Rev. 1

Ready-to-use 3D Human Airway Spheroids
SP3D-HAS

Cat. #SP3D-3210

Product Description

Airway remodeling, chronic inflammation, and bacterial colonization in many airway diseases occur in the lower respiratory tract [1]. Primary bronchial epithelial cells are, therefore, an excellent *in vitro* source for studying airway diseases such as cystic fibrosis, chronic obstructive pulmonary disease (COPD), and severe asthma [1]. The bronchial epithelium is made up of three principle cell types: basal, goblet, and ciliated cells, of which the latter two form a suprabasal columnar structure and are necessary for mucociliary clearance [2]. ScienCell's ready-to-use 3D Human Airway Spheroids (SP3D-HAS) are generated using primary bronchial epithelial cells and are embedded in their own secreted extracellular matrix (ECM). Importantly, immunofluorescence staining and qPCR analysis revealed the presence of basal, ciliated, and goblet cells in ScienCell's airway spheroids (see Figures 1 and 2). ScienCell's ready-to-use airway spheroids (Cat #SP3D-3210) are great models for studying airway diseases in a simple and high throughput manner.

Kit Components (Included)

3D Cell Culture Components				
Cat #	**# of vials**	**Product Name**	**Quantity**	**Storage**
SP-3210	1	Human Bronchial Epithelial Spheroids (SP-HBEpiS)	1×10^4 spheroids	Liquid nitrogen
3D-3211	1	3D-Airway Spheroid Medium(3D-ASpM)	200 mL	2-8 °C
3D-3262	1	3D-Airway Spheroid Supplement (3D-ASpS)	2 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-HAS are tested for the formation of functional and uniform 3D human bronchial epithelial spheroids according to the included protocol. All components are negative for bacterial and fungal contamination.

Product Use

SP3D-HAS 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-3210, 3D-3262, and 0583 are shipped on dry ice. 3D-3211, and (0343 or 0353 or 0383) are shipped at room temperature.

References

[1] Awatade NT, Wong SL, Hewson CK, Fawcett LK, Kicic A, Jaffe A and Waters SA. (2018) "Human Primary Epithelial Cell Models: Promising Tools in the Era of Cystic Fibrosis Personalized Medicine." *Front. Pharmacol.* 9(1429): 1-11.
[2] Velden VH, Versnel HF. (1998) "Bronchial epithelium: morphology, function and pathophysiology in asthma." *Eur. Cytokine Netw.* 9: 585-597.

Procedure:

Step I: Preparing the complete 3D culture medium

1. Thaw 3D-airway spheroid supplement (3D-ASpS; Cat. #3D-3262), and penicillin/streptomycin solution (P/S solution; Cat. #0583) at 37°C. Mix 3D-ASpS and P/S solution into the 3D-airway spheroid medium (3D-ASpM; Cat. #3D-3211) by gently swirling the medium bottle around.
    a. 3D-ASpM is **viscous** and optimized for homogenous spheroid formation.
    b. Warm the complete 3D-ASpM 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 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 airway 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 airway spheroids after thawing (taken at 100X magnification).

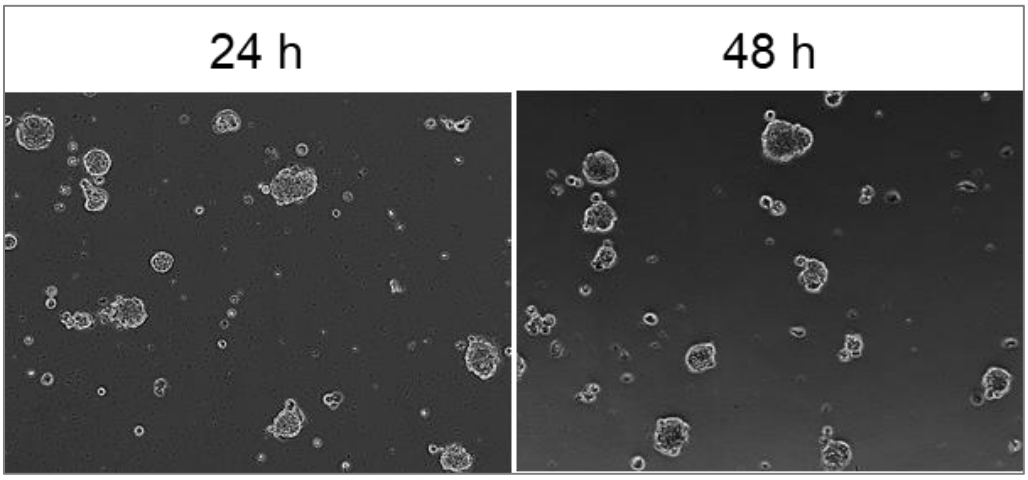

Fig. 2 –Immunostaining analysis of the human airway spheroids showing the presence of markers for basal cells (CK14), ciliated cells (acetylated α-tubulin, αTub), and goblet cells (MUC5AC).

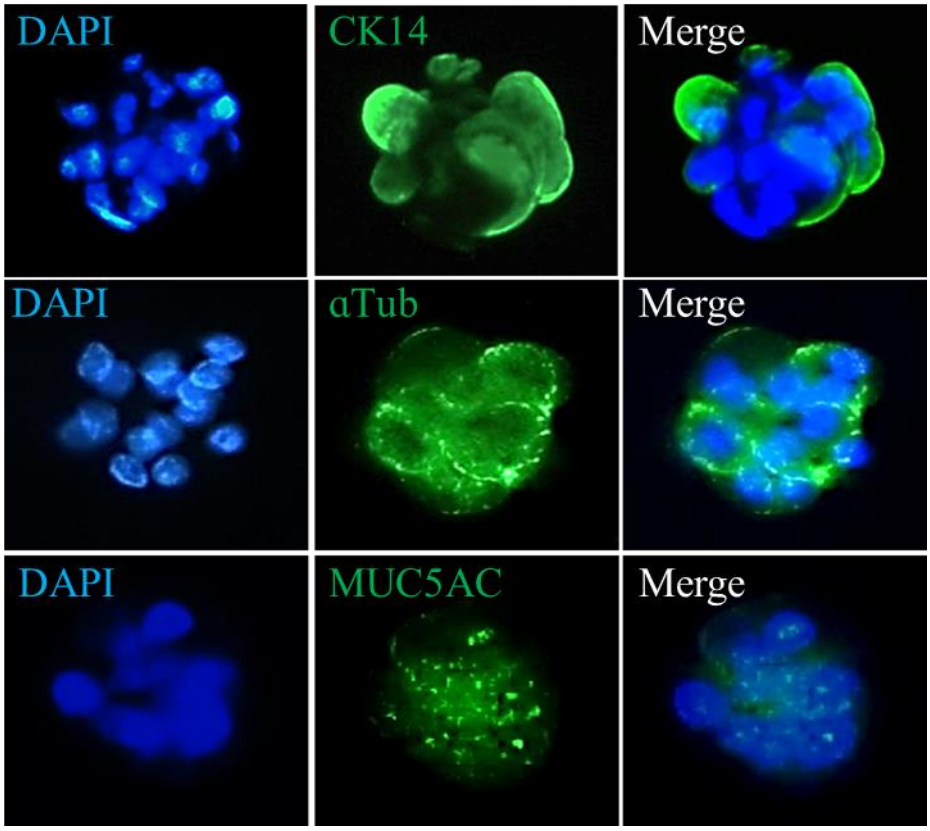

Fig. 3: Quantitative PCR analysis of basal (ITGA6), goblet (MUC5B), and ciliated cell markers (TUBA4A) expressed by bronchial epithelial cells grown in 2D or 3D cell cultures.