



Primary Subcutaneous Pre-adipocytes; Normal, Human

PCS-210-010™

Description

Normal Human Pre-Adipocytes were isolated from the adipose tissue of a donor and are derived from de-differentiated mature adipocytes by ceiling culture and providing an ideal culture model for the study of diabetes, obesity, metabolism, insulin sensitivity, and adipose biology.

Organism: *Homo sapiens*, human

Tissue: Adipose tissue

Age: adult

Gender: Lot-specific

Morphology: Spindle-shaped, fibroblast-like

Growth properties: Adherent

Cells per vial: $\geq 1.0 \times 10^6$

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 1

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.

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: 1. Obtain one Fibroblast Growth Kit-Low Serum from the freezer; make sure that the caps of all components are tight.

2. Thaw the components of the growth kit just prior to adding them to the basal medium.

3. Obtain one bottle of Fibroblast Basal Medium (480 mL) from cold storage.

4. Decontaminate the external surfaces of all growth kit component vials and the basal medium bottle by 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 below, to the bottle of basal medium using a separate sterile pipette for each transfer.

Fibroblast Growth Kit-Low Serum Components (ATCC® PCS-201-041):

rh FGF β , 0.5 mL, 5 ng/mL;

L-glutamine, 18.75 mL, 7.5 mM;

Ascorbic acid, 0.5 mL, 50 μ g/mL;

Hydrocortisone hemisuccinate, 0.5 mL, 1 μ g/mL;

rh Insulin, 0.5 mL, 5 μ g/mL;

Fetal Bovine Serum, 10.0 mL, 2%

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 below.

Addition of Antimicrobials/Antimycotics and Phenol Red (Optional):

Gentamicin-Amphotericin B Solution, 0.5 mL, (Gentamicin: 10 µg/mL, Amphotericin B: 0.25 µg/mL)

Penicillin-Streptomycin-Amphotericin B Solution, 0.5 mL, (Penicillin: 10 Units/mL, Streptomycin: 10 µg/mL, Amphotericin B: 25 ng/mL)

Phenol Red, 0.5 mL, 33 µM

1. 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.
2. 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 30 days.

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-210-010**
3. Trypsin Neutralizing Solution (ATCC PCS-999-004)

Required media: One bottle of Fibroblast Basal Medium (ATCC PCS-201-030) plus one Fibroblast Growth Kit (ATCC PCS-201-041).

Optional media supplements:

1. Adipocyte Differentiation Toolkit (ATCC PCS-500-050)
2. Gentamicin-Amphotericin B Solution (ATCC PCS-999-025)
3. Penicillin-Streptomycin-Amphotericin B Solution (ATCC PCS-999-002)
4. Phenol Red (ATCC PCS-999-001)

Handling Procedure:

1. ***Refer to the batch specific information provided on the last page of the product information sheet for the total number of viable cells recovered from this lot of ATCC® PCS-210-010.***
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-210-010 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. Carefully remove the cap to avoid contamination or spatter. Gently resuspend the cells in the vial using a 1 or 2 mL sterile pipette and quickly conduct cell counting. Do not centrifuge; the cells may be directly plated from the vial. Plate the cells into pre-warmed culture medium in the desired culture vessel(s) at a density of 5,000 cells per cm²
7. Replace the medium after the cells have attached, approximately 4-36 hours after inoculation to remove cryopreservation reagents.

Subculturing procedure:

1. Passage normal adipose-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. Rinse the cell layer one time with 3 to 5 mL D-PBS (ATCC® 30-2200) to remove residual medium.
5. Add pre-warmed trypsin-EDTA solution (1 to 2 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, and then aspirate the excess fluid off of the monolayer.
7. Observe the cells under the microscope. When the cells pull away from each other and round up (typically within 2 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.

8. 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.
9. Transfer the dissociated cells to a sterile centrifuge tube and set aside while processing any remaining cells in the culture flask.
10. 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.
11. Transfer the cell/D-PBS suspension to the centrifuge tube containing the trypsin-EDTA-dissociated cells.
12. Repeat steps 10 and 11 as needed until all cells have been collected from the flask.
13. Centrifuge the cells at 250 x g for 3 to 5 minutes.
14. Aspirate neutralized dissociation solution from the cell pellet and resuspend the cells in 2 to 8 mL fresh, pre-warmed, complete growth medium.
15. Count the cells and seed new culture flasks at a density of 5,000 viable cells per cm².
16. 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.

Culture 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. If not ready to passage, repeat steps 3 and 4 as described above. When cultures have reached approximately 70% to 80% confluence, and are actively proliferating (many mitotic figures are visible), it is time to subculture.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: Primary Subcutaneous Pre-adipocytes; Normal, Human (ATCC PCS-210-010)

References

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

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Revision

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Product Sheet

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