

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® Adipogenic Supplement is a media supplement for the differentiation of human, mouse, or rat mesenchymal stem cells (MSCs) into adipocytes. All the components have been selected and optimized for MSC adipogenesis. This product does not contain antibiotics.

INTENDED USE

The StemXVivo® Adipogenic Supplement is designed for use 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 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.

PRECAUTIONS

This product contains 95% ethanol and is highly flammable. Keep the container tightly closed, and keep it away from sources of ignition.

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 ADIPOGENIC DIFFERENTIATION OF HUMAN, MOUSE, AND RAT MESENCHYMAL STEM CELLS

This protocol describes the adipogenic differentiation of human, mouse, and rat MSCs using StemXVivo® Osteogenic/Adipogenic Base Media (R&D Systems, Catalog # CCM007), 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® 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
- 37°C and 5% CO₂ humidified incubator
- Centrifuge
- Hemocytometer
- Inverted Microscope
- 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® Adipogenic Differentiation Media - Add StemXVivo® Adipogenic Supplement to the completed StemXVivo® Osteogenic/Adipogenic Base Media at a 1:100 dilution. **Note:** *If a precipitate forms, warm the Adipogenic Supplement vial in a 37°C water bath for 5 minutes. Vortex until the precipitate dissolves.*

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 1×10^6 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 2.1×10^4 cells/cm².*
3. Add this cell suspension to a 10 cm tissue culture dish. Incubate overnight in a 37°C and 5% CO₂ incubator. Cells should be 100% confluent after overnight incubation. If they are not confluent, replace medium every 2-3 days with StemXVivo® 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

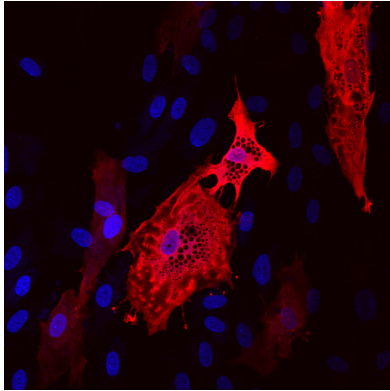


Figure 1: Detection of FABP4 in Human MSC-differentiated Adipocytes.

Human 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 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).

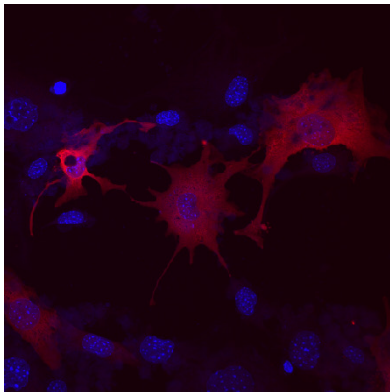


Figure 2. Detection of FABP4 in Mouse MSC-differentiated Adipocytes.

Mouse 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-Mouse FABP4 Antigen Affinity-purified Polyclonal Antibody (R&D Systems, Catalog # AF1443). The cells were stained with NorthernLights™ 557-conjugated Donkey Anti-Goat Secondary Antibody, and the nuclei were counterstained with DAPI (blue).

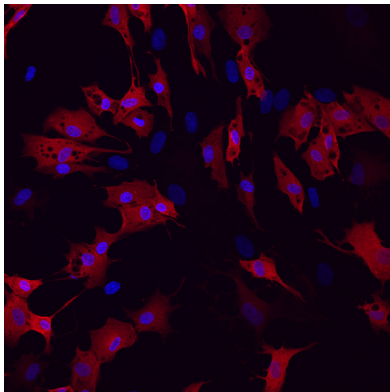


Figure 3. Detection of FABP4 in Rat MSC-differentiated Adipocytes.

Rat MSCs were differentiated for 21 days using the StemXVivo® Osteogenic/Adipogenic Base Media and StemXVivo® Adipogenic Supplement. Mature differentiated adipocytes were detected with Goat Anti-Mouse FABP4 Antigen Affinity-purified Polyclonal Antibody (R&D Systems, Catalog # AF1443). The cells were stained with NorthernLights™ 557-conjugated Donkey Anti-Goat Secondary Antibody, and the nuclei were counterstained with DAPI (blue).

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