Primary Vaginal Epithelial Cells (ATCC® PCS-480-010™)

Please read this FIRST

**Storage Temp.**
liquid nitrogen vapor phase

**Biosafety Level**
1

**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 Vaginal Epithelial Cells (ATCC® PCS-480-010™)

**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 submerged 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.

**Preparation of Complete Growth Medium**
One bottle of Vaginal Epithelial Cell Basal Medium (ATCC PCS-480-030) plus Vaginal Epithelial Cell Growth Kit (ATCC PCS-480-040)

**Handling Procedure for Frozen Cells and Initiation of Culture**
Refer to the batch specific information for the total number of viable cells recovered from this lot of ATCC PCS-480-010
1. Using the total number of viable cells, determine how much surface area can be inoculated to achieve an initial seeding density of between 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 ATCC PCS-480-010 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 – 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.

**Subculturing**
1. Passage normal vaginal epithelial cells when culture has reached approximately 85 to 100% 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
For each flask, carefully aspirate the spent media without disturbing the monolayer.

3. 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.

4. Add pre-warmed trypsin-EDTA solution (2 to 3 mL for every 25 cm²) to each flask.

5. Gently rock each flask to ensure complete coverage of the trypsin-EDTA solution over the cells.

7. 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.

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

12. Centrifuge the cells at 150 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 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. Refer to Maintenance for guidelines on feeding.

**Quality Control Specifications**

**Sterility Testing**

- **Bacteria and yeast:** No growth
- **Mycoplasma:** No growth

**Viral Testing**

- **Hepatitis B:** None detected
- **Hepatitis C:** None detected
- **Human immunodeficiency virus 1:** None detected
- **Human immunodeficiency virus 2:** None detected

**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.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product
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vapor phase

Biosafety Level
1

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

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