Primary Gingival Keratinocytes

PCS-200-014™

Description

Primary gingival keratinocytes were isolated from the jaw and have important applications in antibiotic treatment, dental implants, and many other applications for oral biology research.

Organism: *Homo sapiens*, human
Cell Type: keratinocyte
Tissue: Jaw
Age: lot-specific
Gender: Lot-specific
Morphology: epithelial-like; cobblestone appearance; cells are rounded, not flat; cells display a high mitotic index; at near 80% confluence, the cells will be associated with each other in colonies.
Growth properties: Adherent
Disease: Normal
Cells per vial: Approximately 5.0 x 10^5
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.

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: One bottle of Dermal Cell Basal Medium (ATCC® PCS-200-030™) plus one Keratinocyte Growth Kit (ATCC® PCS-200-040™).
Handling Procedure:

1. **Refer to the batch specific information for the total number of viable cells recovered from this lot of ATCC PCS-200-014.**

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 cm^2.

3. Prepare the desired combination of flasks. Add 5 mL of complete growth medium per 25 cm^2 of surface area. Place the flasks in a 37°C, 5% CO_2 humidified incubator and allow the media to preequilibrate to temperature and pH for 30 minutes prior to adding cells.

4. While the culture flasks equilibrate, remove one vial of ATCC PCS-200-014 from

storage and thaw the cells by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the Oring 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 preequilibrated 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.

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 Keratinocytes (ATCC PCS-200-014)

References

References and other information relating to this material are available at

www.atcc.org.

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