Rev. 1

Ready-to-use 3D Human Retinal Pigment Epithelial Spheroids
SP3D-HRPEpiS

Cat. #SP3D-6540

Product Description

Age-related macular degeneration (AMD) is characterized in its early stages by the presence of extracellular deposits, known as drusen, that accumulate between the basal surface of the retinal pigmented epithelium and Bruch's membrane, an extracellular matrix complex that separates the neural retina from the capillary network in the choroid [1]. Several studies have shown that drusen contains a variety of protein and lipid components [2]. Although liver is the primary biosynthetic site for most of these molecules, retinal pigment epithelial (RPE) cells locally synthesize a number of drusen components [2]. The respective contributions of RPE-derived and plasma-derived molecules to the biogenesis of drusen, and the relevant molecular interactions leading to drusen depositions, however, have not been fully identified. One of the major limitations is that RPE cells, once isolated from the eye, tend to dedifferentiate into myofibroblasts in conventional 2D cell culture. Recent studies have shown that 3D retinal pigment epithelial cell spheroids form and maintain a well-differentiated epithelium in 3D cell culture [3]. ScienCell Research Laboratories, as a result, has developed ready-to-use 3D human retinal pigment epithelial spheroids (SP3D-HRPEpiS). 3D RPE spheroids exhibit the differentiated epithelial cell marker cytokeratin-18 and deposit apolipoprotein ApoE, a prominent drusen constituent. The 3D RPE spheroid model is an ideal way to model drusen *in vitro* and study the pathogenesis of related diseases, such as AMD.

Kit Components (Included)

3D Cell Culture Components				
Cat #	**# of vials**	**Product Name**	**Quantity**	**Storage**
SP-6540	1	Human Retinal Pigment Epithelial Spheroids (SP-HRPEpiS)	1×10^4 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 (FBS)	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-HRPEpiS are tested for the formation of functional and uniform 3D human retinal pigment epithelial spheroids according to the included protocol. All components are negative for bacterial and fungal contamination.

Product Use
SP3D-HRPEpiS 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-6540, 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] Abdelsalam A., Del Priore L., and Zarbin M.A. (1999) "Drusen in age-related macular degeneration: pathogenesis, natural course, and laser photocoagulation-induced regression." *Surv. Ophthalmol.* 44, 1-29.
[2] Hageman G.S., Luthert P.J., Victor Chong N.H., Johnson L.V., Anderson D.H., Mullins R.F. (2001) "An integrated hypothesis that considers drusen as biomarkers of immune-mediated processes at the RPE-Bruch's membrane interface in aging and age-related macular degeneration." *Prog Retin Eye Res.* 20: 705-732.
[3] Sato R., Yasukawa T., Kacza J., Eichler W., Nishiwaki A., Iandiev I., Ohbayashi M., Kato A., Yafai Y., Bringmann A., Takase A., Ogura Y., Seeger J. and Wiedemann P. (2013) "Three-Dimensional Spheroidal Culture Visualization of Membranogenesis of Bruch's Membrane and Basolateral Functions of the Retinal Pigment Epithelium." *IOVS.* 54: 1740-1749.

Procedure:

<u>Step I: Preparing the complete 3D culture medium</u>

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 basal medium (3D-EpiSpM; Cat. #3D-4101) by gently swirling the medium bottle around.
    a. 3D-EpiSpM is **viscous** and optimized for homogenous spheroid formation.
    b. Warm the complete 3D-EpiSpM to **room temperature** before use.
    c. When stored in the dark at 4°C, the complete medium is stable for one month.

<u>Step II: Thawing and maintaining the ready-to-use 3D spheroids</u>

2. One frozen vial contains $\geq 1 \times 10^4$ 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	~ 1000 µL
48-well	~ 500 µL
96-well	~ 250 µL

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

10. Monitor the health of spheroids every day under the microscope. Human retinal pigment epithelial spheroids are recovered and ready for experiments around 48 hours post thawing (see Figure 1).

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.

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Fig. 1 –Phase contrast images of the ready-to-use human retinal pigment epithelial spheroids after thawing (100X magnification).

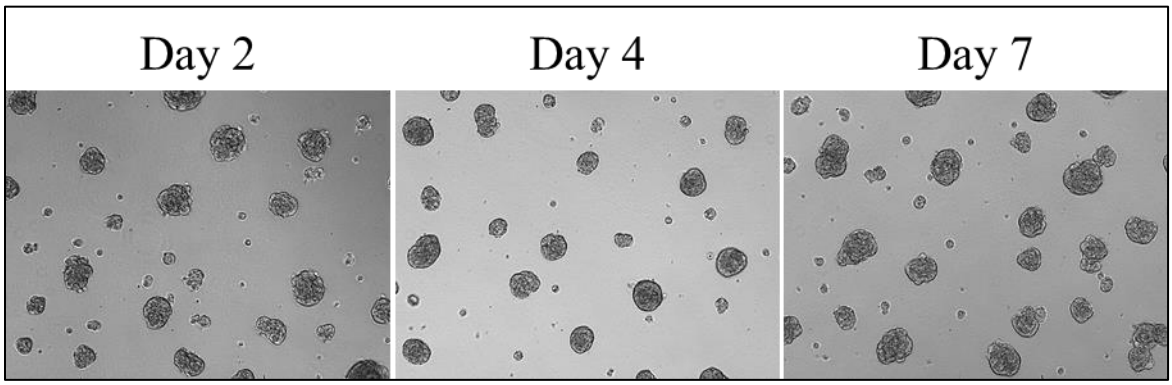

Fig. 2 – Immunostaining of human retinal pigment epithelial cell spheroids. Differentiated epithelial cell marker CK18 is distributed throughout the spheroids. ApoE is localized in a granular pattern on the surface of the spheroids. ApoE deposits are non-uniformly distributed with areas of high deposit density interspersed among areas with few to no detectable deposits (200x magnification).