mesenchymal Stem Cell Growth Kit–Low serum) and support differentiation.
Viability: ≥ 70% when thawed from cryopreservation

Sterility Testing
Bacteria and Yeast: Negative
Mycoplasma: Negative

Viral Testing
Hepatitis B: Negative
Hepatitis C: Negative
HIV-1: Negative
HIV-2: Negative

Specific Staining
Positive for CD29, CD44, CD73, CD90, CD105, and CD166 (greater than 95% of the cell population expresses
these markers by flow cytometry).
Negative for CD14, CD31, CD34, and CD45 (less than 5% of cell population expresses these markers by flow cytometry). Each lot of PCS-500-010 is tested to ensure in vitro differentiation.

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.

All tissues used for isolation are obtained under informed consent and conform to HIPAA standards to protect the privacy of the donor’s personal health 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 HIV. With infectious virus assays or viral antigen assays, even a negative test result may leave open the possible existence of a latent viral genome.

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.

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