3D Renal Tubule Formation Kit

3D-RTF

Cat # 3D-4110

Product Description

The human kidney is frequently exposed to drugs and toxic compounds, which can result in nephrotoxicity. A drug's uptake and clearance from the kidney largely relies on a subset of membrane transporters such as organic anion transporters (OAT) 1/3, organic cation transporter (OCT) 2, and multidrug and toxin extrusion proteins (MATE) 1/2. In 2D monolayer culture, primary renal epithelial cells rapidly lose the expression of OAT1/3, OCT2, and MATE1/2, limiting the accurate predictability of a drug's effect [1]. ScienCell has developed a 3D renal tubule formation kit (3D-RTF) using human primary renal cortical epithelial cells isolated from normal adult kidney tissue, which are then embedded in Collagen I extracellular matrix. We demonstrated that renal tubule structures are formed in a robust and reproducible manner in a 3D collagen-embedded model using the 3D-RTF. 3D cultured renal epithelial cells had elevated expression levels of xenobiotic transporters such as OAT1/3, and MATE1/2 (see Figure 1 and 2). The 3D-RTF provides a useful *in vitro* tool for accurately predicting a drug's effect on kidney function.

Kit Components (Included)

Cat #	# of vials	Product Name	Quantity	Storage
4110	1	Human Renal Cortical Epithelial Cells (HRCEpiC)	5×10^5 cells	Liquid nitrogen
4101	1	Epithelial Cell Medium – Basal	500 mL	2-8 °C
4152	1	Epithelial Cell Growth Supplement	5 mL	-20 °C
0010	1	Fetal Bovine Serum	10 mL	-20 °C
0503	1	Penicillin/Streptomycin Solution	5 mL	-20 °C
8708-a	1	Collagen I from rat tail, 4 mg/mL	10 mL	2-8 °C
8708-b	1	Buffer A, 10X	1.5 mL	2-8 °C
8708-c	1	Buffer B	1 mL	2-8 °C
8708-d	1	Sterile H_2O	5 mL	2-8 °C

Additional Recommended Materials (Not Included)

Cat #	Product Name
0183	0.05% Trypsin/EDTA (T/E)
0303	Dulbecco's Phosphate-Buffered Saline (DPBS)
0413	Poly-L-Lysine (10 mg/mL)
0113	Trypsin Neutralization Solution (TNS)
N/A	Normal TC-treated 48-well plate

Quality Control
3D-RTF is tested for the formation of 3D renal tubule structures according to the included protocol. All components are negative for bacterial and fungal contamination.

Product Use
3D-RTF is for research use only. It is not approved for human or animal use, or application in clinical or *in vitro* diagnostic procedures.

Shipping
4110, 4152, 0010, and 0503 are shipped on dry ice. 4101 is shipped at room temperature. 8708-a, 8708-b, 8708-c, and 8708-d are shipped on gel ice.

References
[1] Secker P, Luks L, Schlichenmaier N, Dietri D. (2018) "Cells Form Highly Differentiated Tubules When Cultured in a 3D Matrix." *Altex* 35(2): 223-234.

Procedure:

A. Initiating cells in 2D culture

Step I: Preparation of the complete EpiCM medium

- Thaw EpiCGS (Cat. #4152), FBS (Cat. #0010), and P/S solution (Cat. #0503) at 37°C. Add EpiCGS, FBS and P/S solution to the EpiCM medium (Cat. #4101) and mix well.
    - Warm the complete EpiCM medium only to room temperature (instead of 37°C) prior to use.
    - When stored in the dark at 4°C, the complete medium is stable for one month.

Step II: Thawing, maintaining and sub-culturing cells in 2D cell culture

- Please see the product sheet Cat. #4110 for thawing, and maintaining ScienCell's primary renal cortical epithelial cells (Cat. #4110) in 2D monolayer culture.

B. Generation of 3D renal tubules in type I collagen gel

Step III: Preparation of cells for 3D culture

Table A: An Example of Suggested Cell Number, and Collagen Gel Volume

# of samples in 48 well plate	Total cell number	Total collagen gel volume
1 sample	1.2×10^5	170 µL
5 samples	6.0×10^5	850 µL
48 samples	5.8×10^6	8.16 mL

- Please see **Table A** for the required number of cells for different sample sizes. A confluent T-75 flask should yield about 4×10^6 cells.
- When desired amount of cells have been achieved in 2D monolayer culture, you can prepare cell pellets for 3D culture as described below.
- Rinse the cells with DPBS.
- Add 10 ml 0.05% T/E solution (Cat. #0183) into flask (in the case of a T-75 flask). Gently rock the flask to ensure complete coverage of cells by T/E solution. Use a microscope to monitor the change in cell morphology.
- Transfer T/E solution from the flask to the 50 ml centrifuge tube (a small percent of cells may detach) and continue to incubate the flask at 37°C for another minute (no solution in the flask at this time).
- At the end of incubation, gently tap the side of the flask to dislodge cells from the surface. Check under a microscope to make sure that all cells detach.
- Add 5 ml of TNS solution or 10% FBS to the flask and transfer detached cells to the 50 ml centrifuge tube. Rinse the flask with another 5 ml of TNS or 10% FBS to collect the residual cells.

- Count cells and aliquot 1.2×10^5 cells (per sample). Please see Table A to scale up appropriately for your experiments.
- Centrifuge at 1,000 rpm for 5 minutes and remove the supernatant.
- Save the cell pellets and proceed to Step IV.

Step IV: Embedding cells in collagen I gel

- Keep all components shown in Table B on ice.

Table B: Preparation of ~1mL Collagen Gel (for 5 samples)

Components	Volume
Collagen I from rat tail, 4 mg/mL	450 µl
Buffer A, 10X	100 µl
Sterile H_2O	450 µl
Buffer B	32 µl

- On ice, mix all components shown in Table B by adding **Buffer B** as the **LAST** component.
- Immediately after the addition of Buffer B, resuspend 1.2×10^5 cells (pellets) in 170 µL of collagen I gel (for one sample) by gently pipetting up and down for ~ 15 times using a p200 pipette.
    - Scale up cell number and collagen gel volume appropriately for your experiment (see Table A).
- Slowly add 170 µl of cells/collagen gel mixture to the center of each well in the normal TC-treated 48 well plates.
- Tilt around the plate to ensure complete coverage of the collagen gel mixture in the well.

Step V: Collagen Gel Polymerization and Sample Incubation

- Incubate the plate at 37°C for 1 hour to allow collagen gel polymerization.
- After 1 hour, slowly add 500 µl of the complete epithelial cell medium (EpiCM) to the side of each well in the cell culture hood.
- Incubate the plates at 37°C in a 5% CO_2 incubator.
- Robust renal tubule formation can be observed after 24 hours post-seeding (Figure 1). Renal tubule formation peaks at 48 hours post-seeding.

Fig. 1 – Development of renal tubule-like structures in collagen I gel over time. Renal tubule-like structures were generated according to the provided protocol. Robust and reproducible renal tubule formation could be observed at 24 - 48 hours post-seeding.

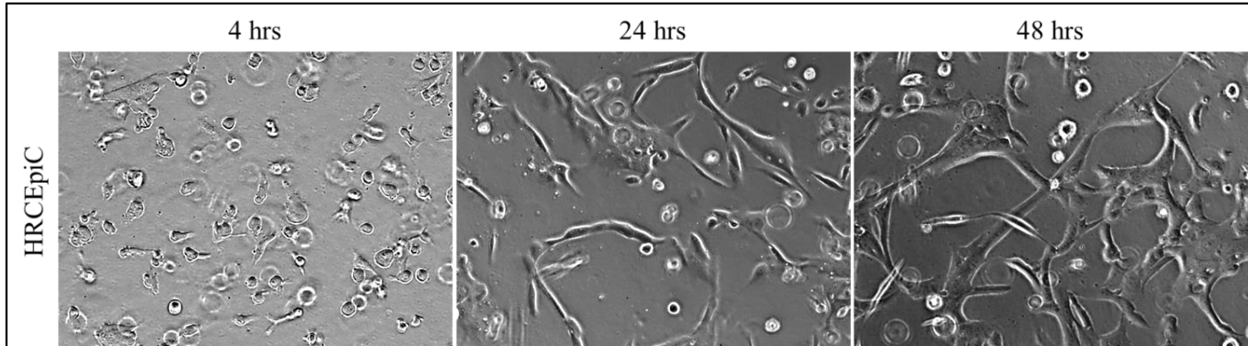
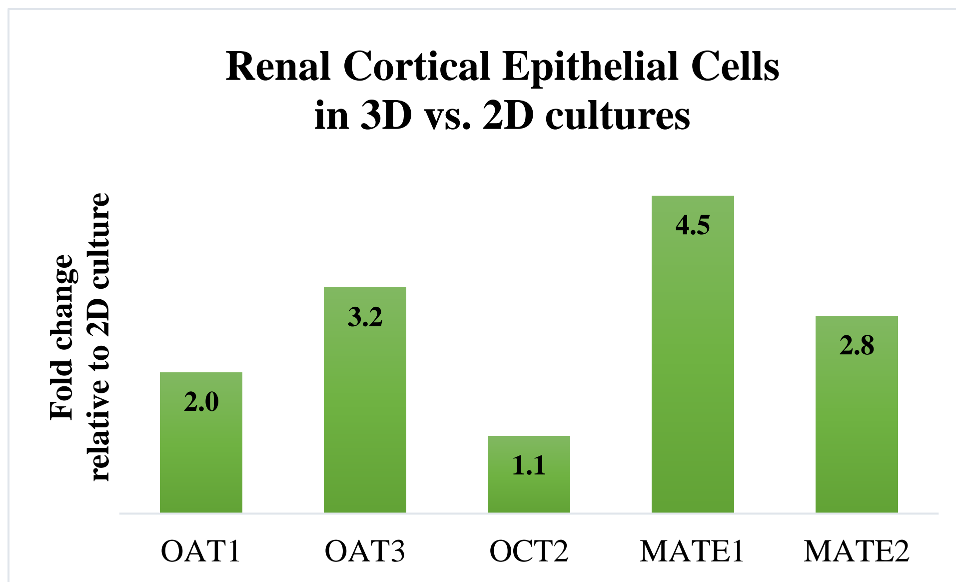


Fig. 2 – Gene expression profile analysis of HRCEpiC (at passage 3) grown in 3D Collagen embedded model vs. 2D monolayer culture. Cells were isolated from 2D and 3D cultures at 48 hours post-seeding. Functionality of renal cortical epithelial cells was assessed by measuring the gene expression levels of xenobiotic transporters such as OAT1/3, OCT2, and MATE1/2.



Troubleshooting Guide

Problem	Possible Cause	Potential Solution
Cells are not forming tubules.	Cells are not proliferating.	- Check cell viability (should be >90%) and cell population doubling. - Reduce extensive sub-culturing in 2D culture.
Cells are not forming tubules.	Collagen gel is floating or is not polymerized well.	- Avoid aggressive addition of renal tubule media to the gel. - Check the pH of the Collagen I solution (should be ~ 7.0 - 7.6).
Cells are assembling as clusters, instead of elongated tubules.	Cells are not resuspended well.	- Obtain single cell suspension in collagen gel by gently pipetting up and down for approximately 15 - 20 times. - Use p200 pipette tips instead of p1000 pipette tips to resuspend the cells in collagen solution.