



Ready-To-Use 3D Human Mesenchymal Stem Cell Spheroids
SP3D-HMSCS
 Cat. #SP3D-7500

Product Description

Mesenchymal stem cells (MSCs) have emerged as a leading candidate for use in cell therapy due to its multifunctional properties. Their ability to differentiate into specific cell types, their immune-suppressive properties, as well as their potent tropic effects during tissue repair make them invaluable in cell therapy [1]. Several lines of evidence has shown that culturing MSC as three-dimensional (3D) spheroids enhances a wide range of biological properties [1-3]. Traditionally, the generation of 3D MSC spheroids can be tedious and rate limiting. In order to facilitate high-throughput MSC studies, ScienCell offers ready-to-use 3D mesenchymal stem cell spheroids (SP3D-HMSCS), which can be thawed and plated directly in multiwell plates. By RT-qPCR analysis, we demonstrate that SP3D-HMSCS have elevated mRNA expression of pluripotency markers such as Sox2, Nanog, and CD90, indicating the enhanced stemness. Furthermore, chemotactic factor (CXCR4), anti-inflammatory molecule (PEG2), as well as other endogenous growth factors (VEGF and FGF2) are highly expressed in 3D MSC spheroids, thereby promoting the migration, colonization, and viability of MSC. SP3D-HMSCS are, therefore, a great model for examining the functions of mesenchymal stem cells in cell therapy and tissue regeneration.

Kit Components (Included)

3D Cell Culture Components				
Cat #	# of vials	Product Name	Quantity	Storage
SP-7500	1	Human Mesenchymal Stem Cell Spheroids (SP-HMSCS)	4 × 10 ³ spheroids	Liquid nitrogen
3D-7501	1	3D-Mesenchymal Stem Cell Spheroid Medium (3D-MSCSpM)	200 mL	2-8 °C
3D-7552	1	Mesenchymal Stem Cell Spheroid Supplement (3D-MSCSpS)	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-HMSCS are tested for the formation of functional and uniform 3D human mesenchymal stem cell spheroids according to the included protocol. All components are negative for bacterial and fungal contamination.

Product Use

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

Shipping

SP-7500, 3D-7552, 0010, and 0583 are shipped on dry ice. 3D-7501, and (0343 or 0353 or 0383) are shipped at room temperature.

References

- [1] Sart S., Tsai A.C., Li Y., Ma T. (2014) “Three-Dimensional Aggregates of Mesenchymal Stem Cells: Cellular Mechanisms, Biological Properties, and Applications.” *Tissue Engineering*. 20 (5): 365-380.
- [2] Kabiri M., Kul B., Lott W., Futrega K., Ghanavi P., Upton Z., Doran M.R. (2012) “3D mesenchymal stem/stromal cell osteogenesis and autocrine signaling.” *Biochemical and Biophysical Research Communications*. 419: 142-147.
- [3] Han H., Asano S., Hsu S. (2019) “Cellular Spheroids of Mesenchymal Stem Cells and Their Perspectives in Future Healthcare.” *Applied Sciences*. 9 (627): 1-13.

Procedure:

Step I: Preparing the complete 3D culture medium

1. Thaw 3D-mesenchymal stem cell spheroid supplement (3D-MSCSpS; Cat. #3D-7552), and penicillin/streptomycin solution (P/S solution; Cat. #0583) at 37°C. Mix 3D-MSCSpS and P/S solution into the 3D-mesenchymal stem cell spheroid basal medium (3D-MSCSpM; Cat. #3D-7501) by gently swirling the medium bottle around.
 - a. 3D-MSCSpM is **viscous** and optimized for homogenous spheroid formation.
 - b. Warm the complete 3D-MSCSpM 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 $\geq 4 \times 10^3$ 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 the spheroid suspension up and down **two times** to disperse potential spheroid aggregates.
5. Gently transfer the spheroid suspension into a fresh 50 mL conical tube.
6. Add 24 mL of 3D culture medium to the above 50 mL conical tube.
7. Resuspend spheroids in 3D culture medium by gently pipetting up and down for ~ 5 times using a serological pipette.

Fig. 2 – Human mesenchymal stem cell spheroids exhibit the stem cell surface antigens CD73 and CD90 throughout the spheroids (200x magnification).

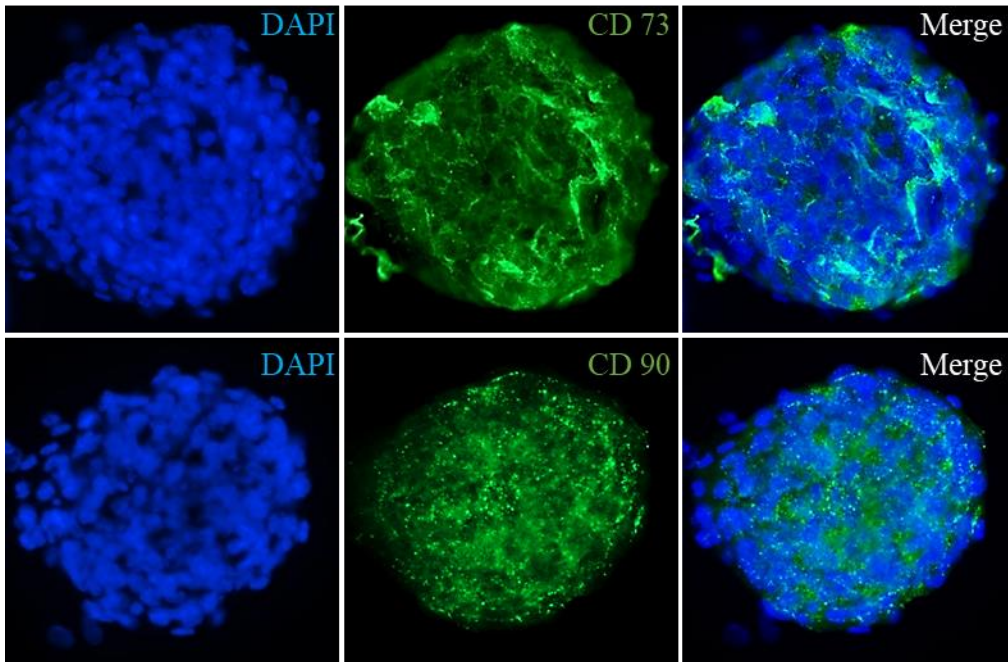


Fig. 3 – qRT-PCR analysis showed that 3D human mesenchymal stem cell spheroids better maintain stem cell properties. In addition, 3D MSC spheroids also secrete a higher level of chemotactic factor, anti-inflammatory molecule, and other endogenous growth factors, compared to 2D-cultured MSC cells.

