Ready-to-use 3D Human Pulmonary Alveolar Epithelial Cell Spheroids
SP3D-HPAEpiS
Cat. #SP3D-3200

Product Description

The lung is a complex organ with a large and highly vascularized epithelial surface area. Gas exchange in the lung occurs in the air-filled alveoli containing type I and II alveolar epithelial cells. Type II cells function as progenitor cells in the alveoli and proliferate and differentiate into type I cells, while type I cells are involved in gas exchange [1]. Type II cells not only proliferate to maintain the epithelium but also play critical roles in respiration and host defense mechanism through production of the pulmonary surfactants (SPs) [1]. Type II cells, however, lose their functional markers within a few days of cultivation in 2D culture. Studies have shown that when alveolar epithelial cells are cultured in three-dimensional (3D) cell culture, cells maintain their epithelial functional markers [2]. To provide a more native *in vitro* lung model, ScienCell offers ready-to-use human pulmonary alveolar epithelial cell spheroids (SP3D-HPAEpiS), which express the surfactant proteins such as SP-A, -B, -C, and –D (Figures 1 and 2). These spheroids are ready for experiments at 24 hours post thawing, and are an excellent *in vitro* model for studying the contribution of the alveolar epithelial cells to alveolar maintenance and repair.

Kit Components (Included)

3D Cell Culture Components				
Cat #	**# of vials**	**Product Name**	**Quantity**	**Storage**
SP-3200	1	Human Pulmonary Alveolar Epithelial Spheroids (SP-HPAEpiS)	1×10^4 spheroids	Liquid nitrogen
3D-3201	1	3D-Alveolar Epithelial Cell Spheroid Medium (3D-AEpiCSpM)	200 mL	2-8 °C
3D-4152	1	3D-Epithelial Cell Spheroid Supplement (3D-EpiCSpS)	4 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-HPAEpiS are tested for the formation of functional and uniform 3D human pulmonary alveolar epithelial spheroids according to the included protocol. All components are negative for bacterial and fungal contamination.

Product Use

SP3D-HPAEpiS 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-3200, 3D-4152, 0004, 0583 are shipped on dry ice. 3D-3201, and (0343 or 0353 or 0383) are shipped at room temperature.

References
[1] Barkauskas CE, Cronce MJ, Rackley CR, Bowie EJ, Keene DR, Stripp BR, Randell SH, Noble PW, Hogan B. (2013) "Type 2 alveolar cells are stem cells in adult lung." *J Clin Invest.* 123(7): 3025-3036.
[2] Takahashi K, Mitsui M, Takeuchi K, Uwabe Y, Kobayashi K, Sawasaki Y, Matsuoka T. (2004) "Preservation of the Characteristics of the Cultured Human Type II Alveolar Epithelial Cells." *Lung* 182: 213-226.

Procedure:

Step I: Preparing the complete 3D culture medium

1. Thaw 3D-epithelial cell spheroid supplement (3D-EpiCSpS; Cat. #3D-4152), fetal bovine serum (FBS; Cat. #0004), and penicillin/streptomycin solution (P/S solution; Cat. #0583) at 37°C. Mix 3D-EpiCSpS, FBS, and P/S solution into the 3D-alveolar epithelial cell spheroid medium (3D-AEpiCSpM; Cat. #3D-3201) by gently swirling the medium bottle around.

    a. 3D-AEpiCSpM is **viscous** and optimized for homogenous spheroid formation.
    b. Warm the complete 3D-AEpiCSpM 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 $\geq 1 \times 10^4$ 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 12 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 pulmonary alveolar epithelial spheroids are recovered and ready for experiments after 24 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 –Brightfield images of the ready-to-use human pulmonary alveolar epithelial cell spheroids after thawing (taken at 100X magnification).

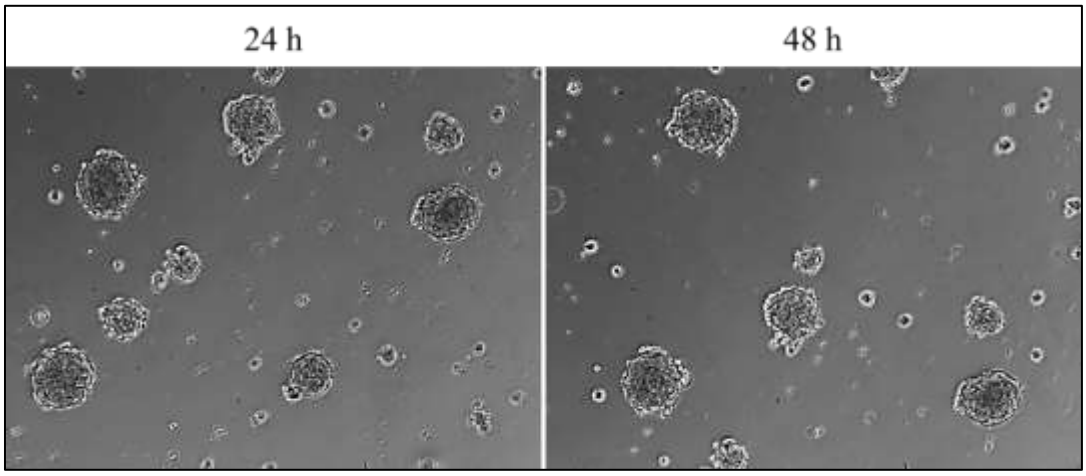

Fig. 2 –Immunostaining of the human pulmonary alveolar epithelial cell spheroids with the epithelial cell marker CK18 and the specific alveolar epithelial cell type II marker SP-C.

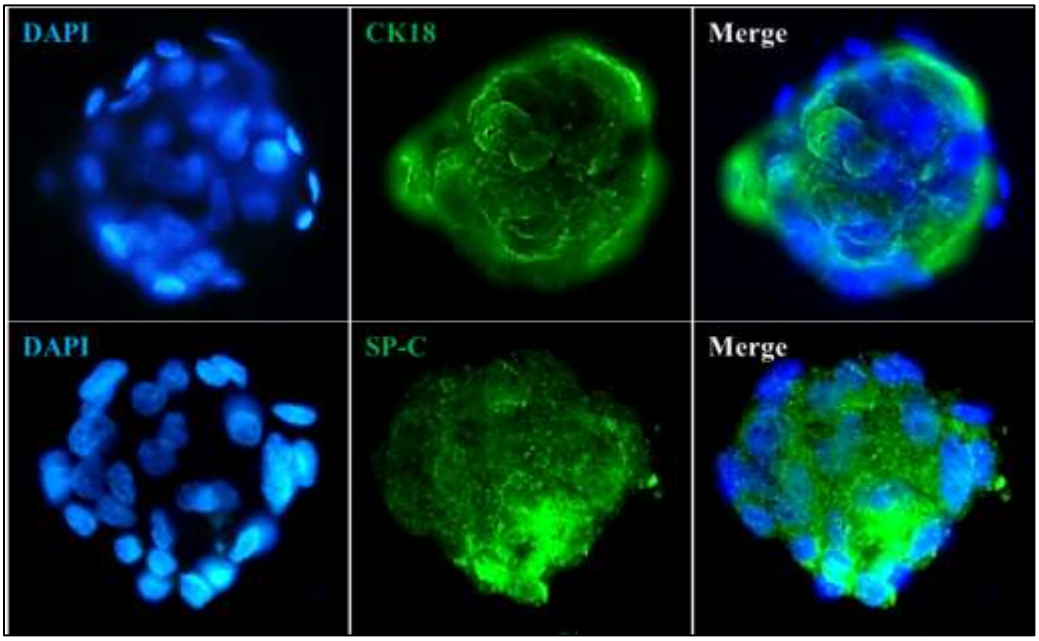

Fig. 3: qPCR analysis of the human pulmonary alveolar epithelial cell spheroids.