Human Preadipocytes-subcutaneous (HPA-s)

Catalog #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 tissue and can proliferate and differentiate into mature adipocytes, contributing to increased adipose tissue mass [2]. *In vitro* studies indicate that various tissue-derived preadipocytes exhibit different lipid accumulations, adipogenic transcription factor expression, and TNFα-induced apoptosis [3]. There is also a close relationship between adipocyte differentiation and many physiological and pathological processes including fat metabolism, obesity, diabetes, hyperlipidemia, and breast cancer.

HPA-s from ScienCell Research Laboratories are isolated from human subcutaneous fat tissue. HPA-s are cryopreserved at passage one and delivered frozen. Each vial contains >1 x 10^6 cells in 1 ml volume. HPA-s are characterized by immunofluorescence with antibodies specific 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 expand for 5 population doublings under the conditions provided by ScienCell Research Laboratories.

Recommended Medium

It is recommended to use Preadipocyte Medium (PAM, Cat. #7211) for culturing HPA-s *in vitro*. Preadipocyte Differentiation Medium (PADM, Cat. #7221) can be used for *in vitro* differentiation of preadipocytes into mature adipocytes, and then followed by Adipocyte Medium (AdM, Cat. #7201), which maintains mature adipocytes after differentiation.

Product Use

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

Storage

Upon receiving, directly and immediately transfer the cells from dry ice to liquid nitrogen and keep the cells in liquid nitrogen until they are needed for experiments.

Shipping

Dry ice.

References

[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 SB. (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.

Instructions for culturing primary cells

Caution: Cryopreserved primary cells are very delicate. Thaw the vial in a 37ºC water bath and return the cells to culture as quickly as possible with minimal handling! Do not centrifuge the cells after thawing as this can damage the cells.

Initiating the culture:

Note: ScienCell primary cells must be cultured in a 37ºC, 5% CO_2 incubator. Cells are only warranted if ScienCell media and reagents are used and the recommended protocols are followed.

1. Prepare a poly-$_L$-lysine-coated culture vessel (2 µg/cm^2, T-75 flask is recommended). To obtain a 2 µg/cm^2 poly-$_L$-lysine-coated culture vessel, 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, Cat. #0413). Leave the vessel in a 37ºC incubator overnight (or for a minimum of one hour).
2. Prepare complete medium. Decontaminate the external surfaces of medium bottle and medium supplement tubes with 70% ethanol and transfer them to a sterile field. Aseptically transfer supplement to the basal medium with a pipette. Rinse the supplement tube with medium to recover the entire volume.
3. Rinse the poly-$_L$-lysine-coated vessel twice with sterile water and then add 15 ml of complete medium. Leave the vessel in the sterile field and proceed to thaw the cryopreserved cells.
4. Place the frozen vial in a 37ºC 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.
5. Carefully remove the cap without touching the interior threads. Gently resuspend and dispense the contents of the vial into the equilibrated, poly-$_L$-lysine-coated culture vessel.

   Note: Dilution and centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of residual DMSO in the culture. It is also important that cells are plated in poly-$_L$-lysine-coated culture vessels to promote cell attachment.
6. Replace the cap or lid of the culture vessel and gently rock the vessel to distribute the cells evenly. Loosen cap, if necessary, to allow gas exchange.
7. Return the culture vessel to the incubator.
8. Do not disturb the culture for at least 16 hours after initiation. Refresh culture medium the next day to remove residual DMSO and unattached cells.

Maintaining the culture:

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

Subculturing:

1. Subculture when the culture reaches 90-95% confluency.
2. Prepare poly-$_L$-lysine-coated culture vessels (2 μg/cm^2) one day before subculture.
3. Warm complete medium, trypsin/EDTA solution, 0.05% (T/E, Cat. #0183), T/E neutralization solution (TNS, Cat. #0113), and DPBS (Ca^{++}- and Mg^{++}-free, Cat. #0303) to **room temperature**. We do not recommend warming reagents and medium in a 37°C water bath prior to use.
4. Rinse the cells with DPBS.
5. Add 5 ml 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.

   Note: We recommend using ScienCell 0.05% T/E solution which is optimized to minimize cell damage due to over trypsinization. If 0.25% T/E solution (Cat. #0103) is used, then 9 ml of DPBS and 1 ml of 0.25% T/E solution should be used.

   Caution: Do NOT use undiluted trypsin when subculturing primary cells.
6. During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine serum (FBS, Cat. #0500).
7. Once cells completely round up, transfer T/E solution from the flask to the 50 ml centrifuge tube (a small percent of cells may detach) and continue to incubate the flask at 37°C for another minute (no solution in the flask at this time).
8. 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.
9. Add 5 ml of TNS solution to the flask and transfer detached cells to the 50 ml centrifuge tube. Rinse the flask with another 5 ml of TNS to collect the residual cells.
10. Examine the flask under a microscope for a successful cell harvest by looking at the number of cells being left behind; there should be less than 5%.
11. Centrifuge the 50 ml centrifuge tube at 1000 rpm for 5 minutes. Gently resuspend cells in culture medium.
12. Count and plate cells in a new poly-$_L$-lysine-coated culture vessel with the recommended cell density. A seeding density of 5,000 cells/cm^2 is recommended.

    Note: We do not recommend cryopreservation of primary cells by the end user. Refreezing cells may damage them and affect cell performance. ScienCell does not guarantee primary cells cryopreserved by the end user.

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 with 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 WE, Polt S. (1988) "Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues." *J Tissue Cult Methods*. 11: 191-9.

Instructions for Preadipocyte Differentiation

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

Undifferentiated Expansion of Human Preadipocytes:

1. 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.
2. 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.
3. Change the medium every other day thereafter, until the culture is ready for subculture.

Induction of Adipocyte Differentiation:

1. Plate the preadipocyte suspension in PAM at a density of 10,000 cells/cm^2 in the coated flask or plate.
2. 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.
3. 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.
4. Replace the medium with fresh PADM every 2-3 days.
5. 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.
6. 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

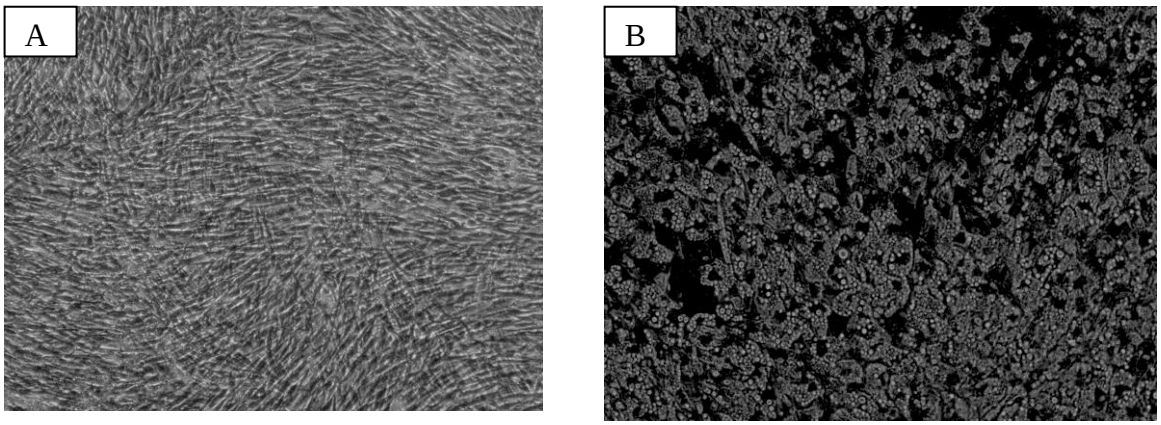
The working solution

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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the universal procedures for handling products of human origin as the minimum precaution against contamination [1].

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