Rev. 0

All-Inclusive Human Mammary Epithelial Spheroid Formation Kit (SP3D-HMEpiC)

Catalog #SP3D-7610

Product Description

The All-Inclusive Human Mammary Epithelial Spheroid Formation Kit provides a convenient, integrated solution for establishing three-dimensional (3D) human mammary epithelial spheroid cultures. Designed to support the formation of multicellular structures in a controlled in vitro environment, this kit combines the essential cell culture components needed to facilitate spheroid generation and streamline 3D mammary cell culture workflows.

By supporting the development of 3D cellular architecture and cell–cell interactions, the kit offers researchers a practical platform for investigating mammary epithelial biology, tissue organization, and cellular responses under physiologically relevant culture conditions [1]. It is suitable for applications in mammary gland research, breast biology, 3D cells culture method development, and preclinical research [2,3].

The all-inclusive format helps simplify experimental setup, reduce the need to source individual reagents separately, and promote greater consistency across experiments. Whether used for exploratory studies or the development of advanced in vitro models, the kit provides a convenient starting point for researchers seeking to incorporate 3D human mammary epithelial spheroid culture into their workflows.

Kit Components (Included)

3D Cell Culture Components				
Cat #	**# of vials**	**Product Name**	**Quantity**	**Storage**
7610	1	Human Mammary Epithelial Cells (HMEpiC)	5 X 10^5 cells	Liquid nitrogen
7630	1	Human Mammary Fibroblast Cells (HMF)	5 × 10^5 cells	Liquid nitrogen
3D-7612	1	3D- Human Mammary Spheroids Medium (3D- MEpiSpM)	200 mL	2-8 °C
3D-7653	1	3D- Human Mammary Spheroids Supplement (3D-MEpiSpS)	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
2D Cell Culture Components				
7611	1	2D-Mammary Epithelial Cell Medium	500 mL	2-8 °C
2301	1	2D-Fibroblast Medium	500 mL	2-8 °C
7652	1	2D-Mammary Epithelial Cell Growth Supplement (2D-MEpiCGS)	5 mL	-20 °C

2352	1	2D-Fibroblast Cell Growth Supplement (2D-FCGS)	5 mL	-20 °C
0025	1	Fetal Bovine Serum (FBS)	25 mL	-20 °C
0503	1	Penicillin/Streptomycin Solution (P/S)	5 mL	-20 °C

Quality Control
SP3D-HMEpic are tested for the formation of functional and uniform 3D human mammary epithelial and fibroblast co-culture spheroids according to the included protocol. All components are negative for bacterial and fungal contamination.

Product Use
SP3D-HMEpic 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
7610, 7630, 3D-7653, 0004, 0583, 7652, 2352, 0025 and 0503 are shipped on dry ice. 3D-7612, 7611, 2301 and (0343 or 0353 or 0383) are shipped at room temperature.

References
[1] Dontu G, Abdallah WM, Foley JM, Jackson KW, Clarke MF, Kawamura MJ, Wicha MS. (2003). In vitro propagation and transcriptional profiling of human mammary stem/progenitor cells.
[2] Chen G, Bouamar H, Sun L-Z. (2019). Isolation, Culture, and Differentiation of Mammary Epithelial Stem/Progenitor Cells from Fresh or Ex Vivo Cultured Human Breast Tissue.
[3] Corinus A, et al. (2026). Human mammary 3D spheroid models uncover the role of filopodia in breaching the basement membrane to facilitate invasion.

Procedure:

<u>Step I: Prepare the complete 2D-mammary epithelial and fibroblast culture medium</u>

1. Thaw 2D-mammary epithelial and mammary fibroblast cell culture growth supplements (2D-MEpiCGS; Cat. #7652 and 2D-FCGS Cat. #2352), fetal bovine serum (FBS; Cat. #0025), and penicillin/streptomycin solution (P/S solution; Cat. #0503) at 37°C.

2. Add 2D-MEpiCGS and P/S solution to the 2D-mammary epithelial cell medium-basal (2D- MEpiCM; Cat. #7611) and mix well.

3. Add 2D-FCGS, FBS and P/S solution to the 2D-fibroblast cell medium-basal (2D- FM; Cat. #2301) and mix well.

   a. Warm the 2D-complete mediums only to room temperature prior to use.
   b. When stored in the dark at 4°C, the complete medium is stable for one month.

<u>Step II: Thaw, maintain and sub-culture cells in 2D cell culture</u>

4. For the human mammary epithelial cells (HMEpiC; Cat. #7610), one cryopreserved vial contains 5×10^5. It is recommended to plate directly into one pLL-coated T-75 flask using the complete 2D-mammary epithelial cell medium (2D- MEpiCM).

5. For the human mammary fibroblast cells (HMF; Cat. #7630), one cryopreserved vial contains 5×10^5. It is recommended to plate directly into one pLL-coated T-75 flask using the complete 2D fibroblast medium (2D-FM).

Note: HMEpiC are guaranteed to further expand for ONLY 10 population doublings under the conditions provided by ScienCell Research Laboratories. Therefore, please use cells for experiments at earliest passage after initial plating.

Note: For detailed instructions on thawing and maintaining the HMEpiC and HMF in 2D culture, please see the product sheets Cat #7610, and #7630, respectively.

B. Establishing 3D spheroid culture

Step III: Prepare the complete 3D spheroid medium

6. Thaw 3D- Human Mammary Spheroids Supplement (3D-MEpiSpS; Cat. #3D-7653), and penicillin/streptomycin solution (P/S solution; Cat. #0583) at 37°C. Mix 3D-MEpiSpS, and P/S solution into the 3D- Human Mammary Spheroids Medium (3D- MEpiSpM; Cat. #3D-7612) by gently swirling the medium bottle around.

a. 3D- MEpiSpM medium is viscous and optimized for homogenous spheroid formation.
b. Warm the complete 3D- MEpiSpM medium only to room temperature before use.
c. When stored in the dark at 4°C, the complete medium is stable for one month.

Step IV: Harvest cells for 3D culture

7. When desired number of cells have been achieved in 2D monolayer culture, you can begin setting up 3D spheroid culture as described below.
8. Rinse the cells with DPBS.
9. Add 8 mL of DPBS and 2 ml 0.05% T/E solution (Cat. #0183) into each flask (in the case of a T-75 flask). Gently rock the flask to ensure complete coverage of cells by T/E solution. Use a microscope to monitor the change in cell morphology.
10. Transfer T/E solution from the flask to the 50 ml centrifuge tube (a small percent of cells may detach) and continue to incubate the flask at 37ºC for another for 2-3 minutes (no solution in the flask at this time).
11. At the end of incubation, gently tap the side of the flasks to dislodge cells from the surface. Check under a microscope to make sure that all cells detach.
12. Add 5 ml of TNS solution to each flask and transfer detached cells to the separate 50 ml centrifuge tubes. Rinse the flasks with another 5 ml of TNS to collect the residual cells.

Step III: Resuspend and seed cells in 3D cell culture medium

13. Count cells using a hemacytometer. Please see **Table A** for the suggested cell numbers for HMEpiC and HMF for different plate formats.

Table A: An Example of Suggested Cell Number and Culture Volume per Sample

1	2	3	4
Plate formats	**HMEpiC cell number per well**	**HMF cell number per well**	**Volume per well**
24-well	2.6 × 10^5 cells	1.3 × 10^5 cells	~ 1000 µL
48-well	9.4 × 10^4 cells	5.0 × 10^4 cells	~ 500 µL
96-well	4.0 × 10^4 cells	2.1 × 10^4 cells	~ 200 µL

14. Aliquot the suggested number of cells for HMEpiC and HMF into a fresh conical tube. **Note:** It is recommended to make **a minimum of 5 mL cell suspension in 3D medium** for easier pipetting due to the viscosity of 3D medium.
15. Centrifuge the tube at 1000 rpm for 5 minutes.
16. Aspirate the supernatant while leaving behind the 100-200 µl supernatant above the pellet in the tube.
17. Resuspend cells in the residual supernatant by pipetting up and down for ~ 10 times to obtain a single cell suspension.
18. Next, add the appropriate volume of the complete 3D-HSpM medium to obtain the suggested density of cell suspension (see **Table A; column 4**).
19. Slowly pipette up and down for ~ 5-7 times and make sure you have uniform cell suspension in 3D medium before proceeding to next step.

Note: 3D culture medium has a high viscosity; thus, pipetting slowly is important to avoid the formation of bubbles.

20. Add the suggested volume of cell mixture (see Table A; column 4) to each well in the provided ultra-low binding plate by using a p1000 pipette. In order to minimize pipette errors, do not use a serological pipette.
21. Incubate the cells at 37°C in a 5% CO2 humidity incubator.
22. Spheroids can be maintained in culture without changing the medium.
23. Monitor the growth and formation of spheroid every day under the microscope. Mature spheroids develop at ~ **4 days** post seeding (Figure 1 and 2).

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Fig. 1 – At 200x magnification, brightfield images of human mammary epithelial and fibroblast cell co-culture spheroids at different days in 3D cell culture.

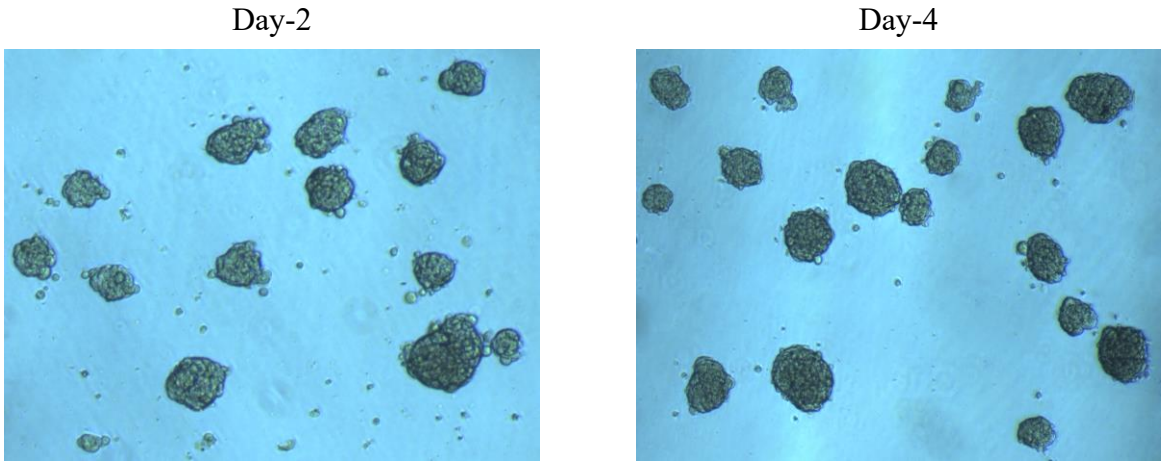

Fig. 2 – At days 6; immunostaining of the human mammary spheroids with the epithelial marker CK18 distributed throughout the spheroids (200x magnification).

