**Important:** Please read these instructions carefully

The viability of these cells is warranted for 30 days from date of shipment when specified reagents and growth conditions are followed as described in this instruction.

These cells are for research use only. Please allow the cells to acclimate before use.

**Table of Contents**

- Product Description
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- Cell Maintenance
- Cell Subculturing
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**Product Description**

Epithelial cells grow as tightly packed cuboidal shaped cells. They form the lining of the surfaces of organs and vessels of the body and are associated with forming a barrier and can also play a role in absorption and secretion, dependent on their tissue of origin. Epithelial cells are isolated from freshly resected tumor, as well as epithelial cells from the normal tissue adjacent to the tumor when available. Cells are cryopreserved after a limited number of passages and are provided as frozen ampoules. All cases are provided with a standard set of clinical data.

**Quality Control**

The cells are grown in antibiotic-free medium and monitored for bacterial contamination. The cell cultures have tested mycoplasma-negative.

**Contents and Storage**

One vial of cells in cryopreservation media (CryoStor® CSS, Sigma Aldrich). Store vials at ≥ -80° C or colder until ready to use.

**Handling Procedures**

Use Biosafety Level 1 safety procedures when handling these primary cells.

**Required Materials**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Recommended Supplier</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMEM or [See BioIVT website Cancer Cell inventory list for details]</td>
<td>Gibco</td>
<td>11965-092</td>
</tr>
<tr>
<td>REGM BulletKit</td>
<td>Lonza</td>
<td>CC-3190</td>
</tr>
<tr>
<td>Fetal Bovine Serum (Qualified)*</td>
<td>Gibco</td>
<td>26140-079</td>
</tr>
<tr>
<td>TrypLE™ Express</td>
<td>Gibco</td>
<td>12605-010</td>
</tr>
</tbody>
</table>

*Refer to lot specific CoAs for tissue specific media.*
Materials
- Sterile Conical Tubes, 50mL
- Cell Culture Treated Flask, T-25
- Cell Culture Treated Flask, T-75
- 70% Ethanol (EtOH)

Equipment
- Biological Safety Cabinet (BSC)
- Centrifuge
- Incubator: 37°C, 5% CO₂
- Water bath, 37°C

Culture Medium Preparation

**Culture Medium:**

1. In Biological Safety Cabinet (BSC):
   a. Refer to the BioIVT website Cancer Cell inventory list to select the appropriate lot specific Culture Medium.
   b. Prepare reagents (refer to manufacturer instructions for recommended protocols).
   c. Prepare the Culture Medium according to the recipe listed in Table 1 below.

2. Store prepared Culture Medium at 2-8°C until ready for use.

### Table 1: Primary Epithelial Cell Culture Medium

<table>
<thead>
<tr>
<th>Component</th>
<th>Stock Concentrations</th>
<th>Final Concentrations</th>
<th>Amount added to 500ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMEM</td>
<td>-</td>
<td>-</td>
<td>450ml bottle</td>
</tr>
<tr>
<td>Fetal Bovine Serum (Qualified), <strong>heat-inactivated</strong></td>
<td>-</td>
<td>10%</td>
<td>50ml</td>
</tr>
<tr>
<td>Fetal Bovine Serum (Qualified)* <strong>heat-inactivated</strong></td>
<td>--</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Or REGM BulletKit

**Antibiotic/Antimycotic usage:** BioIVT does not recommend the use of antibiotics or antimycotics. Use in cell culture media at your own discretion.

Cell Thaw

**Note:** Some liquid nitrogen-stored vials may blow off cap when transferred to warm water due to gas overexpansion. Always wear appropriate PPE when handling frozen vials and perform the following steps as directed.

1. Equilibrate Culture Medium to 37°C.
2. In BSC, transfer 25ml of Culture Medium to a 50ml conical tube.
3. Using sterile technique, twist cell vial cap one-quarter turn. Re-tighten cap.
4. Quickly swirl and thaw vial in 37°C water bath (~2 minutes). Do not submerge vial past cap threads. Immediately remove vial from bath the moment thaw is complete. **Do not allow the suspension to warm.**
5. Disinfect vial with 70% ethanol (or equivalent) and place in BSC.

Cell Culture

1. In BSC:
   a. Quickly transfer thawed contents from cell vial into the 50ml conical containing pre-warmed Culture Medium and rinse pipette tip 3-5 times.
   b. Optional: rinse vial with Culture Medium to collect any remaining cells and transfer to 50ml conical.
   c. Mix entire suspension thoroughly.
2. Centrifuge the cell suspension at approximately 200xg for 5-10 minutes.
3. After centrifugation is complete, transfer conical tube to BSC:
   a. Remove supernatant.
   b. Re-suspend cells with 2-3ml of pre-warmed Culture Medium
   c. Remove sample for counting and viability testing (approximately 50µl).
   d. Transfer cell suspension to appropriately sized culture flask and add media as indicated:

<table>
<thead>
<tr>
<th>Flask</th>
<th>Media Volume</th>
<th>Total # Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-25</td>
<td>5ml</td>
<td>1x10⁶</td>
</tr>
<tr>
<td>T-75</td>
<td>15ml</td>
<td>3x10⁶</td>
</tr>
</tbody>
</table>

4. Gently rock the culture flasks to evenly distribute the cells.
5. Place flask into a 37°C incubator at 5% CO₂.
6. Monitor for 24 hours then perform Post-seeding Evaluation.

### Post-seeding Evaluation

The day after initiating cell culture, evaluate the cell adherence:

- If the majority of cells are adherent, proceed with Cell Maintenance.
- If there is poor adherence, transfer supernatant to a 50mL conical tube and repeat Cell Culture steps 2-6 using Culture Medium. Add cells to a new appropriately sized flask.

### Cell Maintenance

1. Evaluate cells to confirm attachment to culturing vessel and to determine percent confluency (refer to Appendix A Cell Culture Images for visual reference).

   **Note:** Cells do not grow well at low density and should be subcultured at higher densities (see Table 2). The enriched epithelial cells generally have a cuboidal morphology. Some fibroblasts (typically have a spindle-shaped morphology) may be present and can be removed by following Cell Subculturing steps 1-4 (fibroblasts will detach from the flask in approximately half of the time that epithelial cells do), rinsing the flasks with serum-free isotonic solution 2-3 times (to remove fibroblasts) and performing Cell Maintenance steps 2-6.
   a. If cells are less than 90% confluent, perform steps 2-5.
   b. If cells are at least 90% confluent, proceed to Cell Subculturing steps.
2. Equilibrate Culture Medium to 37°C.
3. In BSC:
   a. Remove supernatant.
   b. Add appropriate amount of warmed Culture Medium;
4. Place flask into a 37°C incubator at 5% CO₂.
5. Incubate cells; observe daily and repeat Cell Maintenance steps as necessary.
6. Change media 3 times per week for established cultures.

### Cell Subculturing

1. Equilibrate Culture Medium, TrypLE™ and serum-free isotonic solution (e.g. phosphate buffered saline or equivalent) to 37°C.
2. In BSC:
   a. Remove supernatant.
   b. Rinse flask with 3-5ml pre-warmed serum-free isotonic solution and discard.
   c. Add 2-5ml fresh, pre-warmed TrypLE™.
3. Incubate at 37°C, checking for cell dissociation every 2 minutes, until cells are detached.
4. Once cells have detached, transfer flask to BSC and add a volume of pre-warmed Culture Medium equal to that of the TrypLE™ used (to neutralize TrypLE™).
5. Aspirate and pipette cell suspension a number of times to obtain a single-cell suspension.
6. Transfer the suspension to a 50ml conical tube.
7. Rinse the flask with an additional 3-5ml of Culture Medium to collect residual cells.
8. Pipette and thoroughly mix the suspension in the conical tube.
9. Passage cells at 1:3 split ratio*.
   *This is a general split ratio and may require adjustments for cells isolated from a variety of donors. Time to confluence will also vary by tissue donor.
   a. Passage cells every 3-14 days at 1:3 split ratio (See comment in Step 9 above).
   b. Refer to Table 2 for cell seeding volumes and densities.

**Freezing Cells**

1. Place a controlled rate freezing unit (e.g. Nalgene® Mr. Frosty) at 4°C 1-2 hours prior to expected usage.
3. Proceed to perform Cell Culture steps 2 & 3a-c.
4. When cell counts/ml of suspension has been determined, centrifuge the suspension again at 200xg for 5-10 minutes.
5. After centrifugation is complete, transfer conical tube to BSC.
   a. Remove supernatant.
   b. Gradually add cooled (4°C) cryopreservation medium (CryoStor® 5 or preferred cryopreservation medium) to re-suspend the pelleted cells to the desired concentration.
6. Mix to a homogenous suspension and aliquot to cryopreservation vials.
7. Transfer the vials to the pre-cooled controlled rate freezing unit.
8. Place the controlled rate freezing unit in -80°C freezer for 24 hours.
9. Transfer vials of cells from controlled rate freezing unit to liquid nitrogen vapor phase or -80°C storage.

**APPENDIX A: Cell Culture Images**

*Picture 1: Primary epithelial cells in culture*