Rev. 0

Ready-to-use 3D Human Bronchial Fibroblast Spheroids (SP3D-HBFS)

Catalog #SP3D-3420

Product Description

Human Bronchial Fibroblasts (HBF) are mesenchymal-derived cells and one of the largest cells in the lung. Under physiological conditions and in response to injury, HBF secretes extracellular proteins such as collagen, fibronectin and glycoproteins [1]. During tissue injury and chronic inflammation, a series of molecular signaling events induce the transformation of fibroblasts into myofibroblasts [2]. Myofibroblasts trigger excessive ECM production and may lead to scar tissue formation through a process known as fibrosis [2]. Thickened lung tissue in lung fibrosis is a detrimental condition due to the downstream respiratory defects. To better understand and treat lung fibrosis, *in vitro* models to study HBF is necessary. The conventional method of studying fibroblasts in 2D culture is limited in the ability to mimic their native *in-vivo* state due to the lack of extracellular matrix.

To provide an *in vitro* model that better reflects HBF function physiologically, ScienCell has developed ready-to-use human bronchial fibroblast spheroids (SP-HBFS) comprised of primary HBF. These cells are cultured in three-dimensional (3D) architecture and are embedded in their own ECM, which better reflects the native lung tissue architecture. Furthermore, HBF in these spheroids can be used to model fibrosis through TGF-β1 treatment. These spheroids are ready for experiments within 24-48 hours of thawing, and are an excellent *in vitro* model for studying HBF function and its contribution to pulmonary fibrosis.

Kit Components (Included)

3D Cell Culture Components				
Cat #	**# of vials**	**Product Name**	**Quantity**	**Storage**
SP-3420	1	Human Bronchial Fibroblast Spheroids (SP-HBF)	1×10^4 spheroids	Liquid nitrogen
3D-3421	1	3D-Bronchial Fibroblast Medium (3D-HBFSpM)	200 mL	2-8 °C
3D-3452	1	3D-Bronchial Fibroblast Spheroid Supplement (3D-HBFSpS)	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-HBFS are tested for the formation of functional and uniform 3D human bronchial fibroblast spheroids according to the included protocol. All components are negative for bacterial and fungal contamination.

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Product Use
SP3D-HBFS are for research use only. It is not approved for human or animal use, or for application in clinical or *in vitro* diagnostic procedures.

Shipping
SP-3420, 3D-3452, 0004, and 0583 are shipped on dry ice. 3D-3421 and (0343 or 0353 or 0383) are shipped at room temperature.

References
[1] Kendall RT, Feghali-Bostwich CA. (2014) "Fibroblasts in fibrosis: novel roles and mediators" *Front Pharmacol*. 5: 123
[2] Ortiz-Zapater E, Signes-Costa J, Montero P and Roger Ines. (2022) "Lung Fibrosis and Fibrosis in the Lungs: Is It All about Myofibroblasts?" *Biomedicines.* 10 (6): 1423.

Procedure:

Step I: Preparing the complete 3D culture medium

1. Thaw 3D-Bronchial Fibroblast Spheroid Supplement (3D-HBFSpS; Cat. #3D-3452), Fetal Bovine Serum (FBS; Cat. #0004) and Penicillin/Streptomycin solution (P/S solution; Cat. #0583) at 37°C. Mix 3D-HBFSpS, FBS and P/S solution into the 3D-Bronchial Fibroblast Spheroid Medium (3D-HBFSpM; Cat. #3D-3421) by gently swirling the medium bottle around.

    a. 3D-HBFSpM is **viscous** and optimized for homogenous spheroid formation.
    b. Warm the complete 3D-HBFSpM 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 bronchial fibroblast spheroids are recovered and ready for experiments after 24-48 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 –Brightfield images of the ready-to-use human bronchial fibroblast spheroids after thawing (taken at 100X magnification).

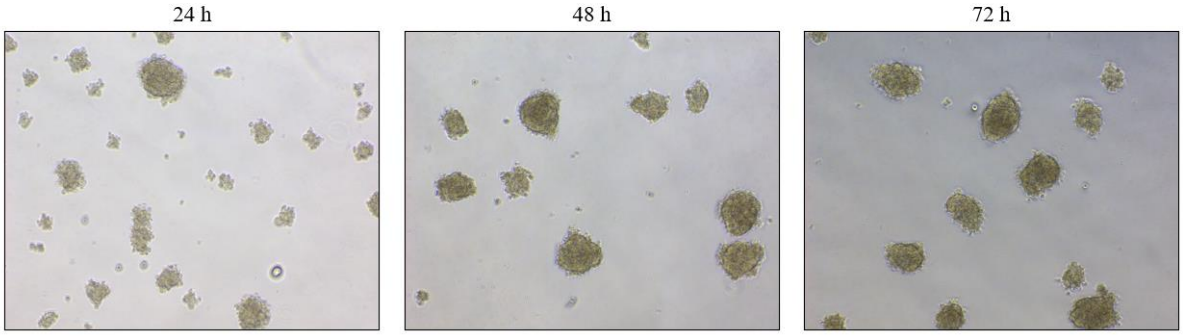

Fig. 2 – Human Bronchial Fibroblast Spheroids express the bronchial fibroblast marker fibronectin (FN) (at 200x magnification).

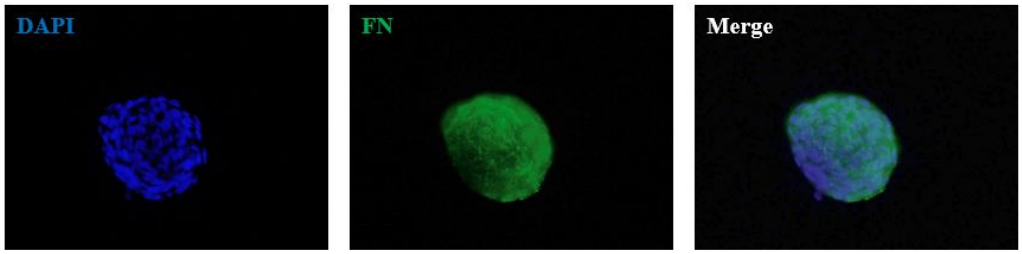

Fig. 3 – Human Bronchial Fibroblast Spheroids demonstrate elevated collagen I (COL1) deposition in response to 72 hours of stimulation with 2ng/mL TGFβ1 (at 200x magnification).