Rev. 1

Ready-to-use 3D Human Mini-Kidney Spheroids
SP3D-HMKS

Cat. #SP3D-4108

Product Description

Kidney proximal tubules make up a significant portion in the human kidney and are composed of specialized proximal tubular epithelial cells [1]. These epithelial cells are involved in transporter activities and immune responses [1]. Notably, most kidney diseases begin in proximal tubules due to their frequent exposure to high concentration of xenobiotics. *In vivo*, the renal proximal tubule epithelial cells (RPTEpiC) are in close proximity with the renal interstitium, the space between the cortical tubules consisting of cells, extracellular matrix, proteoglycans, glycoproteins, and interstitial fluid [2]. The cell types present in the cortical interstitium are fibroblasts, endothelial cells, and immune cells, and they support the function of renal proximal tubular epithelial cells [2]. For example, co-culture of primary proximal tubular epithelial cells with endothelial cells improves RPTEpiC proliferation and differentiation through the paracrine signaling process [3]. Due to the importance of culture proximal tubular epithelial cells with their supporting cells, ScienCell has developed a 3D mini-kidney spheroid model (SP3D-HMKS) comprising human primary renal proximal tubular epithelial cells, mesangial cells, and glomerular endothelial cells enabling more accurate prediction of tissue-level outcomes. These human mini-kidney spheroids allow the functional maintenance of cells over time due to a more native, three-dimensional (3D) architecture. In addition, the 3D mini-kidney model makes it feasible to study kidney diseases that are challenging to model using only epithelial cells such as fibrosis.

Kit Components (Included)

3D Cell Culture Components				
Cat #	**# of vials**	**Product Name**	**Quantity**	**Storage**
SP-4108	1	Human Mini-Kidney Spheroids (SP-HMKS)	4×10^3 spheroids	Liquid nitrogen
3D-4301	1	3D-Kidney Spheroid Medium (3D-KSpM)	200 mL	2-8 °C
3D-4302	1	3D-Kidney Spheroid Supplement (3D-KSpS)	2 mL	-20 °C
0010	1	Fetal Bovine Serum	10 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-HMKS is tested for the formation of functional and uniform 3D human mini-kidney spheroids according to the included protocol. All components are negative for bacterial and fungal contamination.

Product Use
SP3D-HMKS 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-4108, 3D-4302, 0010, and 0583 are shipped on dry ice. 3D-4301, 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] Lemley K. V., and Kriz, W. (1991). "Anatomy of the renal interstitium." *Kidney Int.* 39: 370–381.
[3] Tasnim, F., and Zink, D. (2012). "Cross talk between primary human renal tubular cells and endothelial cells in cocultures." *Am. J. Physiol. Renal Physiol.* 302: 1055–1062.

Procedure:

Step I: Preparing the complete 3D culture medium

1. Thaw 3D-kidney spheroid supplement (3D-KSpS; Cat. #3D-4302), fetal bovine serum (FBS; Cat #0010), and penicillin/streptomycin solution (P/S solution; Cat. #0583) at 37°C. Mix 3D-KSpS, FBS, and P/S solution into the 3D-kidney spheroid medium (3D-KSpM; Cat. #3D-4301) by gently swirling the medium bottle around.
    a. 3D-KSpM medium is **viscous** and optimized for homogenous spheroid formation.
    b. Warm the complete 3D-KSpM 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. Mini-kidney 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 mini-kidney spheroids at different days post thawing.

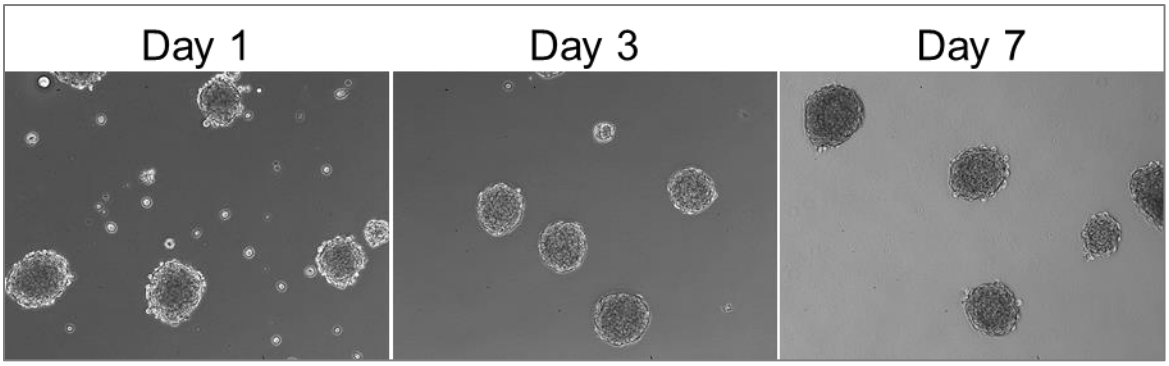

Fig. 2 – 3D human mini-kidney spheroids display the epithelial cell marker CK-18, the endothelial cell marker VWF, and the mesenchymal marker Vimentin. Notably, these spheroids also display high ACE2 expression (200x magnification).

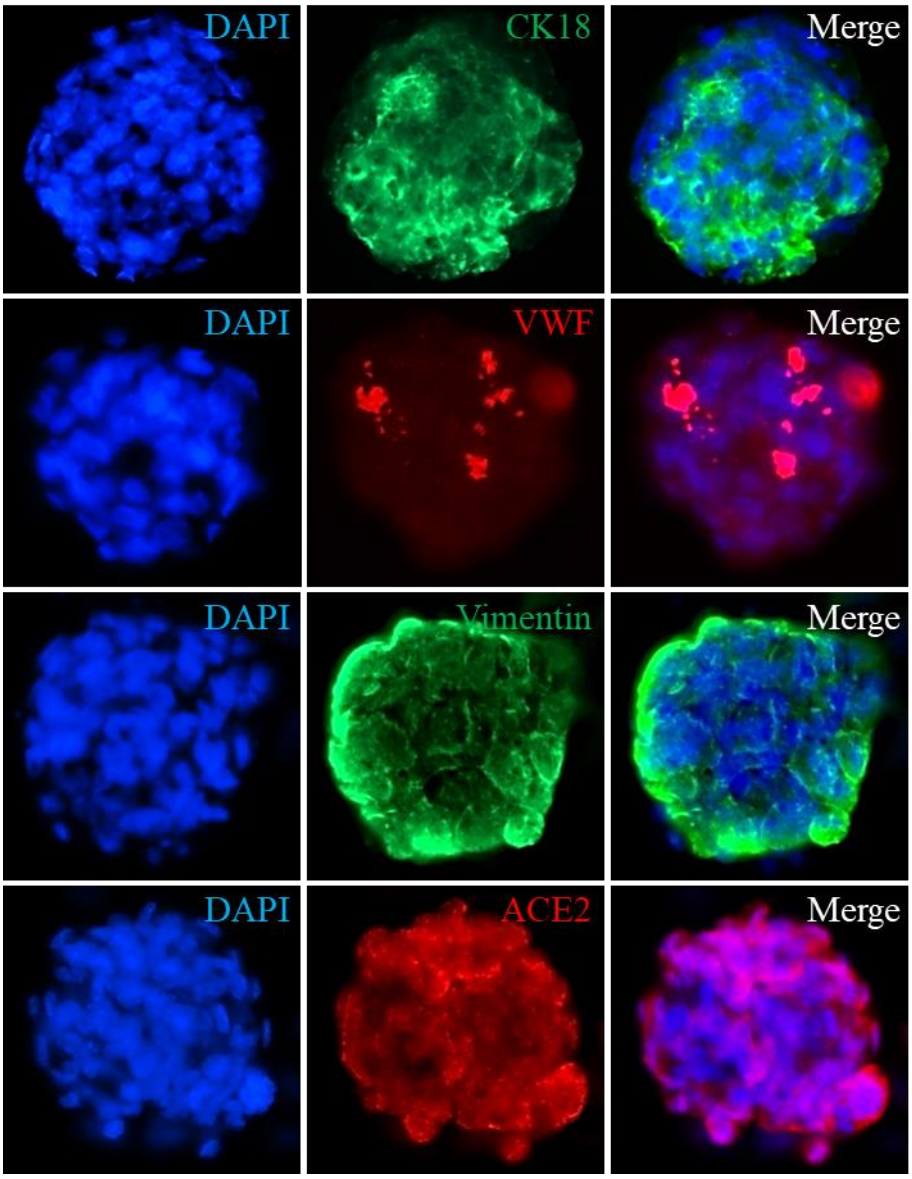

Fig. 3 – qPCR analysis shows the increased mRNA expression of the renal transporters (OAT1/3, OCT2, MATE1/2, and P-gp) and the proximal renal transport molecule (AQP1) in 3D spheroids, compared to 2D culture.