Rev. 2

Ready-to-use 3D Human Renal Proximal Tubular Epithelial Spheroids SP3D-HRPTEpiS

Cat. #SP3D-4100

Product Description

The kidney proximal tubule is a primary site of nephrotoxicity due to its frequent exposure to drugs and toxic compounds [1]. Recent studies have also shown that human renal tubule cells could be the potential host cells targeted by respiratory viruses such as SARS-CoV-2 [2]. Current pre-clinical models using 2D monolayer cultures and animal models are unable to fully recapitulate clinical drug response due to limited *in vitro* lifespan, or species-specific variations. Hence, 3D models of the human proximal tubular epithelial cells have been developed and are proven to maintain the differentiation hallmarks [3]. To serve as a relevant *in vitro* model and overcome the challenges of building 3D spheroids, ScienCell has developed ready-to-use 3D renal proximal tubular epithelial spheroids (SP-HRPTEpiS) generated by co-culturing human renal proximal tubular epithelial cells with human renal mesangial cells at a 4:1 ratio. ScienCell's SP-HRPTEpiS display the differentiated epithelial cell marker CK18. Notably, SP3D-HRPTEpiS also have high ACE2 (angiotensin-converting enzyme 2) expression, a receptor for SARS-CoV and SARS-CoV-2. In addition, our RT-PCR analysis indicate elevated mRNA expression of the renal proximal transport molecule aquaporin-1 AQP1, the organic anion transporters OAT 1/3, the organic cation transporter OCT 2, the multidrug and toxin extrusion proteins MATE 1/2, and P-glycoprotein P-gp in 3D spheroids, compared to 2D monolayer. SP3D-HRPTEpiS, therefore, provide a physiological *in vitro* model to study kidney functions, and immune response of kidney cells to viruses.

Kit Components (Included)

3D Cell Culture Components				
Cat #	**# of vials**	**Product Name**	**Quantity**	**Storage**
SP-4100	1	Human Renal Proximal Tubular Epithelial Spheroids (SP-HRPTEpiS)	4×10^3 spheroids	Liquid nitrogen
3D-4101	1	3D-Epithelial Spheroid Medium (3D-EpiSpM)	200 mL	2-8 °C
3D-4152	1	3D-Epithelial Spheroid Supplement (3D-EpiSpS)	2 mL	-20 °C
0004	1	Fetal Bovine Serum	4 mL	-20 °C
0583	1	Penicillin/Streptomycin Solution (P/S)	2 mL	-20 °C
0343 (or) 0353 (or) 0383	1	Ultra-Low Binding Culture Plates (24-, 48-, or 96- well plate)	1 plate	RT

Quality Control

SP3D-HRPTEpiS is tested for the formation of functional and uniform 3D human renal proximal tubular epithelial spheroids according to the included protocol. All components are negative for bacterial and fungal contamination.

Product Use

SP3D-HRPTEpiS are for research use only. It is not approved for human or animal use, or application in clinical or *in vitro* diagnostic procedures.

Shipping

SP-4100, 3D-4152, 0004, and 0583 are shipped on dry ice. 3D-4101, and (0343 or 0353 or 0383) are shipped at room temperature.

References

[1] Secker P.F. et al. (2017) "RPTEC/TERT1 cells form highly differentiated tubules when cultured in a 3D matrix." *Altex* 35(2): 223-234.
[2] Monteil V. et al. (2020) "Inhibition of SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE2" *Cell* 181: 1-9.
[3] Harari-Steinberg O. et al. (2020) "Ex vivo expanded 3D human kidney spheres engraft long term and repair chronic renal injury in mice." *Cell Reports* 30: 852-869.

Procedure:

Step I: Preparing the complete 3D culture medium

1. Thaw 3D-epithelial spheroid supplement (3D-EpiSpS; Cat. #3D-4152), fetal bovine serum (FBS; Cat #0004), and penicillin/streptomycin solution (P/S solution; Cat. #0583) at 37°C. Mix 3D-EpiSpS, FBS, and P/S solution into the 3D-epithelial spheroid medium (3D-EpiSpM medium; Cat. #3D-4101) by gently swirling the medium bottle around.
    a. 3D-EpiSpM medium is **viscous** and optimized for homogenous spheroid formation.
    b. Warm the complete 3D-EpiSpM medium to **room temperature** before use.
    c. When stored in the dark at 4°C, the complete medium is stable for one month.

Step II: Thawing and maintaining the ready-to-use 3D spheroids

2. One frozen vial contains $\geq 4 \times 10^3$ spheroids, which is sufficient for plating into half of a multi-well plate (e.g. 24-, 48-, and 96-well ultra-low binding culture plate).

3. Place the frozen vial in a 37°C water bath. Hold and rotate the vial gently until the contents completely thaw. Promptly remove the vial from the water bath, wipe it down with 70% ethanol, and transfer it to the sterile field.

4. Carefully remove the cap without touching the interior threads. Gently pipette the spheroid suspension up and down **two times** to disperse potential spheroid aggregates.

5. Gently transfer the spheroid suspension into a fresh 50 mL conical tube.

6. Add 24 mL of 3D culture medium to the above 50 mL conical tube.

7. Resuspend spheroids in 3D culture medium by gently pipetting up and down for ~ 5-7 times using a serological pipette.

   Note: 3D culture medium has a high viscosity; thus, pipetting slowly is important to avoid bubble formation.

8. Aliquot the suggested volumes (see **Table A, column 2**) of spheroid suspension into each well of the ultra-low binding plate (24-, 48- or 96-well plate).

Table A: An Example of Suggested Medium Volumes

1	2
Plate formats	**Volume per well**
24-well	~ 2000 µL
48-well	~ 1000 µL
96-well	~ 500 µL

9. Incubate spheroids at 37°C in a 5% CO_2 incubator.

10. For best results, do not disturb the culture for at least 16 hours after the culture has been initiated.

11. Next day, change 60-70 % of the top layer of the medium using a pipette by hand to remove the residual DMSO (Do not use a vacuum aspirator). After 1st medium change, no additional medium changes are necessary.

    Note: Spheroids are situated at the bottom of the well due to the viscosity of the 3D culture medium. Thus, centrifugation of the plates is not necessary, and spheroid loss will not occur by changing 60-70 % of the top layer of the medium by pipetting.

12. Monitor the health of spheroids every day under the microscope. Renal proximal tubular epithelial spheroids are recovered and ready for experiments after 24 hours post thawing (see Figure 1).

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Fig. 1 – At 100x magnification, phase contrast images of 3D human renal tubular epithelial spheroids at different days post thawing.

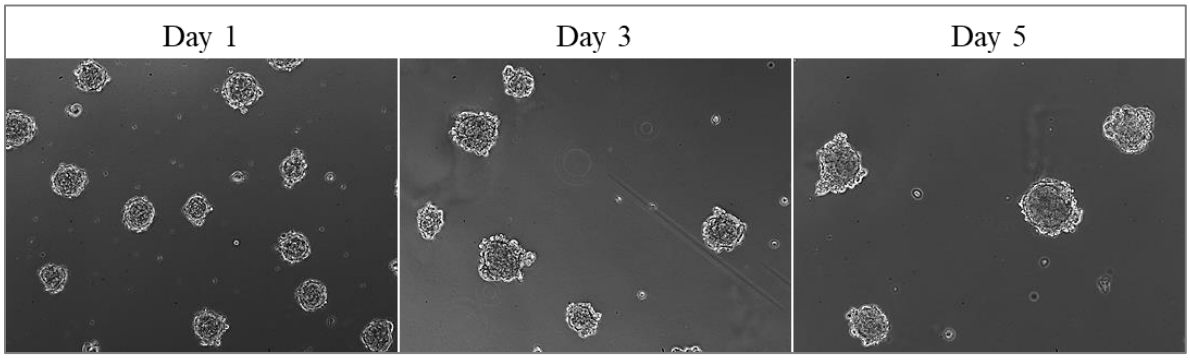

Fig. 2 – Human renal proximal tubular epithelial spheroids exhibit the differentiated epithelial cell marker CK-18. Notably, these spheroids also display high ACE2 expression (200x magnification).

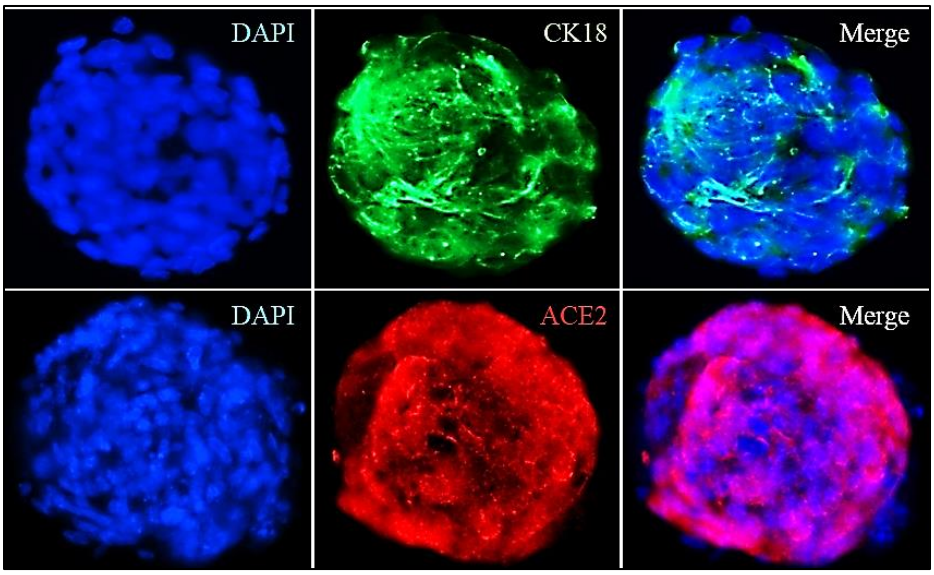

Fig. 3 – qPCR analysis show the increased mRNA expression of the renal transporters (OAT1/3, OCT2, MATE1/2, and P-gp) and the proximal renal transport molecule (AQP1) in 3D spheroids, compared to 2D culture.