StemXVivo® Mouse/Rat Osteogenic Supplement (20X)

Media Supplement for Mouse and Rat MSC Osteogenesis

Catalog Number: CCM009

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® Mouse/Rat Osteogenic Supplement is a media supplement for the differentiation of mouse or rat MSCs into osteocytes. All the components have been selected and optimized for mouse and rat MSC osteogenesis. This product does not contain antibiotics.

INTENDED USE

StemXVivo® Mouse/Rat Osteogenic Supplement is designed to be used with 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 ≤ -20 °C in a manual defrost freezer. The supplement can be thawed at 2-8 °C or at room temperature. Thawed supplement can be aliquoted and stored at ≤ -20 °C in a manual defrost freezer for up to 3 months. Thaw a fresh aliquot with each use. Avoid repeated freeze-thaw cycles.

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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Bio-Techne is a trading name for R&D Systems

PROCEDURE FOR THE OSTEOGENIC DIFFERENTIATION OF MOUSE AND RAT MESENCHYMAL STEM CELLS

The protocol below describes the osteogenic differentiation of mouse and rat MSCs using StemXVivo® Osteogenic/Adipogenic Base Media (R&D Systems®, Catalog # CCM007) and StemXVivo® Osteogenic Supplement (R&D Systems®, Catalog # CCM009).

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
- Pipette 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® Osteogenic Differentiation Media - Add StemXVivo® 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 x 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 x 10^3 cells/cm^2/0.2-0.3 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® 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 EXAMPLES

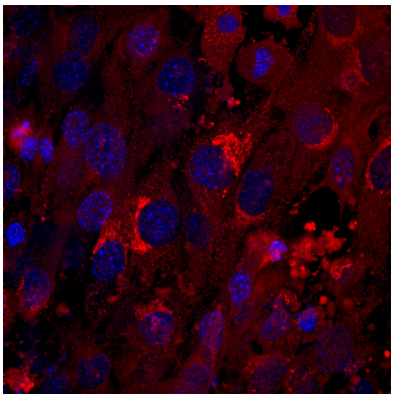

Figure 1. Detection of Osteopontin in Mouse MSC-differentiated Osteocytes. Mouse MSCs were cultured for 21 days using the StemXVivo® Osteogenic/Adipogenic Base Media (R&D Systems®, Catalog # CCM007) and StemXVivo® Mouse/Rat Osteogenic Supplement (R&D Systems®, Catalog # CCM009). Osteocyte differentiation was verified using Goat Anti-Mouse Osteopontin Antigen Affinity-purified Polyclonal Antibody (R&D Systems®, Catalog # AF808). The cells were stained with a NorthernLights™ (NL)557-conjugated Donkey Anti-Goat Secondary Antibody (R&D Systems®, Catalog # NL001; red), and the nuclei were counterstained with DAPI (blue).

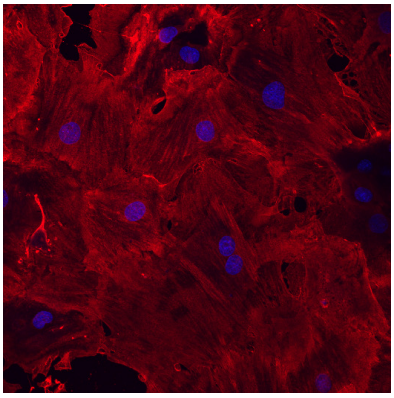

Figure 2: Detection of Osteocalcin in Rat MSC-differentiated Osteocytes. Rat MSCs were cultured for 21 days using the StemXVivo® Osteogenic/Adipogenic Base Media and StemXVivo® Mouse/Rat Osteogenic Supplement. Osteocyte differentiation was verified using Mouse Anti-Human Osteocalcin Monoclonal Antibody (R&D Systems®, Catalog # MAB1419). The cells were stained with a NorthernLights™ (NL)557-conjugated Donkey Anti-Mouse Secondary Antibody (R&D Systems®, Catalog # NL007; red), and the nuclei were counterstained with DAPI (blue).

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