Ready-to-use 3D Osteogenesis-Angiogenesis Coupling Kit

SP3D-OAC
Cat. #SP3D-8748

Product Description

Bone formation and repair is a complex process involving the highly orchestrated interplay between different cell types, such as endothelial cells and osteoblasts. During skeletal development and postnatal bone repair, angiogenesis and osteogenesis must be tightly coupled for physiological bone function, as blood vessels bring a supply of oxygen, nutrients and osteogenic progenitor cells into the osteogenic environment [1]. In fact, the absence of a functional vasculature network can compromise physiological bone healing, leading to osteonecrosis, osteoporosis, and non-union fractures [2]. Thus, elucidation of the molecular crosstalk between angiogenesis and osteogenesis is critical in designing the therapeutic strategies for improving efficient vascularization and bone formation. To study such complex tissue and regulation processes, ScienCell's ready-to-use 3D Osteogenesis-Angiogenesis Coupling kit (SP3D-OAC) offers the cryopreserved osteoblast and endothelial cell co-culture spheroids, serving as a superior model for analysis of complex cellular interactions present in bone tissue. A unique feature of this kit is that researchers can achieve highly functional and homogenous multicellular spheroids in 24 hours after thawing, without requiring any prior experience in 3D cell culture.

Kit Components (Included)

3D Cell Culture Components				
Cat #	**# of vials**	**Product Name**	**Quantity**	**Storage**
SP-8740	1	Human Osteoblasts and Human Dermal Microvascular Endothelial Cell Co-culture Spheroids (SP-HOE)	1×10^4 spheroids	Liquid nitrogen
3D-4621	1	3D-Osteo-Endo Spheroid Medium (3D-OESpM)	200 mL	2-8 °C
3D-4662	1	3D- Osteo-Endo Spheroid Supplement (3D-OESpS)	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 Plates (24-, 48-, or 96- well plate)	1 plate	RT

Quality Control

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

Product Use

SP3D-OAC 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-8740, 3D-4662, 0010, and 0583 are shipped on dry ice. 3D-4621, and [0343 (or) 0353 (or) 0383] are shipped at room temperature.

References
[1] Grosso A, Burger MG, Lunger A, Schaefer DJ, Banfi A, and Maggio ND. (2017) "It Takes Two to Tango: Coupling of Angiogenesis and Osteogenesis for Bone Regeneration." *Front Bioeng Biotechnol* 5 (68): 1-7.
[2] Inglis S, Christensen D, Wilson DI, Kanczler JM, and Oreffo R. (2016) "Human Endothelial and Foetal Femur-derived Stem Cell Co-Cultures Modulate Osteogenesis and Angiogenesis." *Stem Cell Research & Therapy* 7 (13): 1 – 16.

Procedure:

Step I: Preparing the complete 3D spheroid medium

1. Thaw 3D-Osteo-Endo spheroid supplement (3D-OESpS; Cat. #3D-4662), fetal bovine serum (FBS; Cat. #0010), and penicillin/streptomycin solution (P/S solution; Cat. #0583) at 37°C. Gently mix 3D-OESpS, FBS and P/S solution into the 3D-Osteo-Endo spheroid medium (3D-OESpM; Cat. #3D-4621).

    a. 3D-OESpM medium is **viscous** and optimized for homogenous spheroid formation.
    b. Warm the complete 3D-OESpM 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 media by gently pipetting up and down for ~ 8-10 times using a serological pipette.

    Note: 3D culture medium has a high viscosity; thus, pipetting slowly is important to avoid too much 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 other day.

    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-endothelial cells co-culture spheroids are recovered and ready for your experiment after 24 hours post thawing (see Figure 1).

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Fig. 1 –Brightfield images of the ready-to-use 3D osteoblast and endothelial cell co-culture spheroids (taken at 100x magnification).

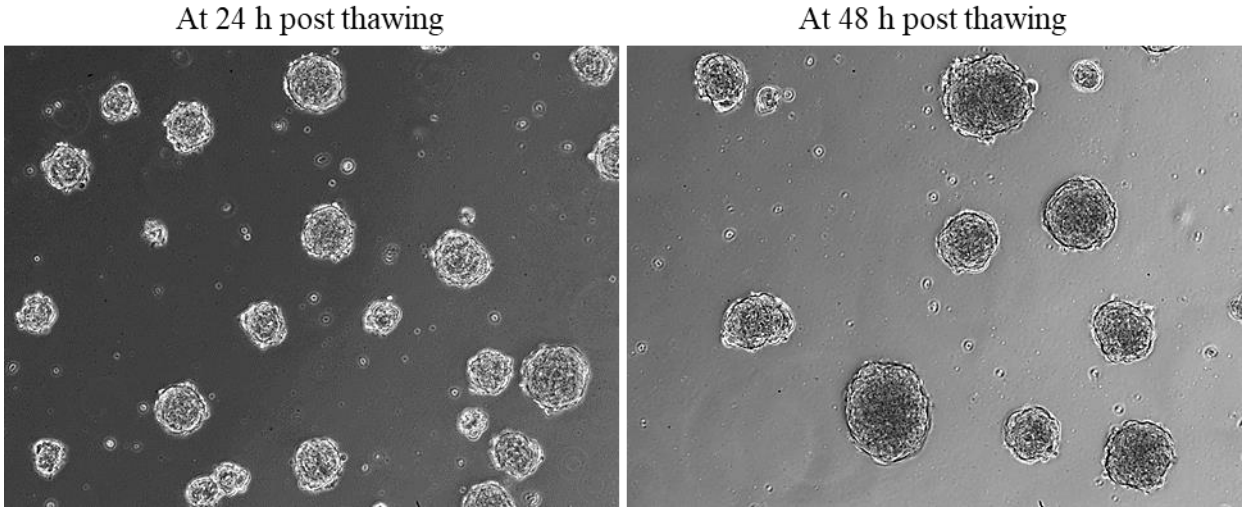
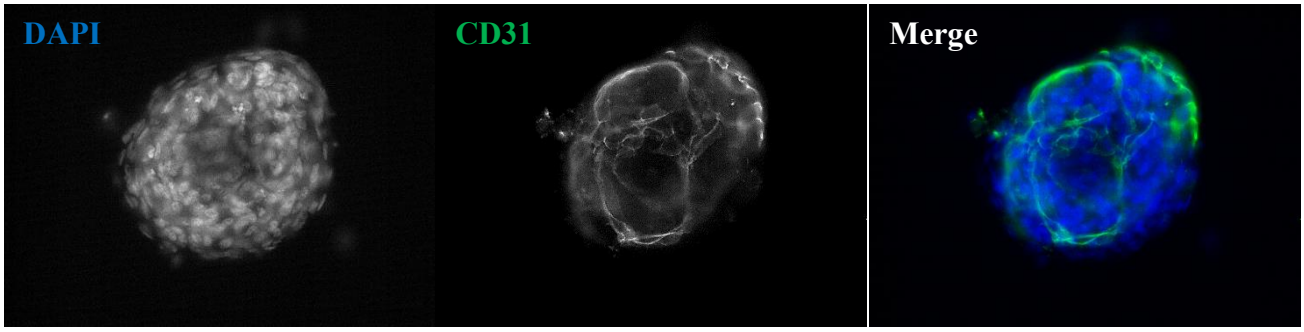


Figure 2 – Day 7; Immunofluorescence staining of osteoblast and endothelial cell co-culture spheroids. IF analysis showed that within 3D spheroids, the CD31+ endothelial cells are directly in contact with the CD31- osteoblasts, mimicking the complex cellular interactions in bone tissue.



At 400x magnification