Rev. 1

Ready-to-use 3D Human Trabecular Meshwork Cell Spheroids
SP3D-HTMCS

Cat. #SP3D-6590

Product Description

Human trabecular meshwork (HTM) is a specialized tissue located next to the cornea. HTM cells play an essential role in regulating aqueous humor outflow resistance by controlling the production of contraction forces and the secretion/degradation of extracellular matrix (ECM) proteins to maintain tissue homeostasis [1, 2]. Abnormal human trabecular meshwork cell function and accumulation of ECM materials in TM tissues contribute to TM stiffening, ultimately leading to the increased AH outflow resistance and elevated intraocular pressure in glaucoma [1, 2]. To investigate the cell behavior and molecular mechanisms involved in glaucoma, it is important to develop reliable *in vitro* human-based models. Most current cellular HTM models, however, do not sufficiently replicate the complex native three-dimensional (3D) cell-ECM interface. To overcome limitations of current models, ScienCell has developed ready-to-use 3D human trabecular meshwork cell spheroids (SP3D-HTMCS), in which TM cells maintain better cell-cell and cell-ECM interactions, thereby preserving the physiological relevance of *in vivo* conditions. More importantly, we demonstrate that the gene expression of myocilin is higher and that of ECM proteins such as collagen type I, and smooth muscle actin (SMA) is lower in 3D HTM spheroids compared to the 2D culture system (Fig. 2 and 3). The size of the spheroids also become smaller over time in 3D culture, suggesting the reduced proliferative activity (Fig.1). In conclusion, the gene expression profile and proliferative activity in 3D spheroid culture resembled those observed in TM tissue. ScienCell's 3D HTMC spheroids can serve as a valuable *in vitro* model for studying the functions of TM cells in glaucoma research.

Kit Components (Included)

3D Cell Culture Components				
Cat #	**# of vials**	**Product Name**	**Quantity**	**Storage**
SP-6590	1	Human Trabecular Meshwork Cell Spheroids (SP-HTMCS)	4×10^3 spheroids	Liquid nitrogen
3D-6591	1	3D-Trabecular Meshwork Spheroid Medium – basal (3D-TMSpM)	200 mL	2-8 °C
0020	1	Fetal Bovine Serum (FBS)	20 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-HTMCS is tested for the formation of functional and uniform 3D human trabecular meshwork cell spheroids according to the included protocol. All components are negative for bacterial and fungal contamination.

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

Shipping
SP-6590, 0020, 0583 are shipped on dry ice. 3D-6591, and (0343 or 0353 or 0383) are shipped at room temperature.

References
[1] Gasiorowski J. Z. and Russell P. (2009) "Biological Properties of Trabecular Meshwork Cells." *Exp Eye Res.* 88(4): 671-675.
[2] Sathiyanathan P., Tay C.Y., and Stanton L.W. (2017) "Transcriptome analysis for the identification of cellular markers related to trabecular meshwork differentiation." *BMC Genomics.* 18: 1-13.

Procedure:

Step I: Preparing the complete 3D culture medium

1. Thaw fetal bovine serum (FBS; Cat. #0020), and penicillin/streptomycin solution (P/S solution; Cat. #0583) at 37°C. Mix FBS, and P/S solution into the 3D-trabecular meshwork spheroid medium (3D-TMSpM medium; Cat. #3D-6591) by gently swirling the medium bottle around.

    a. 3D-TMSpM medium is **viscous** and optimized for homogenous spheroid formation.
    b. Warm the complete 3D-TMSpM 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 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	2 mL
48-well	1 mL
96-well	0.5 mL

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, examine the viability of the spheroids. It is not necessary to perform any medium exchange. However, if the medium exchange is desired, change 60-70 % of the top layer of the medium using a pipette by hand to remove the old medium. (Do not use a vacuum aspirator).

    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. Trabecular meshwork cell spheroids are ready for your experiment after 24 hours post thawing (see Figure 1).

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Fig. 1 – Ready to-use human trabecular meshwork cell spheroids at different days after thawing (taken at 100x magnification).

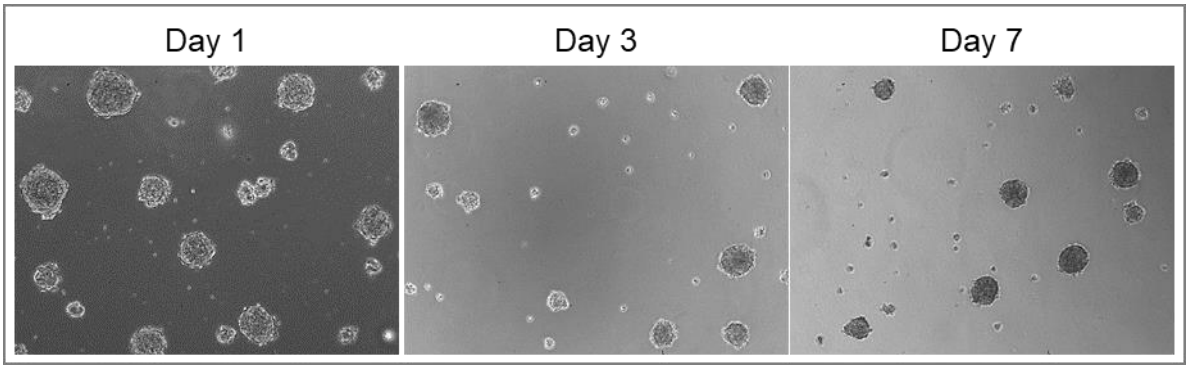

Fig. 2 – At day 1, immunostaining analysis of SP3D-HTMCS with the TM cell marker Myocilin (MYOC), and the ECM marker Fibronectin (FN). Images are taken at 200x magnification.

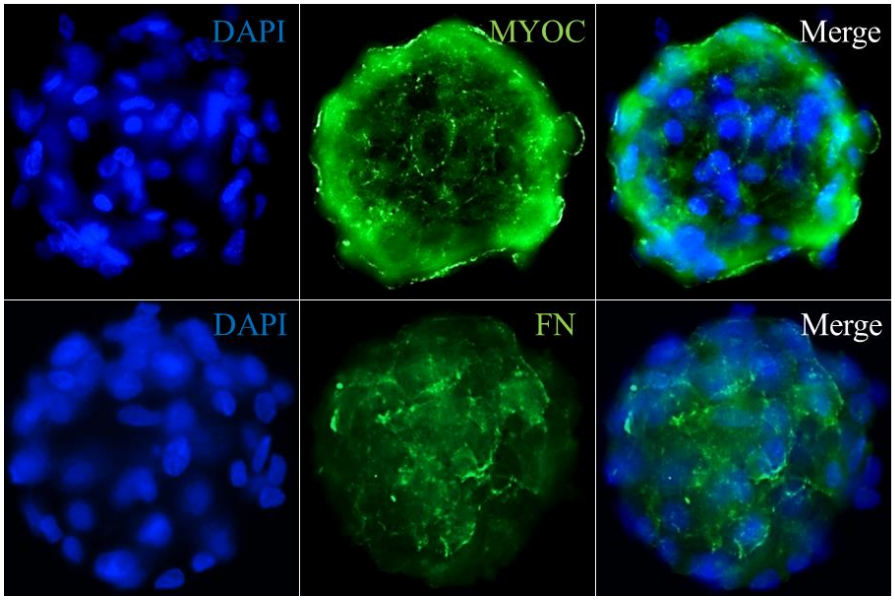

Fig. 3 – qRT-PCR analysis demonstrates that the gene expression of myocilin (MYOC) was higher and that of ECM proteins such as collagen type I (Col1A1), and alpha-smooth muscle actin (ACTA2) were lower in 3D HTM spheroids, compared to the 2D culture system.