Rev. 1

Ready-to-use 3D Human Chondrocyte-articular Spheroid Kit
SP3D-HCaS

Cat. #SP3D-4650

Product Description

The composition and structural integrity of extracellular matrix (ECM) within the cartilage is responsible for enduring the tensile strength of joint biomechanics. Chondrocytes, the main constituent of cartilage, mediate the synthesis and degradation of ECM macromolecules (such as Type II collagen and aggrecan) within the matrix [1]. Type II collagen and aggrecan provide the tensile strength and the osmotic resistance for cartilage, respectively [1]. Chondrocytes in monolayer culture, however, are susceptible to dedifferentiation [2]. Thus, an improved cell culture model that closely mimics the *in vivo* environment is necessary to maintain the features of differentiated chondrocytes. ScienCell has developed a highly innovative ready-to-use 3D chondrocyte spheroid kit (3D-CS) that better approximates the *in vivo* environment. Using ScienCell's Ready-to-use 3D spheroid kit, researchers can obtain highly homogenous and functional 3D spheroids in 24 hours after thawing, without encountering the long and complex workflow of 3D culture.

In 3D spheroid culture, we confirmed that the chondrocytes maintain functional markers such as type II collagen, aggrecan, and Sox9 as determined by qPCR analysis (see Fig. 1 and 2). ScienCell's 3D-CS provides an excellent *in vitro* model for studying normal chondrocyte physiology, mechanism of degenerative joint diseases, and cartilage tissue repair and engineering.

Kit Components (Included)

3D Cell Culture Components				
Cat #	**# of vials**	**Product Name**	**Quantity**	**Storage**
SP-4650	1	Human Chondrocyte Spheroids (SP-HCa)	1×10^4 spheroids	Liquid nitrogen
3D-4651	1	3D-Chondrocyte Spheroid Medium – basal (3D-CSpM)	200 mL	2-8 °C
3D-4682	1	3D-Chondrocyte Spheroid Supplement (3D-CSpS)	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 plate	Ultra-Low Binding Culture Plate (24-, 48-, or 96-well plate)	1 plate	RT

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

Product Use
SP3D-HCaS 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-4650, 3D-4682, 0010, and 0583 are shipped on dry ice. 3D-4651, and [0343 (or) 0353 (or) 0383] are shipped at room temperature.

References
[1] Lin Z, Willers Z, Xu J, and Zheng M. (2006) "The Chondrocyte: Biology and Clinical Application." *Tissue Engineering* 12(7): 1971-1984.
[2] Li J, and Dong S. (2016) "The Signaling Pathways Involved in Chondrocyte Differentiation and Hypertrophic Differentiation." *Stem Cells International* 24: 1-12.

Procedure:

Step I: Preparing the complete 3D culture medium

1. Thaw 3D-chondrocyte spheroid supplement (3D-CSpS; Cat. #3D-4682), fetal bovine serum (FBS; Cat. #0010), and penicillin/streptomycin solution (P/S solution; Cat. #0583) at 37°C. Mix 3D-CSpS, FBS and P/S solution into the 3D-chondrocyte spheroid medium (3D-CSpM medium; Cat. #3D-4651) by gently swirling the medium bottle around.

    a. 3D-CSpM medium is **viscous** and optimized for homogenous spheroid formation.
    b. Warm the complete 3D-CSpM medium 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 multi-well plate (e.g. 24-, 48-, and 96-well ultra-low binding culture 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 ~ 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 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. 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. Chondrocyte 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 chondrocyte spheroids at 24 hours after thawing.

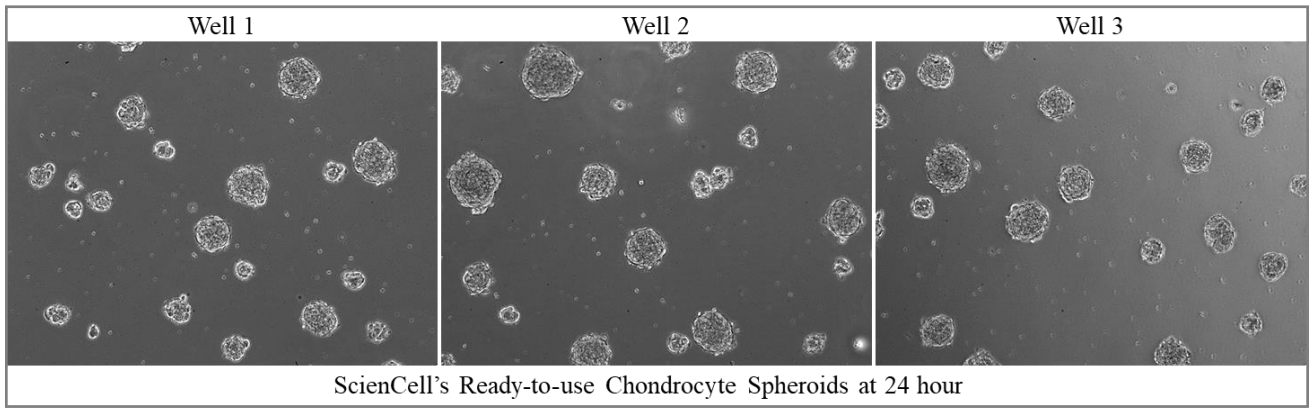

Fig. 2 – Examination of the functional markers of chondrocytes grown in 2D versus 3D spheroid cultures at day 7. Gene expression levels of chondrogenic markers such as **type II collagen, aggrecan, and Sox9** were measured using the ScienCell's GeneQuery Human Chondrocyte Biology qPCR array kit (Cat. #GK079).