Osteoblast Mineralization Medium (ObMM)

Catalog #4611

Product Description

Osteoblast Mineralization Medium (ObMM), when used with Osteoblast Mineralization Supplement (ObMS, Cat #4672) and 25 ml of fetal bovine serum (FBS) is a complete medium designed for the maturation of normal osteoblasts *in vitro*. It is a sterile, liquid medium which contains essential and non-essential amino acids, vitamins, organic and inorganic compounds, hormones, growth factors, trace minerals and a low concentration of fetal bovine serum (5%). 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. The medium is formulated (quantitatively and qualitatively) to provide an optimally balanced nutritional environment that selectively promotes maturation and mineralization of normal osteoblasts *in vitro*.

Components

ObMM consists of 500 ml of basal medium, 25 ml of FBS (Cat #0025), 5 ml of Osteoblast Mineralization Supplement (ObMS, Cat #4672) and 5 ml of penicillin/streptomycin solution (P/S, Cat #0503). *Note: FBS, ObMS and P/S are not pre-mixed in ObMM; they must be added separately to make the complete ObMM.*

Not Included: Additional Recommended Materials

Cat #	Product Name
0103	0.25% Trypsin/EDTA Solution (T/E)
0113	Trypsin Neutralization Solution (TNS)
0223	Alizarin Red S Staining Kit
0303	Dulbecco's Phosphate-Buffered Saline (DPBS)
0403	1mg/ml Poly-L-Lysine (PLL)
0600	Cell Culture Grade Water
4601	Osteoblast Medium
4600 or 4610	Human Calvarial Osteoblasts or Human Osteoblasts
8678	Alizarin Red S Staining Quantification Assay

Product Use
ObMM 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 and the ObMS, FBS and P/S solution at -20°C. Protect from light.

Shipping
Basal medium: room temperature. Supplements: dry ice.

Instructions for Use
Thaw ObMS, FBS and P/S solution at 37°C. Gently tilt the tubes several times to ensure the contents are completely mixed before adding to the medium. Spray the medium bottle and tubes with 70% ethanol, and wipe to remove excess liquid. In a sterile field, remove the caps without touching the interior threads with fingers. Add ObMS, FBS and P/S solution to the medium and mix well. Since several components are light-labile, the medium should not be exposed to light for extended periods. We do not recommend warming medium in a 37°C water bath prior to use. 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.

Instructions for Osteoblast Mineralization

Set Up of Expansion Culture for Differentiation:

1. Primary Osteoblasts (Cat #4600 or 4610) should be expanded with Osteoblast Medium (ObM; Cat #4601) in vessels which have been coated with poly-l-lysine and placed for at least 1 hour in the 37°C incubator. Rinse the poly-l-lysine twice with sterile water before adding culture medium.
2. Change the medium the next morning after establishing a culture from cryopreserved cells following the protocol listed on Cat# 4600 or 4610. 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 Osteogenic Differentiation:

1. Prepare a coated 6-well plate or T-25 flasks with poly-l-lysine (2µg/cm^2). For a 6-well plate, add 144 µl of 1mg/ml 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 1mg/ml poly-l-lysine (Cat #0403) to the flask.
2. Leave the plate or the flask in the 37°C incubator overnight (or at least 1 hour before using).

3. The next day, aspirate the poly-l-lysine from the wells or flask and rinse the vessels twice with sterile water, aspirating in between washes.
4. Plate the cell suspension in Osteoblast Medium (Cat #4601) 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 osteoblast mineralization.

6. When the cells are 100% confluent, carefully replace the osteoblast medium with osteoblast mineralization medium (ObMM, Cat #4611). This medium change counts as differentiation day 1.

Note: It is recommended to treat a sample with Osteoblast Medium (Cat #4601) as a negative control.

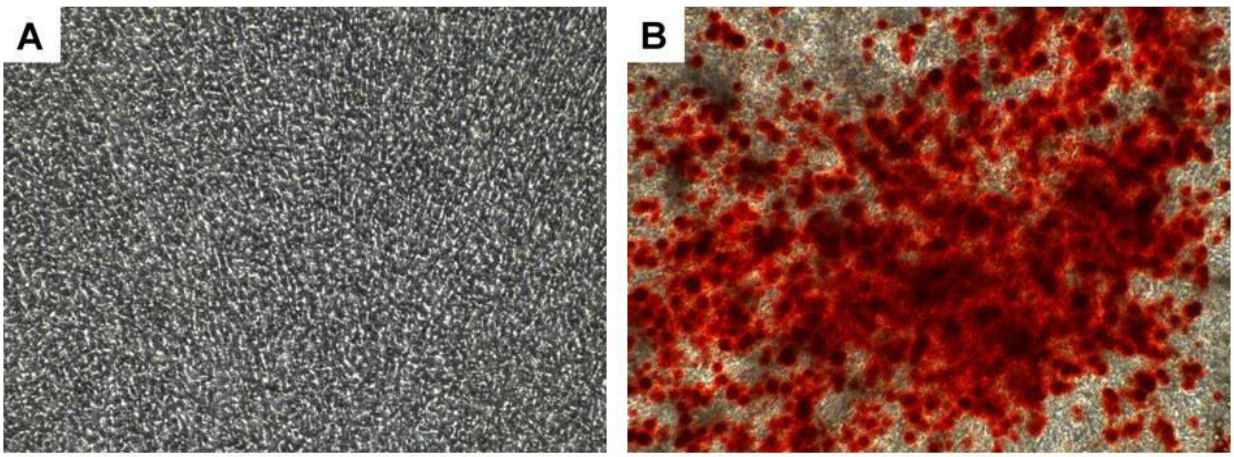
7. Replace the medium with fresh ObMM every 3-4 days.

Note: During the differentiation, cells are easily peeled off from the plates by the medium changes. Be extremely gentle and careful with the cells during medium changes. 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 21 days of differentiation, cells can be fixed and analyzed. For visualization of calcium deposits, use the Alizarin Red S Staining Kit (Cat #0223), or for quantification of calcium deposits, use the Alizarin Red S Staining Quantification Assay (Cat #8678).

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).



A. Human Calvarial Osteoblasts (Cat #4600) were cultivated in Osteoblast Medium (Cat #4601) for 21 days. Alizarin Red staining was not detected.

B. Human Calvarial Osteoblasts (Cat #4600) were cultivated in Osteoblast Mineralization Medium (Cat #4611) for 21 days. The Alizarin Red staining shows calcium deposits throughout the culture.