Primary Gingival Fibroblast; Normal, Human, Adult (HGF)

PCS-201-018™

Description

Primary gingival fibroblast was isolated from the gingiva. The cell has applications in human gingival fibroblasts (hGF) and could potentially be an alternative source of mesenchymal stem cells (MSC) for regenerative medicine studies as they share similar morphology, CD markers, and differentiation lineage.

Organism: *Homo sapiens*, human
Cell Type: fibroblast
Tissue: Gingiva
Age: 60 years
Gender: Female
Morphology: spindle-shaped; cells are bipolar and refractile
Growth properties: Adherent

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

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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.

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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

Temperature: 37°C
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:

1. Obtain one vial of Primary Normal Gingival Fibroblast Cells (ATCC PCS-201-018) from the freezer; make sure that the caps of all components are tight.
2. Thaw the components of the growth kit (ATCC PCS-201-041) just prior to adding them to the basal medium (ATCC PCS-201-030).
3. Obtain one bottle of Fibroblast Basal Medium (485 mL; ATCC PCS-201-030) 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 in "A" below, to the bottle of basal medium using a separate sterile pipette for each transfer.
    A. **If using the Fibroblast Growth Kit-Low Serum, add the indicated volume for each of the following components**
        - rh FGF b, 0.5 mL (Final concentration 5 ng/mL)
        - L-glutamine, 18.75 mL (Final concentration 7.5 mM)
        - Ascorbic acid, 0.5 mL (Final concentration 50 µg/mL)

- Hydrocortisone Hemisuccinate, 0.5 mL (Final concentration 1 µg/mL)
- rh Insulin, 0.5 mL (Final concentration 5 µg/mL)
- Fetal Bovine Serum, 10.0 mL (Final concentration 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 to be added to the complete growth media is summarized below.

B. **Addition of Antimicrobials/Antimycotics and Phenol Red (Optional):**
   - Penicillin-Streptomycin-Amphotericin B Solution, 0.5 mL (Final concentration Penicillin: 10 Units/mL, Streptomycin: 10 µg/mL, Amphotericin B: 25 ng/mL).
   - Phenol Red, 0.5 mL (Final concentration 33 µM)

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 30 days.

Handling Procedure:

To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

1. Thaw the vial 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 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
3. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium. and spin at approximately 125 x *g* for 5 to 7 minutes.
4. Discard supernatant and resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture

recommended dilution ratio). It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6). pH (7.0 to 7.6).

5. Refer to the batch-specific information provided on the Certificate of Analysis for the total number of viable cells recovered from this lot of ATCC PCS-201-018.
6. Using the total number of viable cells reported, determine how much surface area can be inoculated to achieve an initial seeding density of 2,500 to 5,000 cells per cm^2.
7. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

Subculturing procedure:

Volumes used in this protocol are for 75 cm^2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. Corning® T-75 flasks (catalog #430641) are recommended for subculturing this product.

1. Remove and discard culture medium.
2. Rinse the cell layer with DPBS solution for 2 minutes to remove all traces of serum that contains trypsin inhibitor.
3. Add 5.0 to 7.0 mL of Trypsin-EDTA solution to the flask and incubate at 37°C .Observe cells under an inverted microscope until cell layer is dispersed (usually within 4 to 6 minutes).

   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Add 5.0 to 7.0 mL of Trypsin Neutralizing Solution (ATCC® PCS-999-004™) Centrifuge at 125 x g; 10 ± 2 minutes. Discard supernatant and resuspend the cell pellet with 8 mL of complete growth media. Gently break cell pellet by pipetting repeatedly.
4. Count cells. Seed 2,500 to 5,000 viable cells per cm^2. Add appropriate volume of the cell suspension to new culture vessels.
5. Incubate cultures at 37°C.

Change media every 2-3 days

Subculture when cells reach 75-80% confluence. Seeding density should be 2,500 to 5,000 viable cells per cm^2

Culture maintenance:

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).
2. **If the cells are still attached,** aseptically remove all but 5 to 10 mL of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO_2 in air atmosphere until they are ready to be subcultured.
3. **If the cells are not attached,** aseptically remove the entire contents of the flask and centrifuge at 125 x *g* for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 mL of this medium and add to 25 cm^2 flask. Incubate at 37°C in a 5% CO_2 in air atmosphere until cells are ready to be subcultured.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: Primary Gingival Fibroblast; Normal, Human, Adult (HGF) (ATCC PCS-201-018)

References

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

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Contact Information

ATCC
10801 University Boulevard
Manassas, VA 20110-2209
USA
US telephone: 800-638-6597
Worldwide telephone: +1-703-365-2700
Email: tech@atcc.org or contact your local distributor