All-inclusive 3D Human Retinal Pigment Epithelial Spheroid Formation Kit
3D-HRPEpiSF
Cat #3D-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 develops an all-inclusive 3D human retinal pigment epithelial spheroid formation kit (3D-HRPEpiSF). 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**
6540	1	Human Retinal Pigment Epithelial Cells (HRPEpiC)	5×10^5 cells	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
2D Cell Culture Components				
Cat #	**# of vials**	**Product Name**	**Quantity**	**Storage**
4101	1	Epithelial Cell Medium (EpiCM)	500 mL	2-8 °C
4152	1	Epithelial Cell Growth Supplement (EpiCGS)	5 mL	-20 °C
0010	1	Fetal Bovine Serum (FBS)	10 mL	-20 °C
0503	1	Penicillin/streptomycin Solution (P/S)	5 mL	-20 °C

Additional Recommended Materials (Not Included)

Cat #	Product Name
0113	Trypsin Neutralization Solution
0183	0.05% Trypsin/EDTA (T/E)
0303	Dulbecco's Phosphate-Buffered Saline (DPBS)
0413	Poly-L-Lysine (10 mg/mL)

Quality Control

3D-HRPEpiSF 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

3D-HRPEpiSF are for research use only. It is not approved for human or animal use, or application in clinical or *in vitro* diagnostic procedures.

Shipping

6540, 3D-4152, 0004, 0583, 4152, 0010, and 0503 are shipped on dry ice. 3D-4101, 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:

A. Initiating cells in 2D culture

1. Please see the product sheet Cat. #6540 for thawing and maintaining ScienCell's human primary retinal pigment epithelial cells (Cat. #6540) in 2D monolayer culture.

2. For cell expansion in 2D monolayer culture, use the included 2D culture kit components (Cat. #4101, 4152, 0010, and 0503).

B. Establishing 3D spheroid culture

Step I: Prepare the complete 3D culture medium

3. 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; 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 only **to room temperature** before use.
c. When stored in the dark at 4°C, the complete medium is stable for one month.

Step II: Harvest cells for 3D culture

Table A: An Example of Suggested Cell Suspension Density, and Medium Volumes

1	2	3
Plate formats	**Concentration of cell suspension (cells/mL)**	**Volume per well**
24-well	3.4×10^5 cells/mL	~ 1000 µL
48-well	3.0×10^5 cells/mL	~ 500 µL
96-well	3.2×10^5 cells/mL	~ 200 µL

4. Please see **Table A** for the suggested cell densities for different plate formats. A confluent T-75 and T-175 flasks should yield about 5 x 10^6 and 1 x 10^7 cells, respectively.

5. When desired amount of cells have been achieved in 2D monolayer culture, you can begin setting up 3D spheroid culture as described below.

6. Rinse the cells with DPBS.

7. Add 10 mL of 0.05% T/E solution (Cat. #0183) into flask (in the case of a T-75 flask). Gently rock the flask to ensure complete coverage of cells by T/E solution. Use a microscope to monitor the change in cell morphology.

8. During incubation, prepare a 50 ml conical centrifuge tube with 5ml of fetal bovine serum (FBS).

9. Once the cells completely round up, transfer T/E solution from the flask to the 50 ml centrifuge tube (a small percent of cells may detach) and incubate the flask at 37°C for 2-3 minutes (no solution at the flask at this time).

10. At the end of incubation, gently tap the side of the flask to dislodge cells from the surface. Check under a microscope to make sure that all cells detach.

11. Add 5 ml of TNS solution to the flask and transfer detached cells to the 50 ml conical tube. Rinse the flask with another 5 ml of TNS to collect the residual cells.

Step III: Resuspend and seed cells in 3D culture medium

12. Count cells using hemacytometer, and aliquot the appropriate volume of cell suspension into a fresh 50 mL conical tube. Please see **Table A** for the suggested cell densities for different plate formats.

    Note: It is recommended to make a minimum of 5 mL cell suspension in 3D medium for easier pipetting due to the viscosity of 3D medium.

13. Centrifuge the 50 ml conical tube at 1000 rpm for 5 minutes.

14. Aspirate the supernatant while leaving behind the 100-200 µl supernatant above the pellet in the tube.

15. Resuspend cells in the residual supernatant by pipetting up and down for ~ 10 times to obtain a single cell suspension.

16. Next, add the appropriate volume of the 3D-EpiSpM medium to obtain the suggested density of cell suspension (see **Table A; column 2**).

17. Slowly pipette up and down for ~ 10 times and make sure you have uniform cell suspension in 3D medium before proceeding to next step.

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

18. Add the suggested volume of cell mixture (see **Table A; column 3**) to each well in the provided ultra-low binding plate by using a p1000 pipette. Do not use the serological pipette to minimize pipette errors while adding small volumes to the wells.

19. Incubate the cells at 37°C in a 5% CO_2 humidity incubator.

20. Change the 3D culture medium every four days by only changing 60-70% of the top layer of the medium using a pipette by hand (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.

21. Monitor the growth and formation of spheroid every day under the microscope. Mature retinal pigment epithelial spheroids develop at ~ 3-4 days post seeding (Figure 1).

Fig. 1 – Development of human retinal pigment epithelial spheroids over 2 weeks. Phase contrast images are taken at 100x magnification.

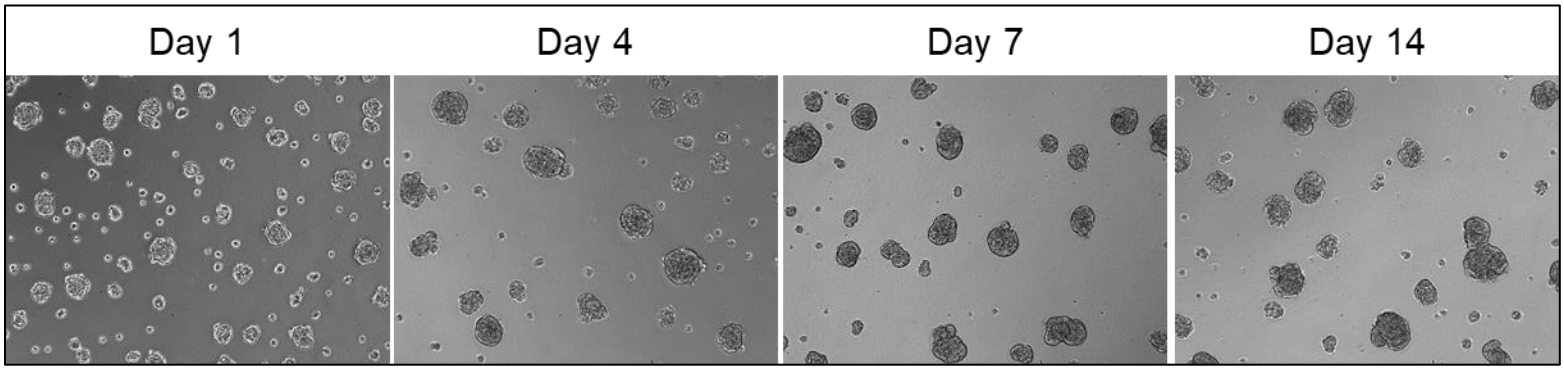

Fig. 2 – Immunostaining of the human retinal pigment epithelial cell spheroids. Epithelial cell marker CK18 is distributed throughout the spheroids. ApoE is localized in a granular pattern on the surface of the spheroids. ApoE deposits are non-uniformly distributed with areas of high deposit density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