# All-inclusive 3D Human Chondrocyte-articular Spheroid Formation Kit

3D-HCaSF
Cat. #3D-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 an all-inclusive 3D chondrocyte spheroid formation kit (3D-HCaSF) enabling the growth of human primary chondrocytes in a more biologically relevant 3D environment. By growing in a proper three-dimensional environment *in vitro*, chondrocytes maintain functional markers such as type II collagen, aggrecan, and Sox9 as validated by the qPCR analysis (see Fig. 1 and 2). ScienCell's 3D-HCaSF 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** |
| 4650 | 1 | Human Chondrocytes-articular (HC-a) | $5 \times 10^5$ cells | 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 | 2 plates | Ultra-Low Binding Culture Plates (24-, 48-, 96-well plates) | 2 plates | RT |
| **2D Cell Culture Components** | | | | |
| **Cat #** | **# of vials** | **Product Name** | **Quantity** | **Storage** |
| 4651 | 1 | Chondrocyte Medium – Basal (CM) | 500 mL | 2-8 °C |
| 4682 | 1 | Chondrocyte Growth Supplement (CGS) | 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-HCaSF is tested for the uniform formation of 3D chondrocyte spheroids according to the included protocol. All components are negative for bacterial and fungal contamination.

**Product Use**

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

**Shipping**

4650, 3D-4682, 4682, 0010, 0025, 0583, and 0503 are shipped on dry ice. 3D-4651, 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:**

**A. Initiating the cells in 2D culture**

1. Please see the product sheet Cat. #4650 for thawing and maintaining ScienCell's human primary chondrocytes (Cat. #4650) in 2D monolayer culture.

2. For expansion in 2D monolayer culture, use the included 2D culture kit components (Cat. #4651, 4682, 0025, 0503).

**B. Establishing 3D spheroid culture**

Step I: Prepare the complete 3D cell culture medium

3. Thaw 3D-chondrocyte spheroid supplement (3D-CSpS; Cat. #3D-4682), fetal bovine serum (FBS; Cat. #0015), and penicillin/streptomycin solution (P/S solution; Cat. #0583) at 37°C. Gently mix 3D-CSpS, FBS and P/S solution into the 3D-chondrocyte spheroid medium (3D-CSpM medium; Cat. #3D-4651) by rotating around the media bottle.

    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: 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 | $2.7 \times 10^5$ cells/mL | ~ 1000 µL |
| 48-well | $2.4 \times 10^5$ cells/mL | ~ 500 µL |
| 96-well | $2.5 \times 10^5$ 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 $5 \times 10^6$ and $1 \times 10^7$ cells, respectively.

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

6. Rinse the cells with DPBS (Cat. #0303).

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 37ºC 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 cell culture medium

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

    ***Note: It is recommended to prepare 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-CSpM 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 a 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.

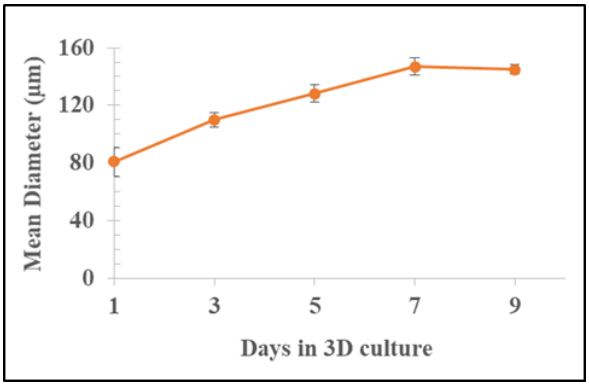
19. Change the 3D culture medium every four days by only changing 60-70% of the top layer of the medium using a pipette by hand (Do not use a vacuum aspirator).

    ***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 chondrocyte spheroids develop at ~ 3-4 days post seeding (Figure 1).

Fig. 1 – Growth of chondrocyte spheroids over time. (A) Spheroids were generated using the human primary chondrocytes according to the provided protocol. (B) Brightfield images of chondrocyte spheroids were taken at 100X magnification at days 1, 3, and 7. Mature spheroids develop around day 3 post seeding.

A.




B.

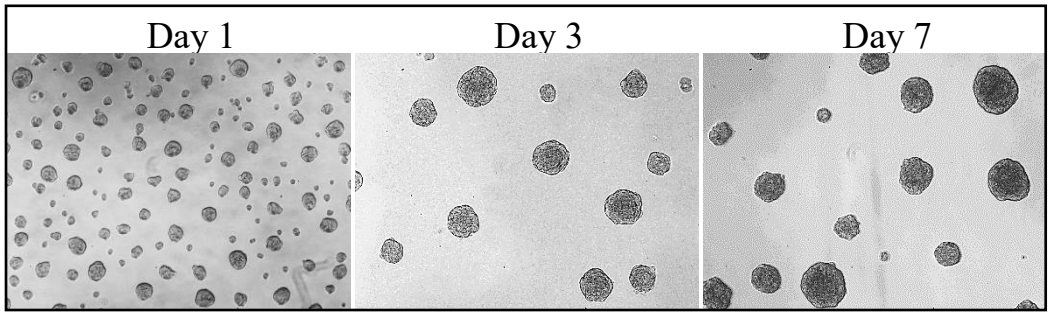
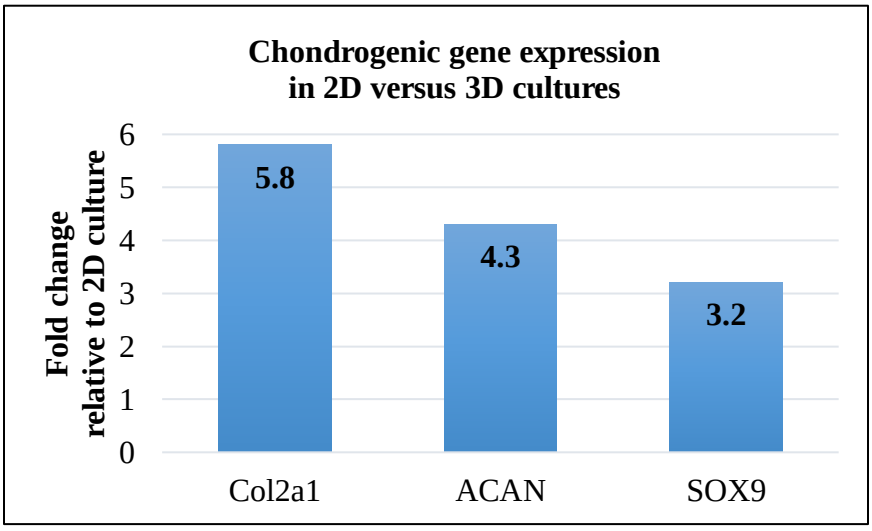



Fig. 2 – Examination of the functional markers of chondrocytes grown in 2D versus 3D spheroid cultures at day 7. Gene expression levels of chondrogenic markers such as **type II collagen, aggrecan, and Sox9** were measured using the ScienCell's GeneQuery Human Chondrocyte Biology qPCR array kit (Cat.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



**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.<br><br>- 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).<br><br>- 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. |