**Primary Prostate Epithelial Cells, Human (HPrEC)**

**CS-440-010™**

**Description**

Normal human primary prostate epithelial cells were isolated from the prostate of a donor and can be used in hormonal regulation of the prostate, regulation control of the secretory function of prostate cells, and as a control for the study of prostate cancer.

- **Organism** Homo sapiens, human
- **Cell Type** epithelial cell
- **Tissue** Prostate
- **Age** lot-specific
- **Gender** Lot-specific
- **Morphology** Epithelial, packed cuboidal
- **Growth properties** Adherent
- **Disease** Normal
- **Cells per vial** $\geq 5.0 \times 10^5$
- **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
Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

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**
  1. Obtain one Prostate Epithelial Cell Growth Kit from the freezer; make sure that the caps of all
components are tight.

2. Thaw the components of the growth kit just prior to adding them to the basal medium. Warm the L-glutamine component in a 37°C water bath and shake to dissolve any precipitates prior to adding to the basal medium.

3. Obtain one bottle of Prostate Epithelial Cell Basal Medium (485 mL) from cold storage.

4. Decontaminate the external surfaces of all growth kit component vials and the basal medium bottle by spraying them with 70% ethanol.

5. Using aseptic technique and working in a laminar flow hood or biosafety cabinet, transfer the indicated volume of each growth kit component, as indicated below, to the bottle of basal medium using a separate sterile pipette for each transfer.

   A. **Prostate Epithelial Cell Growth Kit Components**
      - L-Glutamine, 15 mL (Final concentration 6 mM)
      - Extract P, 2.0 mL (Final concentration 0.4%)
      - Epinephrine, 0.5 mL (Final concentration 1.0 mM)
      - rh TGF-α, 0.5 mL (Final concentration 0.5 ng/mL)
      - Hydrocortisone hemisuccinate, 0.5 mL (Final concentration 100 ng/mL)
      - rh Insulin, 0.5 mL (Final concentration 5 mg/mL)
      - Apo-transferrin, 0.5 mL (Final concentration 5 mg/mL)

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 below.

   B. **Addition of Antimicrobials/Antimycotics and Phenol Red (Optional):**
      - Penicillin-Streptomycin-Amphotericin B Solution, 0.5 mL (Final concentration Penicillin: 10 Units/mL, Streptomycin: 10 μg/mL, Amphotericin B: 25 ng/mL).
      - Phenol Red, 0.5 mL (Final concentration 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.

- **Reagents for subculture:**
  1. D-PBS (ATCC 30-2200)
  2. Trypsin-EDTA for Primary Cells (ATCC PCS-999-003) containing 0.05% Trypsin and 0.02% EDTA. **Note: Do not use other trypsin-EDTA concentrations with ATCC PCS-440-0010.**
  3. Trypsin Neutralizing Solution (ATCC PCS-999-004)

- **Required media** One bottle of Prostate Epithelial Cell Basal Medium (ATCC PCS-440-030) plus one Prostate Epithelial Cell Growth Kit (ATCC PCS-440-040) that contains the following growth supplements: L-Glutamine, Extract P, epinephrine, rh TGF-α, hydrocortisone, rh insulin, and apo-transferrin.

- **Optional media supplements**
  1. Gentamicin-Amphotericin B Solution (ATCC PCS-999-025)
  2. Penicillin-Streptomycin-Amphotericin B Solution (ATCC PCS-999-002)
  3. Phenol Red (ATCC PCS-999-001)

- **Handling Procedure**
  1. **Refer to the batch specific information for the total number of viable cells recovered from**
this lot of ATCC PCS-440-010.

2. Using the total number of viable cells, determine how much surface area can be inoculated to achieve an initial seeding density between 2,500 and 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 pre-equilibrate to temperature and pH for 30 minutes prior to adding cells.

4. While the culture flasks equilibrate, remove one vial of ATCC PCS-440-010 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).

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 pre-equilibrated 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% CO$_2$ atmosphere. Incubate for at least 24 hours before processing the cells further.

**Subculturing procedure**

1. Passage normal Prostate cells when the culture has reached approximately 80% to 90% confluence.

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 the 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. Rinse the cell layer one time with 3 to 5 mL D-PBS (ATCC 30-2200) to remove residual medium.

5. Add pre-warmed trypsin-EDTA solution (1 to 2 mL for every 25 cm$^2$) to each flask.

6. Gently rock each flask to ensure complete coverage of the trypsin-EDTA solution over the cells, and then aspirate the excess fluid off of the monolayer.

7. Observe the cells under the microscope. When the cells pull away from each other and round up (typically within 1 to 3 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 appear to have detached, quickly add an equal volume of the 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 culture flask.

10. Add 3 to 5 mL D-PBS (ATCC 30-2200) to the tissue culture flask to collect any additional cells that might have been left behind.

11. Transfer the cell/D-PBS suspension to the centrifuge tube containing the trypsin-EDTA-dissociated cells.

12. Repeat steps 10 and 11 as needed until all cells have been collected from the flask.
13. Centrifuge the cells at 150 x g for 3 to 5 minutes.

14. Aspirate neutralized dissociation solution from the cell pellet and resuspend the cells in 2 to 8 mL fresh, pre-warmed, complete growth medium.

15. Count the cells and seed new culture flasks at a density of 5,000 viable cells per cm$^2$. Place newly seeded flasks in a 37°C, 5% CO$_2$ incubator for at least 24 to 48 hours before processing the cells further. Refer to Maintenance for guidelines on feeding.

**Culture maintenance**

1. Before beginning, pre-warm complete growth media in a 37°C water bath. This will take between 10 and 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. Carefully remove the spent media without disturbing the monolayer.

3. Add 5 mL of fresh, pre-warmed complete growth medium per 25 cm$^2$ of surface area and return the flasks to the incubator.

4. 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 approximately 95% confluence, and are actively proliferating (many mitotic figures are visible), it is time to subculture. **Note: It is important for the cells to reach near confluence to maintain proper morphology in extended culture.**

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**Material Citation**

If use of this material results in a scientific publication, please cite the material in the following manner: Primary Prostate Epithelial Cells; Normal, Human (HPrEC) (ATCC PCS-440-010)

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**References**

References and other information relating to this material are available at www.atcc.org.

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