Cell Characteristics

Tissue: gingival

Morphology: spindle-shaped; cells are bipolar and refractile

Growth Properties: adherent

Refer to the Certificate of Analysis for batch-specific test results.

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.

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 vial of Primary Normal Gingival Fibroblast Cells (ATCC PCS-201-018) from the freezer; make sure that the caps of all components are tight.
4. Thaw the components of the growth kit (ATCC PCS-201-041) just prior to adding them to the basal medium (ATCC PCS-201-030).
5. Obtain one bottle of Fibroblast Basal Medium (485 mL; ATCC PCS-201-030) from cold storage.
6. Decontaminate the external surfaces of all growth kit component vials and the basal medium bottle by spraying them with 70% ethanol.
7. 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. If using the Fibroblast Growth Kit-Low Serum, add the indicated volume for each of the following components

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>rh FGF b</td>
<td>0.5 mL</td>
<td>5 ng/mL</td>
</tr>
<tr>
<td>L-glutamine</td>
<td>18.75 mL</td>
<td>7.5 mM</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>0.5 mL</td>
<td>50 µg/mL</td>
</tr>
<tr>
<td>Hydrocortisone Hemisuccinate</td>
<td>0.5 mL</td>
<td>1 µg/mL</td>
</tr>
<tr>
<td>rh Insulin</td>
<td>0.5 mL</td>
<td>5 µg/mL</td>
</tr>
<tr>
<td>Fetal Bovine Serum</td>
<td>10.0 mL</td>
<td>2%</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 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>Penicillin-Streptomycin- Amphotericin B Solution</td>
<td>0.5 mL</td>
<td>Penicillin: 10 Units/mL, Streptomycin: 10 µg/mL, Amphotericin B: 25 µg/mL</td>
</tr>
</tbody>
</table>

Please read this FIRST

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: Primary Gingival Fibroblast; Normal, Human, Adult (HGF) (ATCC® PCS-201-018™)
6. **Phenol Red** | 0.5 mL | 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.

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### Handling Procedure for Frozen Cells and Initiation of Culture

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 cm².

7. Incubate the culture at 37°C in a suitable incubator. A 5% CO₂ in air atmosphere is recommended if using the medium described on this product sheet.

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### 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% CO₂ 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 cm² flask. Incubate at 37°C in a 5% CO₂ in air atmosphere until cells are ready to be subcultured.

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### Subculturing

Volumes used in this protocol are for 75 cm² 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 cm². 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 cm²

Quality Control Specifications

Sterility Testing
Bacteria and Yeast: No growth
Mycoplasma: No growth

Viral Testing
Hepatitis B: None detected
Hepatitis C: None detected
HIV-1: None detected
HIV-2: None detected

Specific Staining
TE-7 (+), Pan-Cytokeratin (-)

Biosafety Level: 1

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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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org

Additional information on this culture is available on the ATCC web site at www.atcc.org

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