Rev. 0

Ready-to-use 3D Human Ovarian Fibroblast Spheroids (SP3D-HOFS)

Catalog #SP3D-7330

Product Description

The ovary is one of the most important female reproductive organs in mammalian species. Stromal cells make up 80% of the total cell population in the ovary, with one of the major stromal cells being ovarian fibroblasts [1]. Hormone signaling from neighboring cancer cells can induce activation of normal fibroblasts, transitioning them into cancer-associated fibroblasts (CAF). Increasing evidence is showing the significance of CAF in the tumor microenvironment and the progression of cancer [2]. Aberrant proliferation of ovarian fibroblasts and the accumulation of extracellular matrix can also cause ovarian fibrosis and severely impacts ovarian function [3]. To better understand the contribution of human ovarian fibroblasts (HOF) in these pathophysiological conditions, *in vitro* models to study HOF 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 HOF function physiologically, ScienCell has developed ready-to-use human ovarian fibroblast spheroids (SP-HOFS) comprised of primary HOF. These cells are cultured in three-dimensional (3D) architecture and are embedded in their own ECM, which better reflects the native ovarian tissue architecture. These spheroids are ready for experiments within 24-48 hours of thawing, and are an excellent *in vitro* model for studying HOF function and its contribution to diseases such as ovarian cancer and ovarian fibrosis.

Kit Components (Included)

3D Cell Culture Components				
Cat #	**# of vials**	**Product Name**	**Quantity**	**Storage**
SP-7330	1	Human Ovarian Fibroblast Spheroids (SP-HOFS)	1×10^4 spheroids	Liquid nitrogen
3D-7301	1	3D-Ovarian Fibroblast Spheroid Medium (3D-OFSpM)	200 mL	2-8 °C
3D-7352	1	3D-Ovarian Fibroblast Spheroid Supplement (3D-OFSpS)	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-HOFS are tested for the formation of functional and uniform 3D human ovarian fibroblast spheroids according to the included protocol. All components are negative for bacterial and fungal contamination.

Product Use
SP3D-HOFS 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-7330, 3D-7352, 0004, and 0583 are shipped on dry ice. 3D-7301 and (0343 or 0353 or 0383) are shipped at room temperature.

References
[1] Shahri PAK, Chiti MC and Amorim C. (2019) "Isolation and characterization of the human ovarian cell population for transplantation into an artificial ovary." *Anim Reprod*. 16 (1): 39-44.
[2] Yang D, Liu J, Qian H and Zhuang Q. (2023) "Cancer-associated fibroblasts: from basic science to anticancer therapy." *Exp Mol Med*. 55: 1322-1332.
[3] Gu M, Wang Y and Yu Y. (2024) "Ovarian fibrosis: molecular mechanisms and potential therapeutic targets." *J Ovarian Res*. 17: 139

Procedure:

Step I: Preparing the complete 3D culture medium

1. Thaw 3D-ovarian fibroblast spheroid supplement (3D-OFSpS; Cat. #3D-7352), fetal bovine serum (FBS; Cat. #0004) and penicillin/streptomycin solution (P/S solution; Cat. #0583) at 37°C. Mix 3D-OFSpS, FBS and P/S solution into the 3D-ovarian fibroblast spheroid medium (3D-OFSpM; Cat. #3D-7301) by gently swirling the medium bottle around.

    a. 3D-OFSpM is **viscous** and optimized for homogenous spheroid formation.
    b. Warm the complete 3D-OFSpM 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. Add 12 mL of 3D culture medium into a fresh 50 mL conical tube.

3. 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).

4. 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.

5. Carefully remove the cap without touching the interior threads. Gently pipette the spheroid suspension up and down **two times** to disperse potential spheroid aggregates.

6. Gently transfer the spheroid suspension into the 50 mL conical tube from step 2.

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 mini liver 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 ovarian fibroblast spheroids after thawing (taken at 100X magnification).

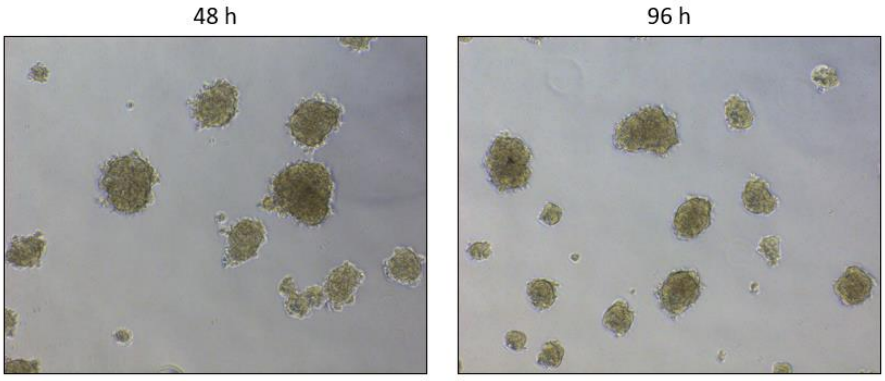

Fig. 2 – Human ovarian fibroblast spheroids express the fibroblast marker fibronectin (FN) (at 200x magnification).