



Mesenchymal Stem Cell Adipogenic Differentiation Medium-phenol red free (MADM-prf)

Catalog Number: 7541-prf

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 quantity of the mesenchymal stem cells. Mesenchymal stem cell Adipogenic Differentiation Medium-phenol red free (MADM-prf) 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% CO₂/95% air.

Components

MADM-prf consists of 500 ml of basal medium, 25 ml of fetal bovine serum (FBS, Cat. #0025), 5 ml of mesenchymal stem cell adipogenic differentiation supplement (MADS, Cat. #7542), 5 ml of penicillin/streptomycin solution (P/S, Cat. #0503).

Note: MADS may be slightly hazy; Haziness does not affect cell culture and differentiation efficiency.

Product Use

MADM-prf 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, FBS, MADS and P/S solution at -20°C. Protect from light.

Shipping

Gel ice.

Prepare for use

Thaw MADS, FBS and P/S solution at 37°C. Gently tilt the MADS 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 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-prf 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 Adipogenic 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 Expansion Culture for Differentiation:

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

1. Primary Mesenchymal Stem Cells (MSCs) should be expanded with MSCM (cat # 7501) 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 ($2\mu\text{g}/\text{cm}^2$). For a 6-well plate, add 144 μl of poly-l-lysine (cat# 0403) 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 50 μl of poly-l-lysine (0403) 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% CO_2 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 #7541). This medium change counts as differentiation day 1.

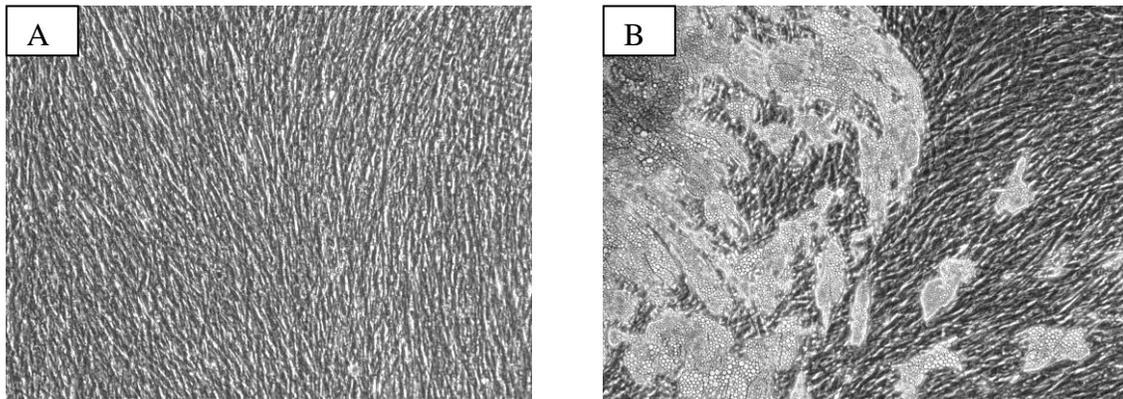
7. Replace the medium with fresh adipogenic differentiation medium every 3-4 days for T-25 flasks, or every 4-5 days for 6 well plates.

Note: During the induction of adipogenesis 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 incubation with differentiation medium. After 18-21 days of differentiation, cells can be fixed and stained with Oil Red O Solution.

Caution: Handling human derived products is potentially biohazardous. Although each cell strain testes 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).



Human mesenchymal stem cells from bone marrow (HMSC-bm, Cat #7500) were observed on a phase contrast microscope.

- A. The cells were cultivated in expansion medium (MSCM, Cat #7501) for 18 days (Control). There were no lipid droplets.
- B. The cells were cultivated in adipogenesis differentiation medium (MADM, Cat #7541) for 18 days. About 45% of mesenchymal stem cells differentiated into mature adipocytes.