StemXVivo™ Osteogenic/Adipogenic Base Media

Base Media for Human, Mouse, and Rat MSC Osteogenesis and Adipogenesis

Catalog Number: CCM007

Volume: 250 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 Osteogenic/Adipogenic Base Media is used for the differentiation of mesenchymal stem cells (MSCs) into osteocytes or adipocytes. All the components, including fetal bovine serum, have been selected and optimized for human, mouse, and rat MSC osteogenesis and adipogenesis. This product is supplemented with sodium bicarbonate but does not contain antibiotics.

INTENDED USE

The StemXVivo Osteogenic/Adipogenic Base Media is designed for use with the StemXVivo Osteogenic Supplement (R&D Systems, Catalog # CCM008 or CCM009) or the StemXVivo Adipogenic Supplement (R&D Systems, Catalog # CCM011) for the desired differentiation application. It may be used with other cytokine/growth factor supplements. The cytokine/growth factor combinations used to differentiate MSCs depend on the experimental design of each researcher.

Note: *Cytokines and growth factors can be obtained from R&D Systems (www.RnDSystems.com/Proteins).*

STABILITY & STORAGE

Upon receipt, this media should be stored at ≤ -20 °C in a manual defrost freezer. The media can be thawed at 2-8 °C or at room temperature. Thawed media can be aliquoted and stored at ≤ -20 °C in a manual defrost freezer for up to 3 months or used within 1 month when stored in the dark at 2-8 °C. Avoid repeated freeze-thaw cycles.

PRECAUTION

When handling biohazardous 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.

PROCEDURE FOR THE CHONDROGENIC DIFFERENTIATION OF HUMAN/MOUSE/RAT MESENCHYMAL STEM CELLS

This protocol describes the osteogenic and adipogenic differentiation of human, mouse, and rat MSCs using StemXVivo Osteogenic/Adipogenic Base Media (R&D Systems, Catalog # CCM007). For use with StemXVivo Osteogenic Supplements (R&D Systems, Catalog # CCM008 and CCM009) and StemXVivo Adipogenic Supplement (R&D Systems, Catalog # CCM011).

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

OTHER MATERIALS REQUIRED

- Bone marrow-derived MSCs
- StemXVivo Human Osteogenic Supplement (R&D Systems, Catalog # CCM008), StemXVivo Mouse/Rat Osteogenic Supplement (R&D Systems, Catalog # CCM009), or StemXVivo Human/Mouse/Rat Adipogenic Supplement (R&D Systems, Catalog # CCM011)
- Penicillin-Streptomycin 100X
- 10 cm tissue culture dishes
- Serological pipettes
- Pipettes and pipette tips
- 37 °C and 5% CO_2 humidified incubator
- Centrifuge
- Hemocytometer
- Water bath

REAGENT PREPARATION

Note: *The reagent and material preparation for completed StemXVivo Osteogenic and Adipogenic Differentiation Media is the same using either the human, mouse, or rat Supplement.*

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.

Completed StemXVivo Adipogenic Differentiation Media - **Note:** *If a precipitate forms, warm the Adipogenic Supplement vial in a 37 °C water bath for 5 minutes. Vortex until the precipitate dissolves.* Add StemXVivo Adipogenic Supplement to the completed StemXVivo Osteogenic/Adipogenic Base Media at a 1:100 dilution.

PROCEDURE FOR OSTEOGENIC DIFFERENTIATION

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.

PROCEDURE FOR ADIPOGENIC DIFFERENTIATION

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 1 x 10^6 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 2.1 x 10^4 cells/cm^2.*
3. Add this cell suspension to a 10 cm tissue culture dish. Incubate overnight in a 37 °C and 5% CO_2 incubator. Cells should be 100% confluent after overnight incubation. If they are not confluent, replace media every 2-3 days with Osteogenic/Adipogenic Base Media until 100% confluency is reached.
4. At 100% confluency, replace the media with 10 mL of pre-warmed completed StemXVivo Adipogenic Differentiation Media to induce adipogenesis.
5. Every 3-4 days remove and discard spent media and replace with 10 mL of freshly prepared, pre-warmed completed StemXVivo Adipogenic Differentiation Media.
6. Differentiation is complete after 7-21 days, at which time adipogenic induced cells will have morphological changes and lipid vacuoles.

DATA EXAMPLES

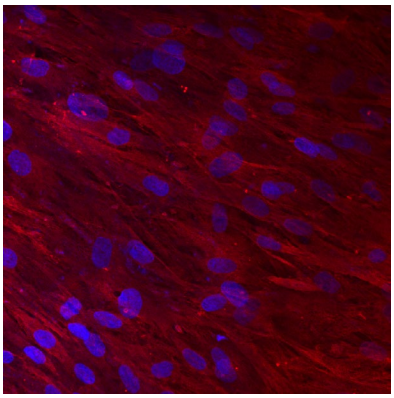

Figure 1: Detection of Osteocalcin in Human MSC-differentiated Osteocytes. Human MSCs were differentiated *in vitro* for 21 days using StemXVivo Osteogenic/Adipogenic Base Media (R&D Systems, Catalog #CCM007) and StemXVivo Human Osteogenic Supplement (R&D Systems, Catalog # CCM008). Osteocyte differentiation 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).

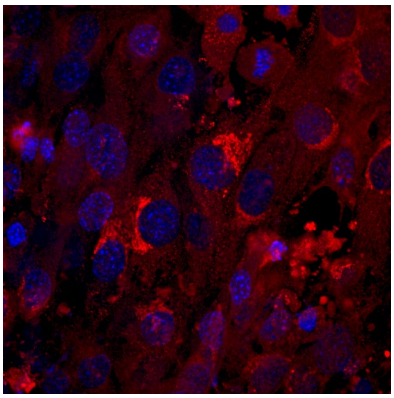

Figure 2: Detection of Osteopontin in Mouse MSC-differentiated Osteocytes. Mouse MSCs were cultured for 21 days using the StemXVivo Osteogenic/Adipogenic Base Media and StemXVivo Mouse/Rat Osteogenic Supplement (R&D Systems, Catalog # CCM009). Osteocyte differentiation was verified using Goat Anti-Mouse Osteopontin Antigen Affinity-purified Polyclonal Antibody (R&D Systems, Catalog # AF808). The cells were stained with a NorthernLights 557-conjugated Donkey Anti-Goat Secondary Antibody (R&D Systems, Catalog # NL001; red), and the nuclei were counterstained with DAPI (blue).

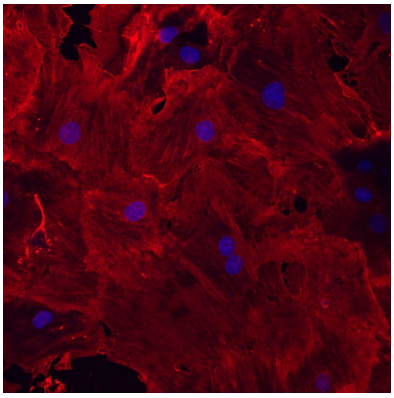

Figure 3: Detection of Osteocalcin in Rat MSC-differentiated Osteocytes. Rat MSCs were cultured for 21 days using the StemXVivo Osteogenic/Adipogenic Base Media and StemXVivo Mouse/Rat Osteogenic Supplement. Osteocyte differentiation was verified using Mouse Anti-Human Osteocalcin Monoclonal Antibody (R&D Systems, Catalog # MAB1419). The cells were stained with a NorthernLights 557-conjugated Donkey Anti-Mouse Secondary Antibody (R&D Systems, Catalog # NL007; red), and the nuclei were counterstained with DAPI (blue).

DATA EXAMPLES *CONTINUED*

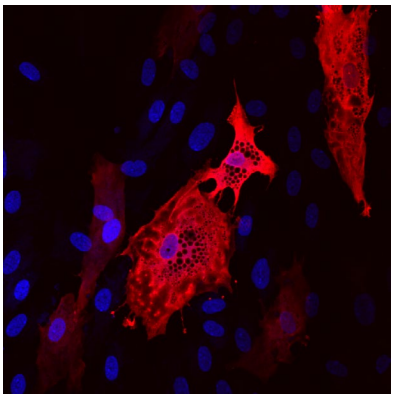

Figure 4: Detection of FABP4 in Human MSC-differentiated Adipocytes.
Human MSCs were differentiated for 21 days using the StemXVivo Osteogenic/Adipogenic Base Media and StemXVivo Adipogenic Supplement. Mature differentiated adipocytes were detected with Goat Anti-Human FABP4 Antigen Affinity-purified Polyclonal Antibody (R&D Systems, Catalog # AF3150). The cells were stained with NorthernLights 557-conjugated Donkey Anti-Goat Secondary Antibody (R&D Systems, Catalog # NL001; red), and the nuclei were counterstained with DAPI (blue).

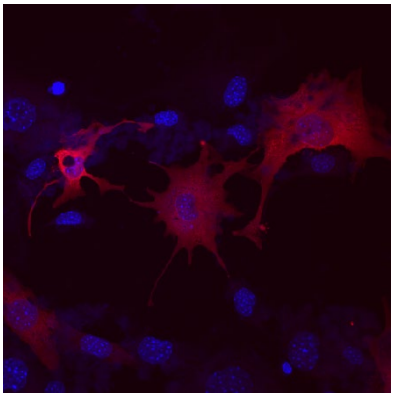

Figure 5: Detection of FABP4 in Mouse MSC-differentiated Adipocytes.
Mouse MSCs were differentiated for 21 days using the StemXVivo Osteogenic/Adipogenic Base Media (R&D Systems, Catalog #CCM007) and StemXVivo Adipogenic Supplement (R&D Systems, Catalog # CCM011). Mature differentiated adipoctyes were detected with a Goat Anti-Mouse FABP4 Antigen Affinity-purified Polyclonal Antibody (R&D Systems, Catalog # AF1443). The cells were stained with a NorthernLights 557-conjugated Donkey Anti-Goat Secondary Antibody, and the nuclei were counterstained with DAPI (blue).

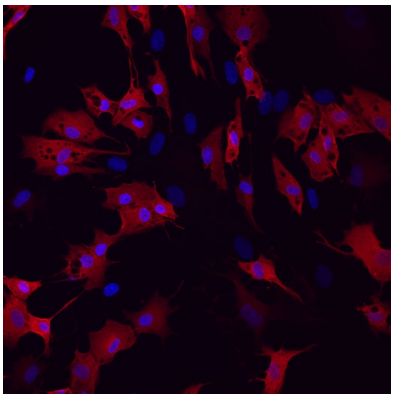

Figure 6: Detection of FABP4 in Rat MSC-differentiated Adipocytes.
Rat MSCs were differentiated for 21 days using the StemXVivo Osteogenic/Adipogenic Base Media and StemXVivo Adipogenic Supplement. Mature differentiated adipoctyes were detected with Goat Anti-Mouse FABP4 Antigen Affinity-purified Polyclonal Antibody. The cells were stained with NorthernLights 557-conjugated Donkey Anti-Goat Secondary Antibody, and the nuclei were counterstained with DAPI (blue).