Rev. 0

Ready-to-use 3D Human Mammary Fibroblast Spheroids (SP3D-HMF)

Catalog #SP3D-7630

Product Description

Human mammary fibroblasts are important stromal cells that contribute to extracellular matrix (ECM) production and remodeling, mammary tissue architecture, and the regulation of epithelial cell behavior. Through paracrine signaling and interactions with mammary epithelial cells and other stromal components, fibroblasts contribute to tissue development, homeostasis, and remodeling [1,2]. Altered fibroblast activity and ECM organization can contribute to pathological conditions, including breast cancer, in which cancer-associated fibroblasts (CAFs) influence tumor progression, invasion, metastasis, and the tumor microenvironment [3,4].

Conventional two-dimensional (2D) cell culture provides a useful platform for studying fibroblast biology, but does not fully reproduce the three-dimensional organization and complex cell–cell and cell–matrix interactions found in native tissues. Three-dimensional (3D) culture models provide alternative approaches for investigating cellular behavior within more complex microenvironments, although their physiological relevance depends on the model design and biological characteristics [5,6].

To support these studies, ScienCell has developed ready-to-use human mammary fibroblast spheroids (SP-HMF), composed of primary human mammary fibroblasts cultured in a three-dimensional configuration. These spheroids provide an *in vitro* platform for investigating mammary fibroblast biology, cellular organization, and stromal functions within a 3D microenvironment. SP-HMF spheroids are designed for experimental use within 24–48 hours after thawing, subject to the recommended recovery and culture protocol. Potential research applications include mammary stromal biology, ECM remodeling, and investigations of fibroblast contributions to the breast cancer microenvironment.

Kit Components (Included)

3D Cell Culture Components				
Cat #	**# of vials**	**Product Name**	**Quantity**	**Storage**
SP-7630	1	Human Mammary Fibroblast Spheroids (SP-HMF)	1×10^4 spheroids	Liquid nitrogen
3D-7631	1	3D- Human Mammary Fibroblast Spheroid Medium (3D-HMFSpM)	200 mL	2-8 °C
3D-7632	1	3D- Human Mammary Fibroblast Spheroid Supplement (3D-HMFSpS)	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-HMF are tested for the formation of functional and uniform 3D human mammary fibroblast spheroids according to the included protocol. All components are negative for bacterial and fungal contamination.

Product Use
SP3D-HMFS 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-7630, 3D-7632, 0004, and 0583 are shipped on dry ice. 3D-7631 and (0343 or 0353 or 0383) are shipped at room temperature.

References
[1] Sumbal J, Belisova D, Koledova Z. (2021). Fibroblasts: The grey eminence of mammary gland development. Seminars in Cell & Developmental Biology, 114, 134–142.
[2] Maller O, Martinson H, Schedin P. (2010). Extracellular matrix composition reveals complex and dynamic stromal-epithelial interactions in the mammary gland. Journal of Mammary Gland Biology and Neoplasia, 15(3), 301–318.
[3] Houthuijzen JM, Jonkers J. (2018). Cancer-associated fibroblasts as key regulators of the breast cancer tumor microenvironment. Cancer and Metastasis Reviews, 37(4), 577–597.
[4] Giorello MB, Borzone FR, Labovsky V, Piccioni FV, Chasseing NA. (2021). Cancer-Associated Fibroblasts in the Breast Tumor Microenvironment. Journal of Mammary Gland Biology and Neoplasia, 26(2), 135–155.
[5] Edmondson R, Broglie JJ, Adcock AF, Yang L. (2014). Three-dimensional cell culture systems and their applications in drug discovery and cell-based biosensors. Assay and Drug Development Technologies, 12(4), 207–218.
[6] Chen Y, et al. (2017). Modeling Physiological Events in 2D vs. 3D Cell Culture. Physiology, 32(4), 266–277.

Procedure:

<u>Step I: Preparing the complete 3D culture medium</u>

1. Thaw 3D-mammary fibroblast spheroid supplement (3D-HMFSpS; Cat. #3D-7632), fetal bovine serum (FBS; Cat. #0004) and penicillin/streptomycin solution (P/S solution; Cat. #0583) at 37°C. Mix 3D-HMFSpS, FBS and P/S solution into the 3D-mammary fibroblast spheroid medium (3D-HMFSpM; Cat. #3D-7631) by gently swirling the medium bottle around.

    a. 3D-HMFSpM is **viscous** and optimized for homogenous spheroid formation.
    b. Warm the complete 3D-HMFpM 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 ≥ 1 × 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 mammary 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 image of the ready-to-use human mammary fibroblast spheroids after thawing (taken at 100X magnification).

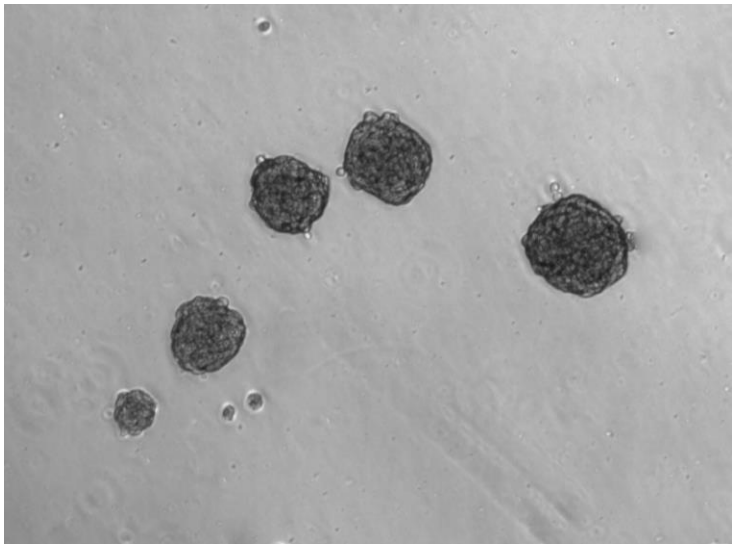


Fig. 2 – Human mammary fibroblast spheroids express the fibroblast marker fibronectin (FN) (at 200x magnification).

