R&D SYSTEMS
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StemXVivo® Human/Mouse Chondrogenic Supplement 100X

Media Supplement for Human and Mouse MSC Chondrogenesis

Catalog Number: CCM006

Volume: 0.5 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/Mouse Chondrogenic Supplement is a media supplement for the differentiation of MSCs into chondrocytes. All the components have been selected and optimized for human and mouse MSC chondrogenesis. This product does not contain antibiotics.

INTENDED USE

The StemXVivo® Human/Mouse Chondrogenic Supplement is designed to be used with the StemXVivo® Chondrogenic Base Media (R&D Systems®, Catalog # CCM005) 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 for each use. Avoid repeated freeze-thaw cycles.

PRECAUTION

When handling bio-hazardous 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 CHONDROGENIC DIFFERENTIATION OF HUMAN AND MOUSE MESENCHYMAL STEM CELLS

The protocol below describes the chondrogenic differentiation of human and mouse MSCs using StemXVivo® Chondrogenic Base Media (R&D Systems®, Catalog # CCM005) and StemXVivo® Human/Mouse Chondrogenic Supplement (R&D Systems®, Catalog # CCM006).

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

OTHER MATERIALS REQUIRED

- Bone marrow-derived MSCs
- StemXVivo® Chondrogenic Base Media (R&D Systems®, Catalog # CCM005)
- Penicillin-Streptomycin (100X)
- 15 mL centrifuge tubes
- Serological pipettes
- Pipettes and pipette tips
- 37 °C and 5% CO_2 humidified incubator
- Centrifuge
- Hemocytometer
- Water bath

REAGENT PREPARATION

StemXVivo® Chondrogenic Base Media - Thaw the Chondrogenic Base Media at 2-8 °C or room temperature.

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

Completed StemXVivo® Chondrogenic Differentiation Media - Add StemXVivo® Chondrogenic Supplement to the completed StemXVivo® Chondrogenic Base Media at a 1:100 dilution. **Note:** *This procedure will use 0.5 mL of completed StemXVivo® Chondrogenic Differentiation Media for each 15 mL conical tube.*

PROCEDURE

1. Pre-warm 5 mL of the completed StemXVivo® Chondrogenic Base Media and 0.5 mL of the completed StemXVivo® Chondrogenic Differentiation Media in a 37 °C water bath.
2. Resuspend 2.5 x 10^5 human MSCs or 1.25 x 10^5 mouse MSCs in 5 mL of the pre-warmed completed StemXVivo® Chondrogenic Base Media.
3. Centrifuge the cells at 200 x g for 5 minutes at room temperature. Remove the media, and resuspend the cells with 0.5 mL of pre-warmed completed StemXVivo® Chondrogenic Differentiation Media.
4. Centrifuge the cells at 200 x g for 5 minutes at room temperature. Do not remove medium. Loosen the cap of the tube to allow gas exchange, and incubate upright at 37 °C and 5% CO_2.
5. After 1-2 days the cell pellet will form a round ball approximately 1-2 mm in diameter. This pellet will remain about the same size for the entire culturing time (see Figure 1).
6. Every 2-3 days remove and discard the spent media and replace with 0.5 mL of pre-warmed completed StemXVivo® Chondrogenic Differentiation Media. **Note:** *Use caution when removing the media to avoid aspirating the pellet.*
7. Chondrogenic pellets can be harvested after 14-28 days in culture and used for desired analysis.

DATA EXAMPLES

Morphology of differentiated MSCs cultured with StemXVivo® Chondrogenic Base Media with StemXVivo® Chondrogenic Supplement.

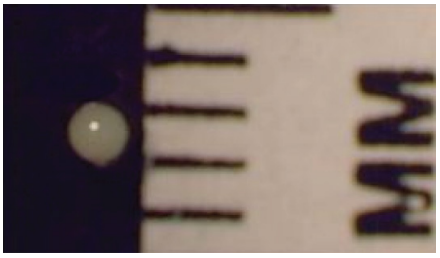

Figure 1: Human MSCs cultured with StemXVivo® Chondrogenic Base Media (R&D Systems®, Catalog # CCM005) and StemXVivo® Human/Mouse Chondrogenic Supplement (R&D Systems®, Catalog # CCM006) formed a chondrogenic pellet (ball) imaged here at day 21 of culture.

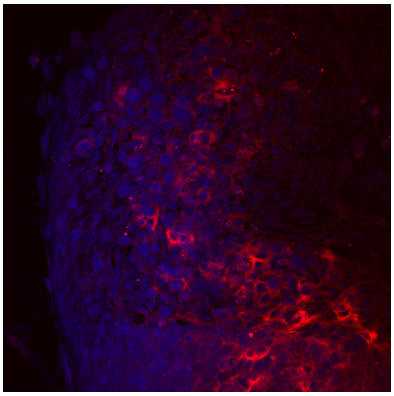

Figure 2: Detection of Aggrecan in a Human MSC-differentiated Chondrogenic Pellet Section. Human MSCs were cultured with StemXVivo® Chondrogenic Base Media and StemXVivo® Human/Mouse Chondrogenic Supplement, and the resulting chondrogenic pellet was cryosectioned. Chondrocyte differentiation was verified using Goat Anti human Aggrecan Antigen Affinity-purified Polyclonal Antibody (R&D Systems®, Catalog # AF1220). The cells were stained using NorthernLights™ 557-conjugated Donkey Anti-goat Secondary Antibody (R&D Systems®, Catalog # NL001; red), and the nuclei were counterstained with DAPI (blue).

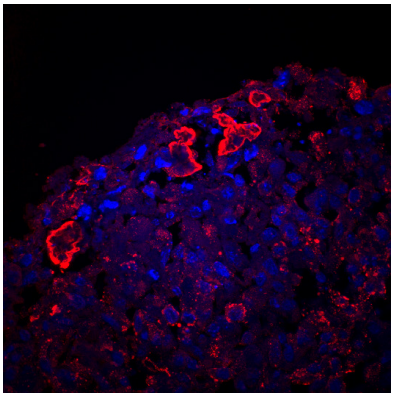

Figure 3: Detection of Collagen II in a Mouse MSC-differentiated Chondrogenic Pellet Section. Mouse MSCs were cultured with StemXVivo® Chondrogenic Base Media and StemXVivo® Human/Mouse Chondrogenic Supplement, and the resulting chondrogenic pellet was cryosectioned. Chondrocyte differentiation was verified using Sheep Anti mouse Collagen II Antigen Affinity-purified Polyclonal Antibody (R&D Systems®, Catalog # AF3615). The cells were stained using NorthernLights™ 557-conjugated Donkey Anti-sheep Secondary Antibody (R&D Systems®, Catalog # NL010; red), and the nuclei were counterstained with DAPI (blue).