Human iPSC-Derived Renal Proximal Tubular Cells
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Human iPSC-Derived Renal Proximal Tubular Cells

Product Information

<table>
<thead>
<tr>
<th>Catalog. No.</th>
<th>Product Name</th>
<th>Format</th>
<th>Stock Conc.</th>
<th>Storage on Arrival</th>
<th>Thawing Instructions</th>
<th>Storage Once Thawed</th>
</tr>
</thead>
<tbody>
<tr>
<td>ax2115</td>
<td>Human iPSC-Derived Renal Proximal Tubular Cells</td>
<td>≥1 million cells/vial</td>
<td>N/A</td>
<td>Liquid Nitrogen</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>ax3534-250</td>
<td>Renal Epithelial Cell Culture Medium</td>
<td>250 mL</td>
<td>1x</td>
<td>Store at -20°C for up to 6 months</td>
<td>Store at 4°C for up to 1 month</td>
<td>Thaw at 4°C or room temperature</td>
</tr>
</tbody>
</table>

Additional Reagents

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Supplier</th>
<th>Product Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth Factor Reduced Matrigel™</td>
<td>Corning</td>
<td>356230</td>
</tr>
<tr>
<td>Recombinant human bone morphogenetic protein 2 (BMP2)</td>
<td>Sigma-Aldrich</td>
<td>H4791-10UG</td>
</tr>
<tr>
<td>Recombinant human bone morphogenetic protein 7 (BMP7)</td>
<td>Gibco (Thermo Fisher Scientific)</td>
<td>PHC9544</td>
</tr>
<tr>
<td>Y-27632 2HCl (ROCK inhibitor)</td>
<td>Selleck Chemicals</td>
<td>S1049</td>
</tr>
</tbody>
</table>

These reagents must be added fresh for each aliquot of medium.
Preparation of Reagents

**Growth Factor Reduced (GFR) Matrigel™**
- Upon receipt, aliquot and store **Growth Factor Reduced (GFR) Matrigel™** at \(-20^\circ\text{C}\), according to manufacturer’s protocol.
- Coat tissue culture plates 45 minutes – 1 hour before thawing **Human iPSC-Derived Renal Proximal Tubular Cells**.

**Recombinant Human Bone Morphogenetic Protein 2 (BMP2)**
- Prepare a 10 μg/mL stock solution of BMP2 by resuspending 10 μg of the lyophilized powder in 1 mL Dulbecco’s-phosphate-buffered saline (D-PBS) with 0.05 % human serum albumin (HSA).

**Recombinant Human Bone Morphogenetic Protein 7 (BMP7)**
- Prepare a 5 μg/mL stock solution of BMP7 by resuspending 10 μg of the lyophilized powder in 2 mL Dulbecco’s-phosphate-buffered saline (D-PBS) with 0.05 % HSA.

<table>
<thead>
<tr>
<th>Hematopoietic Factor</th>
<th>Stock Concentration</th>
<th>Final Concentration</th>
<th>In 50 mL Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMP2</td>
<td>10 μg/mL</td>
<td>10 ng/mL</td>
<td>50 μL</td>
</tr>
<tr>
<td>BMP7</td>
<td>5 μg/mL</td>
<td>2.5 ng/mL</td>
<td>25 μL</td>
</tr>
</tbody>
</table>

**Renal Epithelial Cell Culture Medium**
- Upon receipt, aliquot and store **Renal Epithelial Cell Culture Medium** at or below \(-20^\circ\text{C}\) protected from light.
- When ready to use, thaw an aliquot of **Renal Epithelial Cell Culture Medium** overnight at \(4^\circ\text{C}\) or room temperature in the dark.
Culture of Human iPSC-Derived Renal Proximal Tubular Cells

Coating

- Thaw aliquots (as needed) of GFR Matrigel™ on ice before use.
- Dilute GFR Matrigel™ 1:50 in ice-cold serum-free medium (DMEM or another suitable medium) on ice to make a 1x working solution e.g. 100 μL of GFR Matrigel™ into 5 mL of serum-free medium.
- Coat the surface of your culture vessel with the GFR Matrigel™ 1x working solution. We recommend coating at a volume of 200 μL per cm².
- Incubate the coated cell culture vessel at 37°C, 5% CO₂ in a humidified incubator for 1 hour.

Consult with manufacturer’s protocol for further detailed instructions on coating plates with GFR Matrigel™.

Thawing and Plating

- Prepare a sufficient volume (dependent on the culture vessel format for plating) of Renal Epithelial Cell Culture Medium supplemented with 10 μM Y-27632 2HCl and warm to 37°C prior to use.
- To thaw the cells – transfer the vial of cells from storage by transporting the vial buried in dry ice. Remove the vial from dry ice and transfer it to a 37°C water bath.
- Quickly thaw the vial of cells in a 37°C water bath. Do not completely submerge the vial (only up to 2/3rd of the vial). Remove the vial before the last bit of ice has melted, after 1-2 minutes.
- Do not shake the vial during thawing.
- Take the vial of cells to a biological safety cabinet, spraying the vial and hood thoroughly with 70% ethanol and wiping with an autoclaved paper towel before placing the vial in the hood.
- Using a P1000 pipette, gently add the cell suspension drop-wise into the 15 mL sterile conical tube.
- Slowly add 9 mL of pre-warmed, 37°C, Renal Epithelial Cell Culture Medium + 10 μM Y-27632 2HCl.
- Gently wash the cryogenic vial with 1 mL of Renal Epithelial Cell Culture Medium + 10 μM Y-27632 2HCl to ensure all of the cells are transferred to the 15 mL sterile conical tube.
- Centrifuge the cells at 200 x g for 5 minutes at room temperature.
- Carefully remove the supernatant and resuspend the cell pellet in 1 mL of pre-warmed, 37°C, Renal Epithelial Cell Culture Medium + 10 μM Y-27632 2HCl.
- Perform a cell count to determine the number of viable cells and ensure optimal seeding density.
- Dilute the cells into the required volume of pre-warmed, 37°C, Renal Epithelial Cell Culture Medium + 10 μM Y-27632 2HCl.
- Seed cells into the culture vessel at the recommended seeding density of 50,000 viable cells/cm². The day of seeding the cells is Day 0.
- Incubate the cells at 37°C, 5% CO₂ in a humidified incubator for 2 days.
Maintenance

- On **Day 2** (48 hours after seeding the cells), the culture medium should be replaced with **Renal Epithelial Cell Culture Medium** containing BMP growth factors (BMP2 and BMP7).
- Supplement the required volume of **Renal Epithelial Cell Culture Medium** with **10 ng/mL** BMP2 and **2.5 ng/mL** BMP7. Warm to **37°C** prior to use.
- Remove the spent cell culture medium from the culture vessel and replace with pre-warmed, **37°C**, **Renal Epithelial Cell Culture Medium + 10 ng/mL BMP2 + 2.5 ng/mL BMP7**.
- On **Day 4** (96 hours after seeding the cells), the cells will be ready to use for endpoint assays.

Got any questions? Need help with the protocol? Contact Axol Technical Support at support@axolbio.com
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US phone +1-800-678-AXOL (2965)
Notes