axol

Human iPSC-Derived Renal Proximal Tubular Cells

Table of Contents

Product Information 2

Preparation of Reagents 3

Culture of Human iPSC-Derived Renal Proximal Tubular Cells 4

Product Information

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

Additional Reagents		
Product Name	**Supplier**	**Product Code**
Growth Factor Reduced Matrigel™	Corning	356230
Recombinant human bone morphogenetic protein 2 (BMP2)	Sigma-Aldrich	H4791-10UG
Recombinant human bone morphogenetic protein 7 (BMP7)	Gibco (Thermo Fisher Scientific)	PHC9544
Y-27632 2HCl (ROCK inhibitor)	Selleck Chemicals	S1049

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°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.

Hematopoietic Factor	Stock Concentration	Final Concentration	In 50 mL Medium
BMP2	10 µg/mL	10 ng/mL	50 µL
BMP7	5 µg/mL	2.5 ng/mL	25 µL

Renal Epithelial Cell Culture Medium

- Upon receipt, aliquot and store **Renal Epithelial Cell Culture Medium** at or below **-20°C** protected from light.
- When ready to use, thaw an aliquot of **Renal Epithelial Cell Culture Medium** overnight at **4°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% CO_2** 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% CO_2** 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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