Components: One vial of Bladder Epithelial Cells; Normal, Human (ATCC® No. PCS-420-010) containing a minimum of $5 \times 10^5$ viable cells (provided).

Also Required: One bottle of Prostate Epithelial Basal Medium (ATCC® No. PCS-440-030) supplemented with Corneal Epithelial Growth Kit (ATCC® No. PCS-700-040).

Cell Characteristics

Tissue: Bladder

Morphology: Tightly packed polygonal cells

Growth Properties: Adherent

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

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.

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.
3. Obtain one vial of Primary Bladder Epithelial Cells; Normal, Human (BdEC) (ATCC® PCS-420-010™) from the liquid nitrogen tank; make sure that the caps of all components are tight.
4. Thaw the components of Corneal Epithelial Growth Kit (ATCC® No. PCS-700-040) just prior to adding them to the Prostate Epithelial Basal Medium.
5. Decontaminate the external surfaces of all growth kit component vials and the basal medium bottle by spraying them with 70% ethanol.
6. Using aseptic technique and working in a laminar flow hood or biosafety cabinet, transfer the indicated volume of each growth kit component, as indicated in Table 1, into the bottle of basal medium using a separate sterile pipette for each transfer.

Table 1. Corneal Epithelial Cell Growth Kit; add the indicated volume for each of the following components.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apo-transferrin</td>
<td>0.5 mL</td>
<td>5 mg/mL</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>0.5 mL</td>
<td>1.0 mM</td>
</tr>
<tr>
<td>Extract P</td>
<td>2 mL</td>
<td>0.4%</td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>0.5 mL</td>
<td>100 ng/mL</td>
</tr>
<tr>
<td>Hemisuccinate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Glutamine</td>
<td>15 mL</td>
<td>6 mM</td>
</tr>
<tr>
<td>rh Insulin</td>
<td>0.5 mL</td>
<td>5 µg/mL</td>
</tr>
<tr>
<td>CE Growth Factor</td>
<td>1 mL</td>
<td>Proprietary formulation</td>
</tr>
</tbody>
</table>

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 in Table 2.
Product Sheet
Primary Bladder Epithelial Cells (A/T/N); Normal, Human (BdEC) (ATCC® PCS-420-010™)

Please read this FIRST

Storage Temp.
-130°C or below

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 Bladder Epithelial Cells (A/T/N); Normal, Human (BdEC) (ATCC® PCS-420-010™)

Handling Procedure for Frozen Cells and Initiation of Culture

1. Refer to the batch specific information for the total number of viable cells recovered from this lot of (ATCC® No. PCS-420-010).
2. Using the total number of viable cells, determine how much surface area can be inoculated to achieve an initial seeding density of between 3,000 and 5,000 cells per cm².
3. Prepare the desired combination of media. 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.
4. While the culture flasks equilibrate, remove one vial of (ATCC® No. PCS-420-010) 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 media [volume = (1 mL x number of flask 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 with a 5% CO₂ atmosphere. Incubate for at least 24 hours before processing the cells further.

Table 2. Addition of Antimicrobials/Antimycotics and Phenol Red (Optional)

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin-Streptomycin-Amphotericin B Solution</td>
<td>0.5 mL</td>
<td>Penicillin: 10 Units/mL, Streptomycin: 10 µg/mL, Amphotericin B: 25 µg/mL</td>
</tr>
<tr>
<td>Phenol Red</td>
<td>0.5 mL</td>
<td>33 µM</td>
</tr>
</tbody>
</table>

- 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.
- Complete media should be stored in the dark at 2°C to 8°C (do not freeze). When stored under these conditions, complete media is stable for 30 days.

Maintenance

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 use 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 or subculture, repeat steps 3 and 4 as described above. When cultures have reached approximately 80% to 85% confluence, and are actively proliferating, it is time to subculture. Human bladder epithelial cells are not contact inhibited. However, ATCC® recommends that epithelial be passaged before reaching confluence since post-confluent cells will exhibit slower proliferation.

Note: Cells are typically ready to passage after 6 to 7 days in culture when inoculated with 3,000 cells/cm².

Subculturing

1. Passage normal bladder epithelial cells when culture has reached approximately 80% confluence.
2. Warm both the Trypsin-EDTA for Primary Cells (ATCC® No. PCS-999-003) and the Trypsin Neutralizing Solution (ATCC® No. 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. Rinse the cell layer twice with 3 to 5 mL DPBS (ATCC® No. 30-2200) to remove residual traces of serum.
5. Add pre-warmed trypsin-EDTA solution (1 to 2 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, 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 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® No. 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 DPBS (ATCC® No. 30-2200) to the flask to collect any additional cells that might have been left behind.
11. Transfer the cell/DPBS 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 the neutralized dissociation solution from the cell pellet and re-suspend the cells in 2 to 8 mL fresh, pre-warmed, complete growth medium.
15. Count the cells and seed new flasks at a density of 3,000 to 5,000 cells per cm².
16. Place newly 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.
Primary Bladder Epithelial Cells (A/T/N); Normal, Human (BdEC) (ATCC® PCS-420-010™)

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