



# Primary Cervical Epithelial Cells

PCS-480-011™

## Description

Primary Cervical Epithelial Cells were isolated from the cervix and can provide an ideal in vitro model for studying the pathophysiology of cervical polyps, HPV, and cervical cancer.

**Organism:** *Homo sapiens*, human

**Tissue:** Uterus; Cervix

**Age:** lot-specific

**Gender:** Lot-specific

**Morphology:** polygonal, cobblestone appearance

**Growth properties:** Adherent

**Disease:** Normal

**Cells per vial:**  $\geq 5.0 \times 10^5$

**Volume:** 1.0 mL

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## Storage Conditions

**Product format:** Frozen

**Storage conditions:** Vapor phase of liquid nitrogen

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## 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.

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**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.

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](http://www.atcc.org).

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### Growth Conditions

**Temperature:** 37°C

**Atmosphere:** 95% Air, 5% CO<sub>2</sub>

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### Handling Procedures

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## 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  $-130^{\circ}\text{C}$ , preferably in liquid nitrogen vapor, until ready for use.

## Complete 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.

**Handling Procedure:** Refer to the batch specific information for the total number of viable cells recovered from this lot of ATCC<sup>®</sup> 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  $\text{cm}^2$ .
2. Prepare the desired combination of flasks. Add 5 mL of complete growth media per  $25 \text{ cm}^2$  of surface area. Place the flasks in a  $37^{\circ}\text{C}$ , 5%  $\text{CO}_2$ , 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  $37^{\circ}\text{C}$  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  $\text{cm}^2$  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  $37^{\circ}\text{C}$  with a 5%  $\text{CO}_2$  atmosphere. Incubate for at least 48 hours before processing the cells further.

## Subculturing procedure:

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

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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 cm<sup>2</sup>) 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 per cm<sup>2</sup>.
15. Place freshly seeded flasks in a 37°C, 5% CO<sub>2</sub> 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.

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### Material Citation

# Primary Cervical Epithelial Cells

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If use of this material results in a scientific publication, please cite the material in the following manner: Primary Cervical Epithelial Cells (ATCC PCS-480-011)

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## References

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

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## Primary Cervical Epithelial Cells

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