All-inclusive 3D Human Calvarial Osteoblast Spheroid Formation Kit

3D-HCOSF
Cat. #3D-4600

Product Description

Bone is a living organ maintained by osteoblasts and osteoclasts. Osteoblasts, the bone-forming cells, secrete bone matrix such as type I collagen. Osteoblasts function as an organized tight epithelium to separate the bone extracellular matrix from the general extracellular fluid [1]. As the matrix grows, osteoblasts are incorporated into the matrix as osteocytes. Osteocytes communicate through cellular processes that run within canaliculi in the matrix [1]. The complex interactions between cell-cell and cell-ECM are critical for the process of bone formation and mineralization [2]. Since these complex interactions are strictly dependent on the three-dimensional (3D) environment, ScienCell has developed a 3D osteoblast spheroid formation kit (3D-HCOSF) in which cells maintain direct cell-cell and cell-ECM interactions in all three dimensions. Osteoblasts grown in ScienCell's 3D spheroid culture preserve their functionality and have elevated expression levels of osteogenic genes, compared to those grown in 2D culture (see Figures 1 and 2). 3D-HCOSF is a more physiologically relevant *in vitro* model for studying the bone physiology and the drug effects on bone metabolism.

Kit Components (Included)

3D Cell Culture Components				
Cat #	**# of vials**	**Product Name**	**Quantity**	**Storage**
4600	1	Human Calvarial Osteoblasts (HCO)	5×10^5 cells	Liquid nitrogen
3D-4601	1	3D-Osteoblast Spheroid Medium - basal (3D-OSpM)	200 mL	2-8 °C
3D-4652	1	3D-Osteoblast Spheroid Supplement (3D-OSpS)	2 mL	-20 °C
0010	1	Fetal Bovine Serum (FBS)	10 mL	-20 °C
0583	1	Penicillin/Streptomycin Solution (P/S)	2 mL	-20 °C
0343 (or) 0353 (or) 0383	2	Ultra-Low Binding Culture Plates (24-, 48-, or 96- well plate)	2 plates	RT
2D Cell Culture Components				
Cat #	**# of vials**	**Product Name**	**Quantity**	**Storage**
4601	1	Osteoblast Medium – Basal (ObM)	500 mL	2-8 °C
4652	1	Osteoblast Growth Supplement (ObGS)	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

Additional Recommended Materials (Not Included)

Cat #	Product Name
0113	Trypsin Neutralization Solution
0183	0.05% Trypsin/EDTA (T/E)
0303	Dulbecco's Phosphate-Buffered Saline (DPBS)
0413	Poly-L-Lysine (10 mg/mL)

Quality Control

3D-HCOSF is tested for the uniform formation of 3D osteoblast spheroids according to the included protocol. All components are negative for bacterial and fungal contamination.

Product Use

3D-HCOSF is for research use only. It is not approved for human or animal use, or application in clinical or *in vitro* diagnostic procedures.

Shipping

4600, 3D-4652, 4652, 0010, 0025, 0583, and 0503 are shipped on dry ice. 3D-4601, 4601, and [0343 (or) 0353 (or) 0383] are shipped at room temperature.

References

[1] Blair H, Larrouture Q, Li Y, Lin H, Beer-Stoltz D, Liu L, Tuan R, Robinson L, Schlesinger P, Nelson D. (2017) "Osteoblast Differentiation and Bone Matrix Formation In Vivo and In Vitro." *Tissue Engineering Part B: Reviews* 23(3): 268-280.

[2] Deegan A, Aydin H, Hu B, Konduru S, Kuiper J, Yang Y. (2014) "A facile in vitro model to study rapid mineralization in bone tissues." *BioMedical Engineering Online* 13 (136): 1 – 17.

Procedure:

A. Initiating cells in 2D culture

1. Please see the product sheet Cat. #4600 for thawing and maintaining ScienCell's human primary osteoblasts (Cat. #4600) in 2D monolayer culture.

2. For expansion in 2D monolayer culture, use the included 2D culture kit components (Cat. #4601, 4652, 0025, 0503).

B. Establishing 3D spheroid culture

Step I: Prepare the complete 3D culture medium

3. Thaw 3D-osteoblast spheroid supplement (3D-OSpS; Cat. #3D-4652), fetal bovine serum (FBS; Cat. #0010), and penicillin/streptomycin solution (P/S solution; Cat. #0583) at 37°C. Mix 3D-OSpS, FBS and P/S solution into the 3D-osteoblast spheroid medium (3D-OSpM; Cat. #3D-4601) by gently swirling the medium bottle around.

   a. 3D-OSpM medium is **viscous** and optimized for homogenous spheroid formation.
   b. Warm the complete 3D-OSpM 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 II: Harvest cells for 3D culture

Table A: An Example of Suggested Cell Suspension Density, and Medium Volumes

1	2	3
Plate formats	**Concentration of cell suspension (cells/mL)**	**Volume per well**
24-well	3.4×10^5 cells/mL	~ 1000 µL
48-well	3.0×10^5 cells/mL	~ 500 µL
96-well	3.2×10^5 cells/mL	~ 200 µL

4. Please see **Table A** for the suggested cell densities for different plate formats. A confluent T-75 and T-175 flasks should yield about 5×10^6 and 1×10^7 cells, respectively.

5. When desired amount of cells have been achieved in 2D monolayer culture, you can begin setting up 3D spheroid culture as described below.

6. Rinse the cells with DPBS.

7. Add 5 mL of DPBS and 5 ml 0.05% T/E solution (Cat. #0183) into 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.

8. Transfer T/E solution from the flask to the 50 ml conical tube (a small percent of cells may detach) and continue to incubate the flask at 37°C for another minute (no solution in the flask at this time).

9. At the end of incubation, gently tap the side of the flask to dislodge cells from the surface. Check under a microscope to make sure that all cells detach.

10. Add 5 ml of TNS solution to the flask and transfer detached cells to the 50 ml conical tube. Rinse the flask with another 5 ml of TNS to collect the residual cells.

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

11. Count cells using hemacytometer, and aliquot the appropriate volume of cell suspension into a fresh 50 mL conical tube. Please see **Table A** for the suggested cell densities for different plate formats.

    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.

12. Centrifuge the 50 ml conical tube at 1000 rpm for 5 minutes.

13. Aspirate the supernatant while leaving behind the 100-200 µl supernatant above the pellet in the tube.

14. Resuspend cells in the residual supernatant by pipetting up and down for ~ 10 times to obtain a single cell suspension.

15. Next, add the appropriate volume of the 3D-OSpM medium to obtain the suggested density of cell suspension (see **Table A; column 2**).

16. Slowly pipette up and down for ~ 10 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.

17. Add the suggested volume of cell mixture (see **Table A; column 3**) to each well in the provided ultra-low binding plate by using a p1000 pipette. Do not use the serological pipette to minimize pipette errors while adding small volumes to the wells.

18. Incubate the cells at 37°C in a 5% CO_2 humidity incubator.

19. Change the 3D culture medium every four days by only changing 60-70% of the top layer of the medium using a pipette by hand (Do not use a vacuum aspirator).

    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.

20. Monitor the growth and formation of spheroid every day under the microscope. Mature osteoblast spheroids develop at ~ 3 days post seeding (Figure 1).

Fig. 1 – Growth of osteoblast spheroids over 7 days. (A) Spheroids were generated using primary osteoblasts according to the provided protocol. (B) Brightfield images of osteoblast spheroids were taken at 100X magnification at days 1, 3, and 7.

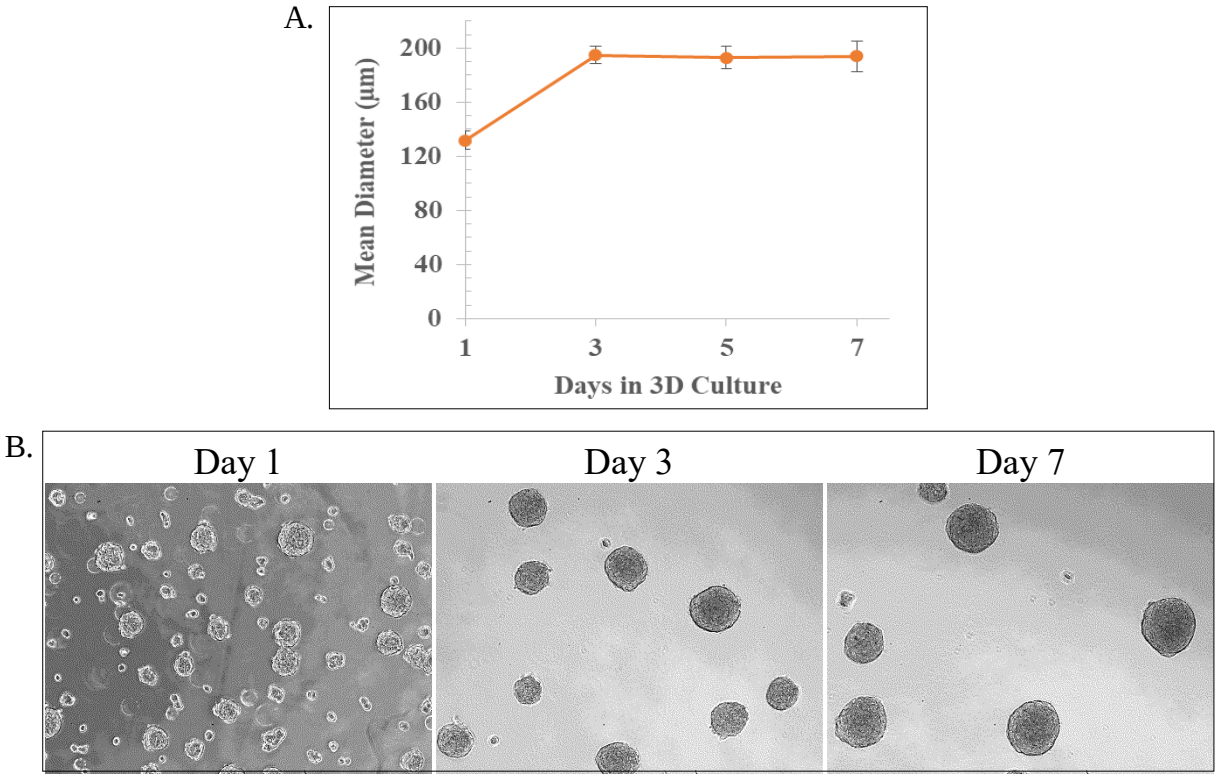
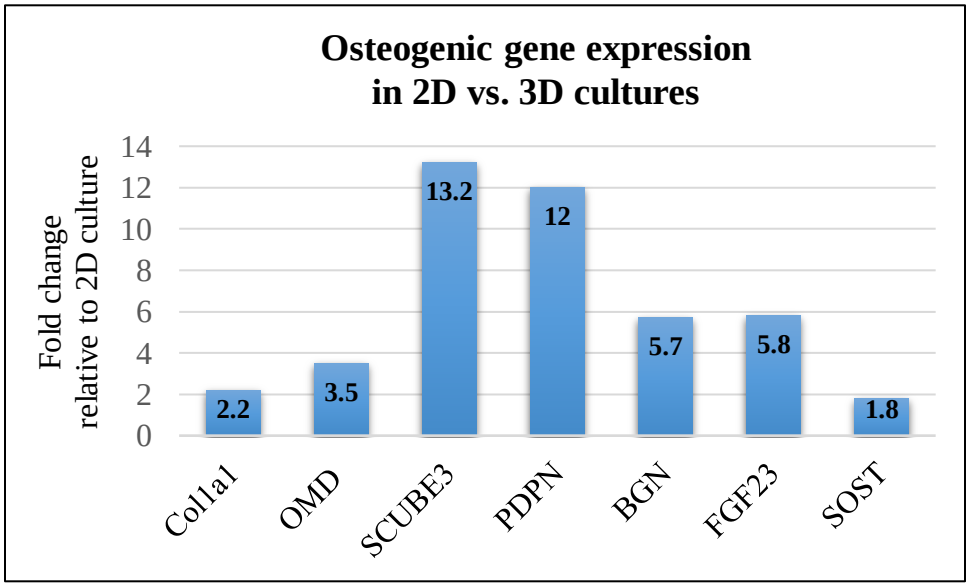


Fig. 2 – Examination of the functional markers of osteoblasts grown in 2D versus 3D spheroid cultures at day 7. Expression levels of osteogenic genes were measured using the ScienCell's GeneQuery Human Osteogenic Differentiation qPCR array kit (Cat. #GK080).



Troubleshooting Guide

Problem	Possible Cause	Potential Solution
Cells do not form spheroids.	Cells are not healthy.	- Check cell viability (should be >90%) and cell proliferation using trypan blue. - Reduce extensive sub-culturing in 2D culture.
Spheroid formation is not homogenous.	1. Cells are not resuspended well.	- First, obtain single cell suspension in the residual supernatant by gently pipetting up and down for approximately 10-15 times (see step 14). - Next, obtain uniform cell suspension in 3D culture medium by pipetting up and down for approximately 10 – 15 times. Additionally, you can rotate the tube around to help mixing cells in 3D medium (see step 16).
	2. Shelves in the cell culture incubator are not level.	- Level your shelves of the CO_2 incubators.