Human Preadipocytes-subcutaneous (HPA-s)

Catalog Number: 7220

Cell Specification

Adipocytes play an important role in energy storage and metabolism. Adipocyte differentiation is a developmental process that is critical for metabolic homeostasis and nutrient signaling. It is controlled by complex actions involving gene expression and signal transduction [1]. Preadipocytes are present throughout adult life in adipose tissues and can proliferate and differentiate into mature adipocytes according to the energy balance [2]. The proliferation and differentiation of these preadipocytes contribute to increases in adipose tissue mass. *In vitro* study indicates that different tissue-derived preadipocytes exhibit differently in lipid accumulation, adipogenic transcription factor expression, and TNFα-induced apoptosis [3]. It has also been demonstrated that there is a close relationship between adipocyte differentiation and many physiological and pathological processes including fat metabolism, energy balance, obesity, diabetes, hyperlipidemia and breast cancer.

HPA-s from ScienCell Research Laboratories are isolated from human subcutaneous fat tissue. HPA-s are cryopreserved at primary culture and delivered frozen. Each vial contains >1 x 10^6 cells in 1 ml volume. HPA-s are characterized by immunofluorescent method with antibodies to CD44, CD90 and lipid staining after differentiation. HPA-s are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi. HPA-s are guaranteed to further culture at the conditions provided by ScienCell Research Laboratories.

Recommended Medium

It is recommended to use Preadipocyte Medium (PAM, Catalog No. 7211) for culturing HPA-s *in vitro*. Preadipocyte Differentiation Medium (PADM, Catalog No. 7221) can be use for *in vitro* differentiation of preadipocytes into mature adipocytes, and follows by Adipocyte Medium (AdM, Catalog No. 7201) which maintains mature adipocytes after differentiation.

Product Use

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

Storage

Directly and immediately transfer cells from dry ice to liquid nitrogen upon receiving and keep the cells in liquid nitrogen until cell culture is needed for experiments.

Shipping

Dry ice.

Reference

[1] Tominaga, K., Johmura, Y., Nishizuka, M., Imagawa, M. (2004) Fad24, a mammalian homolog of Noc3p, is a positive regulator in adipocyte differentiation. *J Cell Sci*. 117(Pt 25):6217-26.

[2] Reue, K., Glueck, S. B. (2001) Accumulating evidence for differences during preadipocyte development: Focus on "Differential gene expression in white and brown preadipocytes". *Physiol Genomics.* 7(1):1-2.

[3] Tchkonia, T. et al. (2005) Abundance of Two Human Preadipocyte Subtypes with Distinct Capacities for Replication, Adipogenesis, and Apoptosis Varies among Fat Depots. *Am J Physiol Endocrinol Metab*. 288(1):E267-77.

Instruction for culturing cells

Caution: Cryopreserved cells are very delicate. Thaw the vial in a 37°C waterbath and return them to culture as quickly as possible with minimal handling!

Set up culture after receiving the order:

1. Prepare a poly-L-lysine coated flask (2 μg/cm^2, T-75 flask is recommended). Add 10 ml of sterile water to a T-75 flask and then add 15 μl of poly-L-lysine stock solution (10 mg/ml, ScienCell cat. no. 0413). Leave the flask in incubator overnight (minimum one hour at 37°C incubator).
2. Prepare complete medium: decontaminate the external surfaces of medium and medium supplements with 70% ethanol and transfer them to sterile field. Aseptically open each supplement tube and add them to the basal medium with a pipette. Rinse each tube with medium to recover the entire volume.
3. Rinse the poly-L-lysine coated flask with sterile water twice and add 20 ml of complete medium to the flask. Leave the flask in the hood and go to thaw the cells.
4. Place the vial in a 37°C waterbath, hold and rotate the vial gently until the contents are completely thawed. Remove the vial from the waterbath immediately, wipe it dry, rinse the vial with 70% ethanol and transfer it to a sterile field. Remove the cap, being careful not to touch the interior threads with fingers. Using a 1 ml eppendorf pipette gently re-suspend the contents of the vial.
5. Dispense the contents of the vial into the equilibrated, poly-L-lysine coated culture vessels. A seeding density of 5,000 cells/cm^2 is recommended.
   Note: Dilution and centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of DMSO residue in the culture. It is also important that cells are plated in poly-L-lysine coated flask that promotes cell attachment and growth.
6. Replace the cap or cover, and gently rock the vessel to distribute the cells evenly. Loosen cap if necessary to permit gas exchange.
7. Return the culture vessels to the incubator.

8. For best result, do not disturb the culture for at least 16 hours after the culture has been initiated. Change the growth medium the next day to remove the residual DMSO and unattached cells, then every other day thereafter.

Maintenance of Culture:

1. Change the medium to fresh supplemented medium the next morning after establishing a culture from cryopreserved cells.
2. Change the medium every three days thereafter, until the culture is approximately 70% confluent.
3. Once the culture reaches 70% confluence, change medium every other day until the culture is approximately 90% confluent.

Subculture:

1. Subculture the cells when they are over 90% confluent.
2. Prepare poly-L-lysine coated cell culture flasks (2 μg/cm^2).
3. Warm medium, trypsin/EDTA solution (T/E, cat. no. 0103), trypsin neutralization solution (TNS, cat. no. 0113), and DPBS (Ca^{++} and Mg^{++} free, cat. no. 0303) to **room temperature**. We do not recommend warming the reagents and medium at 37°C waterbath prior to use.
4. Rinse the cells with DPBS.
5. Add 8 ml of DPBS first and then 2 ml of trypsin/EDTA solution into flask (in the case of T-75 flask); gently rock the flask to make sure cells are covered by trypsin/EDTA solution; incubate the flask at 37°C incubator for 1 to 2 minutes or until cells are completely rounded up (monitored with inverted microscope). During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine serum (FBS, cat. no. 0500); transfer trypsin/EDTA solution from the flask to the 50 ml centrifuge tube (a few percent of cells may detached); continue incubate the flask at 37°C for 1 or 2 minutes more (no solution in the flask at this moment); at the end of trypsinization, one hand hold one side of flask and the other hand gently tap the other side of the flask to detach cells from

attachment; check the flask under inverted microscope to make sure all cells are detached, add 5 ml of trypsin neutralization solution to the flask and transfer detached cells to the 50 ml centrifuge tube; add another 5 ml of TNS to harvest the residue cells and transfer it to the 50 ml centrifuge tube. Examine the flask under inverted microscope to make sure the cell harvesting is successful by looking at the number of cells left behind. There should be less than 5%.

Note: Use ScienCell Research Laboratories' trypsin/EDTA solution that is optimized to minimize the killing of the cells by over trypsinization.

6. Centrifuge the 50 ml centrifuge tube (harvested cell suspension) at 1000 rpm (*Beckman Coulter* Allegra 6R centrifuge or similar) for 5 min; re-suspend cells in growth medium.
7. Count cells and plate cells in a new, poly-L-lysine coated flask with cell density as recommended.

Caution: Handling human derived products is potentially biohazardous. Although each cell strain tests negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions must be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination [1].

[1]. Grizzle, W. E., and Polt, S. S. (1988) Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues. J Tissue Culture Methods. 11(4).

Instruction for Preadipocyte Differentiation

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Set up of Undifferentiated Expansion of Human Preadipocytes:

4. Primary Human Preadipocytes (HPAs) should be expanded with PAM (cat # 7211) in T-25 or T-75 flasks, which have been coated with poly-L-lysine and placed for at least 1 hour in the 37°C incubator.
5. Change the medium to fresh supplemented medium the next morning after establishing a culture from cryopreserved cells. For subsequent subcultures, change medium 48 hours after establishing the subculture.
6. Change the medium every other day thereafter, until the culture is ready for subculture.

Induction of Adipocyte Differentiation:

8. Plate the preadipocyte suspension in PAM at a density of 10,000 cells/cm^2 in the coated flask or plate.
9. Incubate the cells at 37°C in a 5% CO_2 humidified incubator for 1-2 days.

Note: Cells should reach 100% confluence before initiating adipocyte induction.

10. When the cells are 100% confluent, carefully replace the PAM with Preadipocyte Differentiation Medium (PADM, Cat # 7221). This medium change counts as differentiation day 1.
11. Replace the medium with fresh PADM every 2-3 days.
12. The process of differentiation to mature adipocytes is complete after 5-12 days. Mature adipocytes can be fixed and stained with Oil Red O Solution. Lipid droplets can be observed after 3 days.
13. Mature adipocytes can be maintained in Adipocyte Medium (AdM, Cat. #7201) up to 6 days.

Oil Red O Staining Protocol:

Stock Oil Red O solution

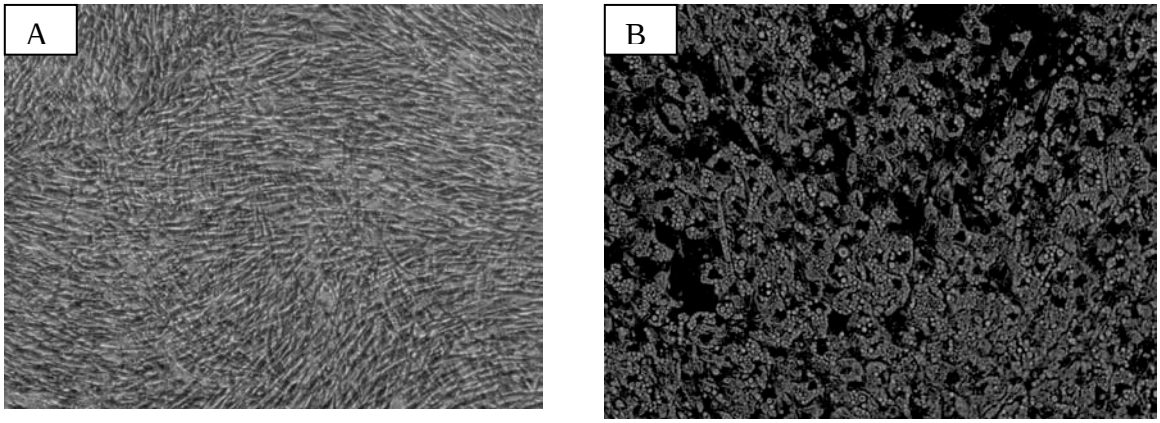
0.3g Oil Red O in 100 ml isopropanol .This solution is stable for up to 1 year

The working solution

1. 3 parts of stock Oil Red O solution and 2 parts of distilled water. (*Note: Let it sit at room temperature for 10 min.)*
2. Filter the working solution completely through the filter funnel.
3. This solution is stable only up to 2 hours. Make it freshly every time you use it.

Procedure

1. Remove media; rinse cells 2X with PBS.
2. Fix the cells by covering 10% formaldehyde.
3. Let plates/flasks sit at least for 15 min (or overnight) at room temperature.
4. Make the working solution as described above.
5. Remove fixative solution (10% formaldehyde); gently rinse tissue culture vessels with H_2O.
6. Remove the water; add Oil Red O filtered working solution slowly along the side of culture vessels. Ensure even spreading throughout the wells/flasks.
7. Sit > 10 min (1 hour or longer) at room temperature.
8. Rinse with tap water until the water runs clear.
9. View the plates on a phase contrast microscope. Lipids will appear red.



Human Preadipocytes-visceral (HPA-v, Cat. # 7210) were observed under a phase contrast microscope.

A. The cells were cultivated in Preadipocyte Medium (PAM, Cat # 7211) for 5 days (Control). There were no lipid droplets (10X).

B. The cells were cultivated in Preadipocyte Differentiation Medium (PADM, Cat # 7221) for 5 days. Lipid droplets were detected under microscope (20X).

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[1]. Grizzle, W. E., and Polt, S. S. (1988) Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues. J Tissue Culture Methods. 11(4).