REF: P60171

MESENCHYMAL STEM CELL ADIPOGENIC DIFFERENTIATION MEDIUM

Product Type: Mesenchymal Stem Cell Adipogenic Differentiation Medium
Catalog Number: P60171

Product Description

Our adipogenesis differentiation medium has been specifically developed and optimized for in vitro mesenchymal stem cell adipogenesis study. Generally, with this medium, we obtain >35% mature adipocytes from human bone marrow, >50% from rat bone marrow, and >60% from human mesenchymal stem cell isolated from adipose tissue. Efficiency of adipogenic differentiation depends on the quanlity of the mesenchymal stem cells. Mesenchymal stem cell Adipogenic Differentiation medium (MADM) 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

- 500 ml of Basal Medium
- 25 ml Fetal Bovine Serum
- 5 ml of Preadipocyte Differentiation Supplement (PDS).
- 5 ml of penicillin/streptomycin solution (P/S solution)

Prepare for use

Thaw MADS, FBS and P/S solution at 37oC. Gently tilt the MADS tube several times during thawing to help the contents dissolve. Make sure the contents of the supplement are completely dissolved into solution before adding to the medium. 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 MADS, 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 MADM 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 37oC. When stored in the dark at 4oC, the reconstituted medium is stable for one month.

Product Use

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in vitro diagnostic or clinical procedure.

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INSTRUCTIONS FOR ADIPOGENIC DIFFERENTIATION

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

Set up of Expansion Culture for Differentiation:

Note: It is recommended to use cells of low passage (≤3 passages) because the efficiency of differentiation deceases as the number of passages increases.

1. Primary Mesenchymal Stem Cells (MSCs) should be expanded with MSCM (cat # 60115) 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.

4. In general, human MSCs can be subcultured every 3 to 4 days, and rat MSCs can be passaged every 4 to 6 days.

Induction of Adipogenesis Differentiation:

1. Prepare a coated 6-well plate or T-25 flask with poly-l-lysine (2µg/cm2). For a 6-well plate, add 144µl of poly-l-lysine (1 mg/ml) to 9ml of sterile water. Add 1.5ml of this diluted poly-l-lysine to each well of the 6-well plate. For a T-25 flask, add 5ml of sterile water containing 50µl of poly-l-lysine (1 mg/ml) to the flask.

2. Leave the plate or the flask in the 37°C incubator for overnight (or at least 1 hour before using).

3. The next day, aspirate the poly-l-lysine dilution from the wells or flask and rinse the vessels twice with sterile water, aspirating in between washes.

4. Plate the cell suspension in MSCM at a density of 10,000 cells/cm^2 in the coated flask or plate.

5. Incubate the cells at 37°C in a 5% CO2 humidified incubator for 1-2 days.

Note: Cells should reach 100% confluence before initiating adipogenic induction

6. When the cells are 100% confluent, carefully replace the MSCM with adipogenic differentiation medium (MADM, Cat # 60171). This medium change counts at differentiation day 1.

7. Replace the medium with fresh adipogenic differentiation medium every 3-4 days for T-25 flasks, or every 4-5 days for 6 well plates.

Note: During the induction of adipogensis differentiation, cells are easily peeled off from the plates by the medium changes. Be extremely gentle and careful with the cells during medium change to avoid disrupting the lipid droplets.

8. Lipid droplets can be seen as early as 6 days incubated with differentiation medium. After 18-21 days of differentiation, cells can be fixed and stained with Oil Red O Solution

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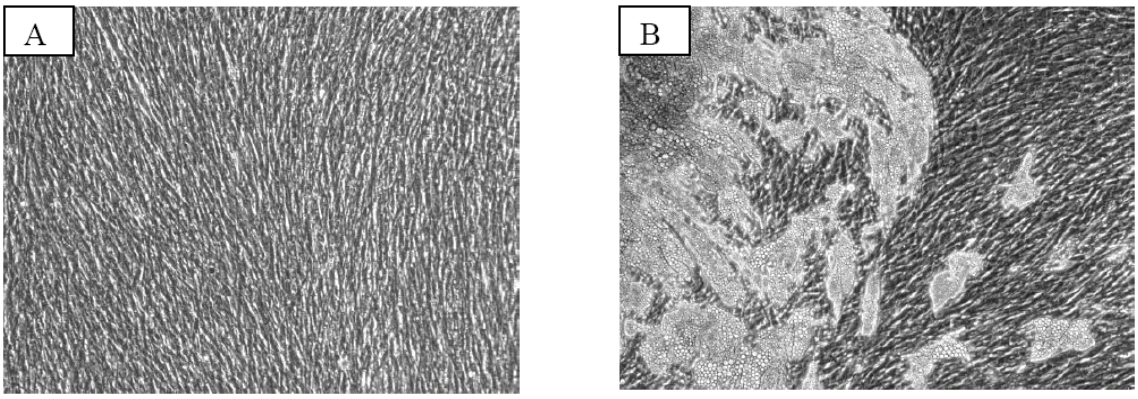

Human mesenchymal stem cells from bone marrow (HMSC-bm/P10576) were observed on a phase contrast microscope.

A. The cells were cultivated in expansion medium (MSCM, Cat #P60115) for 18 days (Control). There were no lipid droplets.

B. The cells were cultivated in adipogenesis differentiation medium (MADM-S, Cat # P60171), for 18 days. It is about 45% of mesenchymal stem cells differentiated into mature adipocytes.

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