



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] Sathiyathan 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 3D-trabecular meshwork spheroid supplement (3D-TMSPS; Cat. #3D-6592), 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 spheroid suspension up and down for **two times** to disperse potential spheroid aggregates.
5. Gently transfer the spheroid suspension into a fresh 50 mL conical tube.
6. Add the 24 mL of 3D culture media to the above 50 mL conical tube.
7. Resuspend spheroids in 3D culture media 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.

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.

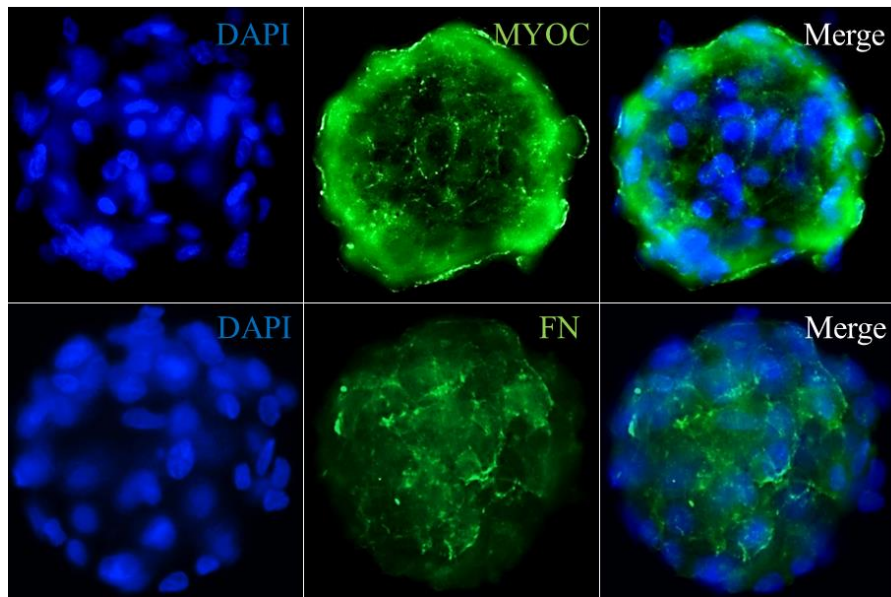


Fig. 3 – qRT-PCR analysis demonstrates that the gene expression of myocilin (MYOC) was higher and that of ECM proteins such as collagen type I (Col1A1), and alpha-smooth muscle actin (ACTA2) were lower in 3D HTM spheroids, compared to the 2D culture system.

