ScienCell™
Research Laboratories

Preadipocyte Differentiation Medium (PADM)

Catalog Number: 7221

Product Description

Our Preadipocyte Differentation Medium (PADM) has been specifically developed and optimized for *in vitro* differentiation of preadipocytes into mature adipocytes. PADM is a sterile, liquid medium which contains essential and non-essential amino acids, vitamins, organic and inorganic compounds, hormones, growth factors, trace minerals. The medium is HEPES and bicarbonate buffered and has a pH of 7.4 when equilibrated in an incubator with an atmosphere of 5% CO_2/95% air.

Components

PADM consists of 500 ml of basal medium, 25 ml of fetal bovine serum (FBS, Cat. No. 0025), 5 ml of Preadipocyte Differentiation Supplement (PAdDS, Cat. No. 7232), 5 ml of penicillin/streptomycin solution (P/S, Cat. No. 0503).

PADM is for research use only. It is not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

Storage

Store the basal medium at 4°C, the FBS, PAdDS and the P/S solution at -20°C. Protect from light.

Shipping

Dry ice.

Prepare for use

Thaw PAdDS, FBS and P/S solution at 37°C. Gently tilt the PAdDS tube several times during thawing to help the contents dissolve. Rinse the bottle and tubes with 70% ethanol, and then wipe to remove excess. Remove the cap, being careful not to touch the interior threads with fingers. Add PAdDS, FBS and P/S solution into basal medium in a sterile field, mix well and then the reconstituted medium is ready for use. Since several components of PADM are light-labile, it is recommended that the medium not be exposed to light for lengthy periods of time. If the medium is warmed prior to use, do not exceed 37°C. When stored in the dark at 4°C, the reconstituted medium is stable for one month.

Caution: If handled improperly, some components of the medium may present a health hazard. Take appropriate precautions when handling it, including the wearing of protective clothing and eyewear. Dispose of properly.

Instruction for Preadipocyte Differentiation

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 of 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

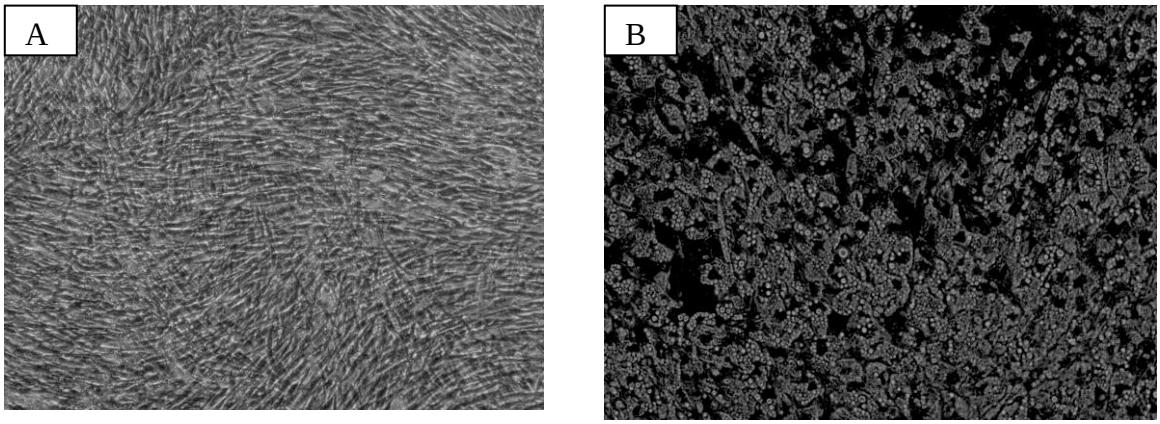
The working solution

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).

Caution: Handling human derived products is potentially bioharzadous. Although each cell strain testes negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions

mush 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).