Rev. 0

Ready-to-use 3D Human Preadipocyte Spheroids

SP3D-HPA
Catalog #SP3D-7220

Product Description

Adipocytes are specialized cells that play a critical role in various metabolic events. Under physiological conditions, adipocytes serve as an energy storage reservoir, as well as exert endocrine effects through the secretion of cytokines and adipokines (1). Dysregulation in the homeostatic function of adipose tissue results in a plethora of metabolic diseases such as type II diabetes and cardiovascular diseases (2, 3). Obesity is a risk factor for type II diabetes as hypertrophy of adipose tissue may contribute to insulin resistance (2). To better understand the physiology of adipocytes in the diseased state and to evaluate potential therapeutic targets for these events, models that appropriately resemble fat tissue is vital. 2D monolayer culture of adipocytes *in vitro* is useful but is limited in its inability to reflect the complexity of human fat tissue. To provide a more native *in vitro* adipose model, ScienCell offers a ready-to-use human preadipocyte spheroid (SP3D-HPA) that can be easily differentiated into mature adipocytes in culture. Upon differentiation, these adipocytes accumulate large lipid droplets confirmed by Oil Red O staining. This spheroid model serves as an excellent tool for drug discovery and the study of adipocyte biology.

Kit Components (Included)

3D Cell Culture Components				
Cat #	**# of vials**	**Product Name**	**Quantity**	**Storage**
SP-7220	1	Human Preadipocyte Spheroids (SP-HPA)	1 x 10^4 spheroids	Liquid nitrogen
3D-7211	1	3D-HPA Spheroid Medium – basal (3D-HPASpM)	200 mL	2-8 °C
3D-7252	1	3D-HPA Spheroid Supplement (3D-HPASpS)	2 mL	-20 °C
3D-7221	1	3D-HPA Spheroid Differentiation Medium (3D-PADM)	200 mL	2-8 °C
3D-7232	1	3D-HPA Spheroid Differentiation supplement (3D-PAdDS)	2 mL	-20 °C
0010	2	Fetal Bovine Serum (FBS)	10 mL	-20 °C
0583	2	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-HPA is tested for the formation of functional and uniform 3D preadipocyte spheroids according to the included protocol. All components are negative for bacterial and fungal contamination.

Product Use

SP3D-HPA 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-7220, 3D-7252, 3D-7232, 0010 and 0583 are shipped on dry ice. 3D-7211, 3D-7221, and [0343 (or) 0353 (or) 0383] are shipped at room temperature.

References

[1] Ahima RS and Lazar MA. (2008) "Adipokines and the peripheral and neural control of energy balance." *Mol Endocrinol*. 22: 1023–31.

[2] Burhans MS, Hagman, DK, Kuzma JN, Schmidt KA and Kratz M. (2018) "Contribution of adipose tissue inflammation to the development of type 2 diabetes mellitus." *Compr Physiol*. 9 (1): 1-58.

[2] Oikonomou EK and Antoniades C. (2019) "The role of adipose tissue in cardiovascular health and disease." *Nat Rev Cardiol.* 16 (2): 83-99.

Procedure:

Recovery of SP-HPA

Step I: Preparing the complete 3D spheroid medium

1. Thaw 3D-HPA spheroid supplement (3D-HPASpS; Cat. #3D-7252), fetal bovine serum (FBS; Cat. #0010), and penicillin/streptomycin solution (P/S solution; Cat. #0583) at 37°C. Mix 3D-HPASpS, FBS and P/S solution into the 3D-HPA spheroid medium (3D-HPASpM; Cat. #3D-7211) by gently swirling the medium bottle around.

    a. 3D-HPASpM medium is **viscous** and optimized for homogenous spheroid formation.
    b. Warm the complete 3D-HPASpM 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 ≥ 1×10^4 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 **one time** to disperse potential spheroid aggregates.

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

6. Add 12 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 ~ 5-7 times using a serological pipette.

    Note: 3D culture medium has a high viscosity; thus, pipetting slowly is important to avoid the formation of bubbles.

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 Per Well

1	2	3
Plate formats	**Volume per well**	**Total number of wells**
24-well	~ 1000 µL	12 wells
48-well	~ 500 µL	24 wells
96-well	~ 250 µL	48 wells

9. Incubate spheroids at 37°C in a 5 % CO_2 incubator.

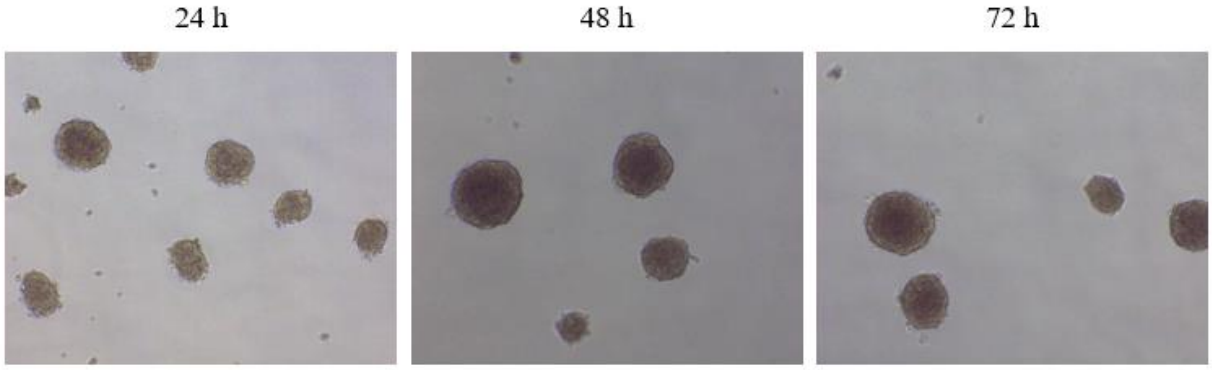
10. For best results, do not disturb the culture for at least 16 hours after the culture has been initiated.

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. Human preadipocyte spheroids are recovered and ready for your experiment after 2-3 days post thawing (see Figure 1).

Figure 1 – Ready-to-use human preadipocyte spheroids at 24-72 hours after thawing (taken at 100x magnification)



Differentiation of SP-HPA

Step III: Prepare the complete 3D preadipocyte differentiation medium

13. Thaw 3D-HPA spheroid differentiation supplement (3D-PAdDS; Cat. #3D-7232), fetal bovine serum (FBS; Cat. #0010), and penicillin/streptomycin solution (P/S solution; Cat. #0583) at 37°C. Mix 3D-PAdDS, FBS and P/S solution into the 3D-HPA spheroid differentiation medium (3D-PADM; Cat. #3D-7221) by gently swirling the medium bottle around.

    a. 3D-PADM medium is **viscous** and optimized for homogenous spheroid formation.
    b. Warm the complete 3D-PADM 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 IV: Induction of preadipocyte spheroid differentiation

14. Once spheroids have recovered (generally by 1-2 days post thawing), gently remove 50-60% of the top layer of the medium using a pipette by hand and replace medium with complete 3D Preadipocyte Differentiation Medium (3D-PADM, Cat #7221). This medium change counts as differentiation day 1.

15. After 1st medium change, change 50-60% of the top layer of the medium daily.

16. The process of differentiation to mature adipocyte spheroids is complete after 5-7 days. Mature adipocyte spheroids can be fixed and stained with Oil Red O Solution.

    Note: It is not recommended for prolonged culture of mature 3D adipocyte spheroids due to the tendency of the necrotic core to form when spheroids grow larger in size.

Figure 2 - Oil Red O Staining of human preadipocyte spheroid after 5 days of culture in complete 3D-human preadipocyte spheroid medium (left) and complete 3D preadipocyte differentiation medium (right).