Rev.1

Mesenchymal Stem Cell Osteogenic Differentiation Medium-phenol red free (MODM-prf)

Catalog #7531-prf

Product Description

Mesenchymal Stem Cell Osteogenic Differentiation Medium-phenol red free (MODM-prf) is a complete medium designed for the optimal osteogenic differentiation of mesenchymal stem cell (MSCs) *in vitro*. It is a sterile, liquid, phenol red free medium which contains essential and non-essential amino acids, vitamins, organic and inorganic compounds, hormones, growth factors, and 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. The medium is formulated (quantitatively and qualitatively) to provide a defined and optimally balanced nutritional environment that selectively promotes osteogenic differentiation of normal human mesenchymal stem cells *in vitro*.

Components

MODM-prf consists of 500 ml of basal medium, 25 ml of fetal bovine serum (FBS, Cat. #0025), 5 ml of mesenchymal stem cell osteogenic differentiation supplement (MODS, Cat. #7532), and 5 ml of penicillin/streptomycin solution (P/S, Cat. #0503).

Product Use

MODM-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^{o}C, the FBS, MODS and the P/S solution at -20^{o}C. Protect from light.

Shipping

Gel ice.

Prepare for use

Thaw MODS, FBS and P/S solution at 37^{o}C. Gently tilt the MODS 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 MODS, 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 MODM 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^{o}C. When stored in the dark at 4^{o}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 Osteogenic Differentiation

Caution: Cryopreserved cells are very delicate. Thaw the vial in a 37°C water bath 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 Osteogenic Differentiation:

1. Prepare a coated 6-well plate or T-25 flask with poly-l-lysine (2µg/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 50µl of poly-l-lysine (Cat. #0403) to the flask.

Note: Even though some studies suggest that coating tissue culture plastic-ware with vitronectin and collagen I may promote osteogenic differentiation over non-coated plates, the results obtained at ScienCell Research Laboratories indicate that ScienCell's MSC products can easily differentiate into osteoblasts when plastic-vessels coated with poly-L-lysine are used.

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 osteogenic induction.

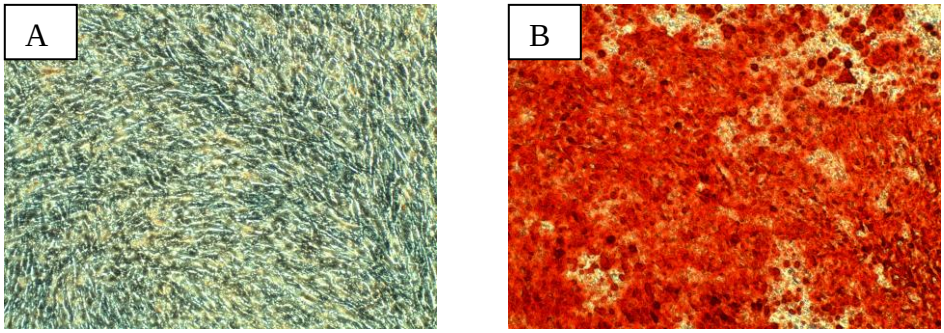
6. When the cells are 100% confluent, carefully replace the MSCM with osteogenic differentiation medium (MODM, Cat. #7531). This medium change counts at differentiation day 1.

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

Note: During the induction of osteogenic differentiation, cells are easily peeled off from the plates by the medium changes. Be extremely gentle and careful with the cells during medium change. The cells are more easily detached when a smaller vessel area is used. Therefore 6 well plates are the smallest multi-well plates recommended for use.

8. After 14-16 days of differentiation, cells can be fixed and stained with Alizarin Red Solution.

Note: after 6-8 days of incubation with osteogenic differentiation medium, dark brown cell multilayers will be observed under microscope (4X). White colonies may also be seen with the naked eye.



A. Human mesenchymal stem cells from bone marrow (HMSC-bm/Cat. #7500) were cultivated in expansion medium (Cat. #7501) for 16 days. Alizarin Red staining was not detected.

B. HMSC-bm cells were cultivated in osteogenic differentiation medium (Cat. #7531) for 16 days. The Alizarin Red staining demonstrates that calcium deposit throughout the culture.