Ready-to-use 3D Human Calvarial Osteoblast Spheroid Kit

SP3D-HCOS
Cat. #SP3D-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, while they remain exclusively as an organized tight epithelium to separate the bone extracellular matrix from general extracellular fluid [1]. As matrix grow, osteoblasts are incorporated into the matrix as osteocytes, which communicate with each other and surface layer osteoblasts through cell 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]. Furthermore, these complex interactions are strictly dependent on the three-dimensional (3D) environment. To better approximate the *in vivo* condition, ScienCell has developed a highly innovative ready-to-use 3D osteoblast spheroid kit (SP3D-HCOS) in which cells maintain direct cell-cell and cell-ECM interactions in all three dimensions. With ScienCell's Ready-to-use 3D spheroid kit, researchers can generate more physiologically relevant data from 3D spheroid culture in 24 hours after thawing (see Fig. 1 and 2), without encountering the long and complex workflow of 3D culture.

Kit Components (Included)

3D Culture Components				
Cat #	**# of vials**	**Product Name**	**Quantity**	**Storage**
SP-4600	1	Human Calvarial Osteoblast Spheroids (SP-HCO)	1×10^4 spheroids	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	1	Ultra-Low Binding Culture Plate (24-, 48-, or 96-well plate)	1 plate	RT

Quality Control

SP3D-HCOS is tested for the homogenous formation of the 3D osteoblast spheroids in 24 hours after thawing. All components are negative for bacterial and fungal contamination.

Product Use

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

Shipping

SP-4600, 3D-4652, 0010, and 0583 are shipped on dry ice. 3D-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:

Step I: Preparing the complete 3D spheroid medium

1. 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: 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 multiwell plate** (e.g. 24-, 48-, and 96-well ultra-low binding 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 spheroid suspension up and down for **two times** to disperse potential spheroid aggregates.

5. Gently transfer the spheroid suspension into a fresh 50 mL conical tube.

6. Add the 12 mL of 3D culture media to the above 50 mL conical tube.

7. Resuspend spheroids in 3D culture medium by gently pipetting up and down for ~ 5-7 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 included ultra-low binding plate (24-, or 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. For best results, do not disturb the culture for at least 16 hours after the culture has been initiated.

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, change 60-70% of the top layer of the medium every 3-4 days.

    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.

12. Monitor the health of spheroids every day under the microscope. Osteoblast spheroids are recovered and ready for your experiment after 24 hours post thawing (see Figure 1).

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Fig. 1 – At 100x magnification, brightfield images of ready-to-use 3D osteoblast spheroids at 24 hours after thawing.

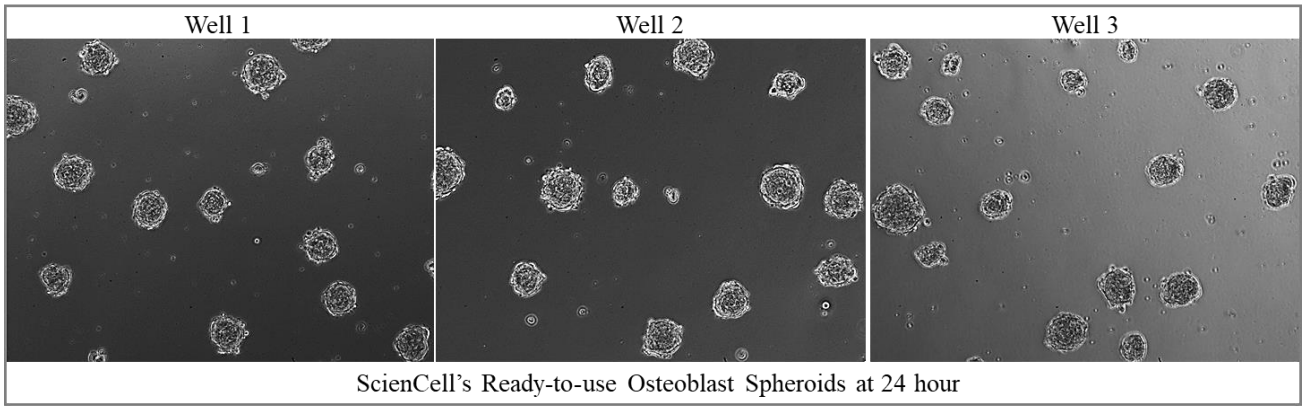

Fig. 2 – Examination of the functional markers of osteoblasts grown in 2D versus 3D spheroid cultures at Day 7. Expression levels of osteogenic genes were measured using the ScienCell's GeneQuery Human Osteogenic Differentiation qPCR array kit (Cat. #GK080).