Primary Cervical Epithelial Cells (ATCC® PCS-480-011™)

Please read this FIRST

Storage Temp.
liquid nitrogen vapor phase

Biosafety Level
1

Description
Organism: Homo sapiens, human
Tissue: cervix
Disease: normal
Age: lot-specific
Gender: lot-specific
Morphology: polygonal, cobblestone appearance
Growth Properties: adherent
Virus Susceptibility: Pan-Cytokeratin (+), TE-7 (-)

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

SAFETY PRECAUTION
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.

Unpacking & 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.

Handling Procedure for Frozen Cells
Refer to the batch specific information for the total number of viable cells recovered from this lot of ATCC® PCS-480-011™.

1. Using the total number of viable cells, determine how much surface area can be inoculated to achieve an initial seeding density of 5000 cells per cm².
2. Prepare the desired combination of flasks. Add 5 mL of complete growth media 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.
3. While the culture flasks equilibrate, remove one vial of PCS-480-011 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).
4. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by spraying with 70% ethanol. All operations from this point onward should be carried out under strict aseptic conditions.
5. Add 5 mL of complete growth media – 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.
6. Count the cells. Plate 5,000 cells per cm² into each of the pre-equilibrated culture flasks prepared in steps 1 to 3 of Handling Procedure for Frozen Cells and Initiation of Culture. Gently rock the culture vessel from side to side and front to back to evenly distribute the cells within the vessel.
7. Place the seeded culture flasks in the incubator at 37°C with a 5% CO₂ atmosphere. Incubate for at least 48 hours before processing the cells further.

Handling Procedure for Flask Cultures
1. Pre-warm complete growth media in a 37°C water bath. This will take between 10 to 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 to 36 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 media 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 85%...
Passage normal cervical epithelial cells when culture has reached approximately 85 to 90% confluence, and are actively proliferating. 

1. Passage normal cervical epithelial cells when culture has reached approximately 85 to 90% confluence, and are actively proliferating.

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 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. Briefly rinse the cell layer with 3 to 5 mL DPBS (ATCC 30-2200) to remove residual traces of serum and then aspirate and discard the DPBS.

5. Add pre-warmed trypsin-EDTA solution (2 to 3 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.

7. Observe the cells under the microscope. When the cells pull away from each other and round up (typically within 3 to 5 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 are detached, quickly add an equal volume of 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 flask.

10. Add 3 to 5 mL Trypsin Neutralizing Solution to the flask to collect any remaining dissociated cells. Transfer remaining cells into the centrifuge tube.

11. Repeat step 10 as needed until all cells have been collected from the flask.

12. Centrifuge the cells at 270 x g for 3 to 5 minutes.

13. Carefully aspirate the neutralized dissociation solution from the cell pellet and re-suspend the cells in 5 to 8 mL fresh, pre-warmed, complete growth medium.

14. Count the cells and seed new flasks at a density of 5,000 cells/cm².

15. Place freshly seeded flasks in a 37°C, 5% CO₂ incubator for at least 24 to 48 hours before processing the cells further.

Every alternate day, remove medium and feed 5 mL of supplemented medium. However, when cultures reach 50% (or greater) confluence, remove medium and feed with 5 to 8 mL of supplemented medium daily.

### Comments

Characterization: Pan-Cytokeratin (+), TE-7 (-)

### References

References and other information relating to this product are available online at [www.atcc.org](http://www.atcc.org).

### 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.

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Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Complete Growth Medium

The basal medium for this primary cell is Cervical Epithelial Cell Basal Medium (ATCC PCS-480-032). To make the complete medium add the contents of Cervical Epithelial Cell Growth Kit (ATCC PCS-480-042) to the basal medium.

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 Cervical Epithelial Cells (ATCC® PCS-480-011™)