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 $5 \times 10^5$ viable cells (provided).

**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: Umbilical Cord-Derived Mesenchymal Stem Cells; Normal, Human (ATCC® PCS-500-010™)

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spraying them with 70% ethanol.

5. Using aseptic technique and working in a laminar flow hood or biosafety cabinet, transfer the indicated volume of each growth kit component, as indicated in Table 1, to the bottle of basal medium using a separate sterile pipette for each transfer.

Table 1. Mesenchymal Stem Cell Growth Kit for Adipose and Umbilical-derived MSCs - Low serum Components

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSC Supplement</td>
<td>10 mL</td>
<td>2% FBS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 ng/mL rhFGF basic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 ng/mL rhFGF acidic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 ng/mL rhEGF</td>
</tr>
<tr>
<td>L-Alanyl-L-Glutamine</td>
<td>6 mL</td>
<td>2.4 mM</td>
</tr>
</tbody>
</table>

Antimicrobials and phenol red are not required for proliferation but may be added if desired. The recommended volume of either of the optional components (GA solution or PSA solution) to be added to the complete growth media is summarized in Table 2.

Table 2. Addition of Antimicrobials/Antimycotics and Phenol Red (Optional)

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicin-Amphotericin B Solution</td>
<td>0.5 mL</td>
<td>Gentamicin: 10 µg/mL Amphotericin B: 0.25 µg/mL</td>
</tr>
<tr>
<td>Penicillin-Streptomycin-Amphotericin B Solution</td>
<td>0.5 mL</td>
<td>Penicillin: 10 Units/mL Streptomycin: 10 µg/mL Amphotericin B: 25 ng/mL</td>
</tr>
<tr>
<td>Phenol Red</td>
<td>0.5 mL</td>
<td>33 µM</td>
</tr>
</tbody>
</table>

6. Tightly cap the bottle of complete growth medium and swirl the contents gently to assure a homogeneous solution. Do not shake forcefully to avoid foaming. Label and date the bottle.

7. Complete growth media should be stored in the dark at 2°C to 8°C (do not freeze). When stored under these conditions, complete growth media is stable for two weeks.

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) 1 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.**
Umbilical Cord-Derived Mesenchymal Stem Cells; Normal, Human (ATCC® PCS-500-010™)

Please read this FIRST

**Storage Temp.**
-130°C or below

**Biosafety Level**
1

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### 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. Gently rock each flask to ensure complete coverage of the trypsin-EDTA solution over the cells, and then aspirate the excess fluid off of the monolayer.
6. Observe the cells under the microscope. When the cells pull away from each other and round up (typically within 1 to 3 minutes), remove the flask from the microscope and gently tap it from several sides to promote detachment of the cells from the flask surface.
7. When the majority of cells appear to have detached, quickly add an equal volume of the Trypsin Neutralizing Solution (ATCC PCS-999-004) to each flask. Gently pipette or swirl the culture to ensure all of the trypsin-EDTA solution has been neutralized.
8. Transfer the dissociated cells to a sterile centrifuge tube and set aside while processing any remaining cells in the culture flask.
9. Centrifuge the cells at 150 x g for 3 to 5 minutes.
10. Add 3 to 5 mL D-PBS (ATCC 30-2200) to remove residual medium.
11. Transfer the dissociated cells to a sterile centrifuge tube and set aside while processing any remaining cells in the culture flask.
12. Centrifuge the cells at 150 x g for 3 to 5 minutes.
13. Aspirate neutralized dissociation solution from the cell pellet and resuspend the cells in 2 to 8 mL fresh, pre-warmed, complete growth medium.
14. Place newly 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.

### 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 ≥ 70% when thawed from cryopreservation
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.

Human Material Precaution
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.

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.

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