StemXVivo® Chondrogenic Base Media

Base Media for Human, Mouse, and Rat MSC Chondrogenesis

Catalog Number: CCM005

Volume: 50 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® Chondrogenic Base Media is a base media for the differentiation of MSCs into chondrocytes. All the components have been selected and optimized for human, mouse, and rat MSC chondrogenesis. This product is supplemented with sodium bicarbonate but does not contain antibiotics.

INTENDED USE

The StemXVivo Chondrogenic Base Media is designed for use with StemXVivo Chondrogenic Supplements (R&D Systems®, Catalog # CCM006 or CCM020) for the desired differentiation application. It may also 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.

PRECAUTIONS

The human origin-derived components used in this product were purified from donor plasma and tested at the donor level using an FDA licensed method and found to be non-reactive for anti-HIV-1/2 and Hepatitis B surface antigen.

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.

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

The protocol below describes the chondrogenic differentiation of human, mouse, and rat MSCs using StemXVivo® Chondrogenic Base Media (R&D Systems®, Catalog # CCM005) and StemXVivo Human/Mouse Chondrogenic Supplement (R&D Systems, Catalog # CCM006) or StemXVivo Rat Chondrogenic Supplement (R&D Systems, Catalog # CCM020).

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

OTHER MATERIALS REQUIRED

- Bone marrow-derived MSCs
- StemXVivo® Human/Mouse Chondrogenic Supplement (R&D Systems, Catalog # CCM006) or StemXVivo Rat Chondrogenic Supplement (R&D Systems, Catalog # CCM020).
- 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 StemXVivo 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 Chondrogenic Differentiation Media for each 15 mL conical tube.*

PROCEDURE

1. Pre-warm 5.0 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 MSCs in 5.0 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.

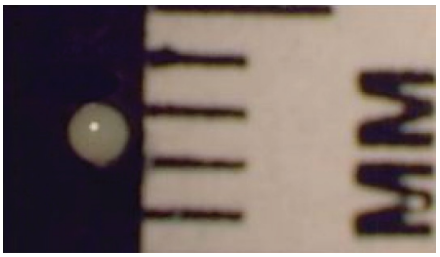

Figure 1: Human MSCs cultured with StemXVivo Chondrogenic Base Media (R&D Systems®, Catalog # CCM005) and StemXVivo Human/Mouse Chondrogenic Supplement (R&D Systems, Catalog # CCM006) formed a chondrogenic pellet (ball) imaged here at day 21 of culture.

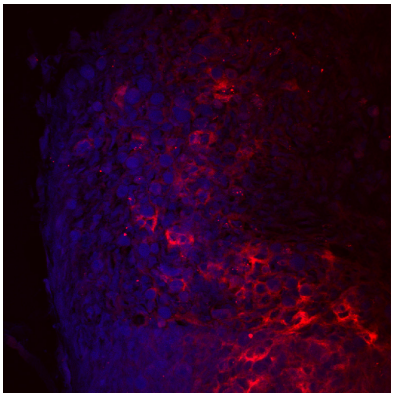

Figure 2: Detection of Aggrecan in a Human MSC-differentiated Chondrogenic Pellet Section. Human MSCs were cultured with StemXVivo Chondrogenic Base Media and StemXVivo Human/Mouse Chondrogenic Supplement, and the resulting chondrogenic pellet was cryosectioned. Chondrocyte differentiation was verified 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).

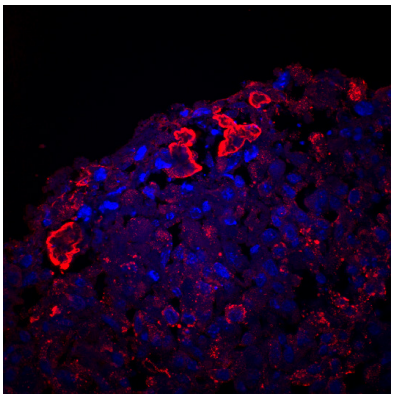

Figure 3: Detection of Collagen II in a Mouse MSC-differentiated Chondrogenic Pellet Section. Mouse MSCs were cultured with StemXVivo Chondrogenic Base Media and StemXVivo Human/Mouse Chondrogenic Supplement, and the resulting chondrogenic pellet was cryosectioned. Chondrocyte differentiation was verified 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).

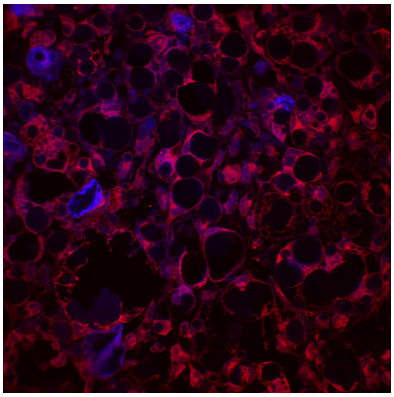

Figure 4: Detection of Aggrecan in a Rat MSC-differentiated Chondrogenic Pellet Section. Rat MSCs were cultured with StemXVivo Chondrogenic Base Media and StemXVivo Rat Chondrogenic Supplement, and the resulting chondrogenic pellet was cryosectioned. Chondrocyte differentiation was verified using Goat 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).