



StemXVivo™ Human Osteogenic Supplement (20X)

Media Supplement for Human MSC Osteogenesis

Catalog Number: CCM008

Volume: 12.5 mL

PRODUCT DESCRIPTION

Mesenchymal stem cells (MSCs) are functionally defined by their capacity to self renew and their ability to differentiate into multiple cell types including adipocytes, chondrocytes and osteocytes (1, 2). The StemXVivo Human Osteogenic Supplement is a media supplement for the differentiation of human mesenchymal stem cells (MSCs) into osteocytes. All the components have been selected and optimized for human MSC osteogenesis. This product does not contain antibiotics.

INTENDED USE

The StemXVivo Human Osteogenic Supplement is designed to be used with the StemXVivo Osteogenic/Adipogenic Base Media (R&D Systems, Catalog # CCM007) for the desired differentiation application. It may be used with other base media to differentiate MSCs depending on the experimental design of each researcher.

STABILITY & STORAGE

Upon receipt, this supplement should be stored at $\leq -20^{\circ}\text{C}$ in a manual defrost freezer. The supplement can be thawed at $2-8^{\circ}\text{C}$ or at room temperature before use. Thawed supplement can be aliquoted and stored at $\leq -20^{\circ}\text{C}$ in a manual defrost freezer for up to 3 months. Thaw a fresh aliquot for each use. Avoid repeated freeze-thaw cycles.

PRECAUTION

When handling biohazard materials such as human cells, safe laboratory procedures should be followed and protective clothing should be worn.

LIMITATIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The safety and efficacy of this product in diagnostic or other clinical uses has not been established.
- This reagent should not be used beyond the expiration date indicated on the label.
- Results may vary due to variations among MSC/progenitor cells derived from different donors.

REFERENCES

1. Gronthos, S. *et al.* (1995) *Blood* **85**:929.
2. Pittenger, M.F. *et al.* (1999) *Science* **284**:143.

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PROCEDURE FOR THE OSTEOGENIC DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS

This protocol describes the osteogenic differentiation of human MSCs using StemXVivo Osteogenic/Adipogenic Base Media (R&D Systems, Catalog # CCM007) and StemXVivo Human Osteogenic Supplement (R&D Systems, Catalog # CCM008).

Note: This protocol must be read in its entirety before using this product.

OTHER MATERIALS REQUIRED

- Bone marrow-derived MSCs
- StemXVivo Osteogenic/Adipogenic Base Media (R&D Systems, Catalog # CCM007)
- Penicillin-Streptomycin 100X
- 10 cm tissue culture dishes
- 15 mL centrifuge tubes
- Serological pipettes
- Pipettes and pipette tips
- Water bath

REAGENT PREPARATION

StemXVivo Osteogenic/Adipogenic Base Media - Thaw the StemXVivo Osteogenic/Adipogenic Base Media at 2-8 °C or room temperature.

Completed StemXVivo Osteogenic/Adipogenic Base Media - Add Penicillin-Streptomycin to the StemXVivo Osteogenic/Adipogenic Base Media at a 1:100 dilution. **Note:** If Penicillin-Streptomycin is not needed for the experiment, it can be omitted.

Completed StemXVivo Human Osteogenic Differentiation Media - Add StemXVivo Human Osteogenic Supplement to the completed StemXVivo Osteogenic/Adipogenic Base Media at a 1:20 dilution.

PROCEDURE

1. Pre-warm the completed StemXVivo Osteogenic/Adipogenic Base Media in a 37 °C water bath. This procedure uses 10 mL for each 10 cm tissue culture dish used.
2. Resuspend $2.3-2.5 \times 10^5$ MSCs in 10 mL of the pre-warmed completed StemXVivo Osteogenic/Adipogenic Base Media.
Note: If using another size tissue culture vessel, seed cells at approximately 4.2×10^3 cells/cm²/0.2-0.3 mL media.
3. Add this cell suspension to a 10 cm tissue culture dish. The cells should be 50-70% confluent in 1-2 days.
4. At 50-70% confluency, replace the media with 10 mL of pre-warmed completed StemXVivo Osteogenic Differentiation Media to induce osteogenesis.
5. Every 3-4 days remove and discard spent media and replace with 10 mL of pre-warmed completed StemXVivo Human Osteogenic Differentiation Media.
Note: Dispense media down the side of the dish so as not to disrupt cells.
6. After 2-3 weeks osteogenic induced cells will have morphological changes and calcium deposition.

DATA EXAMPLE

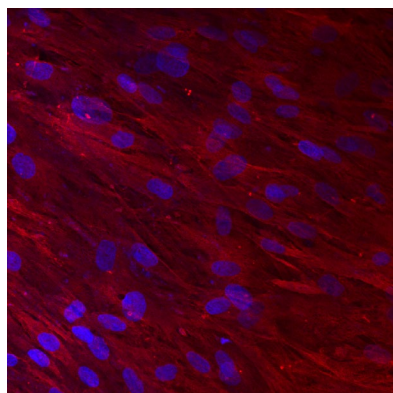


Figure 1: Detection of Osteocalcin in Human MSC-differentiated Osteocytes. Human MSCs were differentiated *in vitro* for 21 days using StemXVivo Osteogenic/Adipogenic Base Media (R&D Systems, Catalog # CCM007) and StemXVivo Human Osteogenic Supplement (R&D Systems, Catalog # CCM008). Osteocyte differentiation was verified using Mouse Anti-Human Osteocalcin Monoclonal Antibody (R&D Systems, Catalog # MAB1419). The cells were stained with NorthernLights™ 557-conjugated Donkey Anti-Mouse Secondary Antibody (R&D Systems, Catalog # NL007; red), and the nuclei were counterstained with DAPI (blue).