StemXVivo™ Xeno-Free Human MSC Expansion Media

Media for Human Mesenchymal Stem Cell Expansion

Catalog Number: CCM021

Volume: 500 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). MSCs are also phenotypically characterized as $CD73^+$, $CD105^+$, $CD90^+$, and $CD45^-$. The StemXVivo Xeno-Free Human MSC Expansion Media is a complete media formulated and optimized for the maintenance and expansion of purified human MSCs. This product does not contain antibiotics.

INTENDED USE

StemXVivo Xeno-Free Human MSC Expansion Media is ready to use or it may be used with additional cytokine/growth factor supplements for the desired cell culture application. MSCs grown under xeno-free conditions should be grown on extracellular matrix (ECM) protein-coated plates. Types and amounts of ECM protein are dependent on the experimental design of each individual investigator.

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

STABILITY & STORAGE

Upon receipt, store the media at ≤ -20 °C in a manual defrost freezer. Thaw 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

This product contains components derived from human plasma, which has been tested and found negative for antibodies to HIV-1/2, hepatitis B surface antigen (HBsAg), and hepatitis C virus (HCV). However, the media should be handled as if potentially infectious. Safe laboratory procedures should be followed and protective clothing should be worn when handling this media. The acute and chronic effects of over-exposure to this media are unknown.

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 cells derived from different donors.

REFERENCES

1. Pittenger, M.F. *et al.* (1999) Science **284**: 143.
2. Dominici, M. *et al.* (2006) Ann. N.Y. Acad. Sci. **677**: 167.

OTHER MATERIALS REQUIRED

- Recombinant Human Fibronectin, ACFP (R&D Systems, Catalog # ACFP4305)
- Penicillin-Streptomycin (100X)
- TrypLE™ Express (Invitrogen® or equivalent)
- Phosphate-Buffered Saline (PBS)
- Bone marrow-derived MSCs
- 75 cm^2 tissue culture flasks
- 15 mL centrifuge tubes
- Serological pipettes
- Pipettes and pipette tips
- 37 °C and 5% CO_2 humidified incubator
- Centrifuge
- Hemocytometer
- Inverted microscope
- 37 °C water bath

REAGENT PREPARATION

Note: *Sterile technique is required when handling the reagents.*

StemXVivo Xeno-Free Human MSC Expansion Media - Thaw the StemXVivo Xeno-Free Human MSC Expansion Media at 2-8 °C or room temperature.

Note: *If needed, Penicillin-Streptomycin can be added to StemXVivo Xeno-Free Human MSC Expansion Media at a 1:100 dilution.*

PROCEDURE FOR THE XENO-FREE EXPANSION OF MESENCHYMAL STEM CELLS

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

Culturing of Mesenchymal Stem Cells

1. Before plating the cells, coat plates with Recombinant Human Fibronectin. Gently dilute the Fibronectin in PBS to a final concentration of 5 µg/mL. Coat the required number of T75 flasks by adding 6 mL of Fibronectin solution to each flask, and incubate for 3 hours at room temperature or overnight at 2-8 °C.

   Note: *MSCs grown in StemXVivo Xeno-Free MSC Expansion Media must be on extracellular matrix (ECM) protein-coated plates. Types and amounts of ECM protein are dependent on the experimental design of each individual investigator.*

2. Pre-warm the required amount of StemXVivo Xeno-Free Human MSC Expansion Media to room temperature. This procedure uses 20 mL for each T75 flask used.

3. Resuspend 4.5-5.0 x 10^5 cells in 20 mL of the pre-warmed media.

   Note: *If using a different size tissue culture vessel, seed cells at approximately 6000 cells/cm^2/0.2-0.3 mL of media.*

4. Gently pipette and remove the Fibronectin solution from the flasks. Slowly add the cell suspension to the T75 flask(s). Be careful to avoid scraping the coated surface.

5. Incubate the cells at 37 °C and 5% CO_2 in a humidified incubator. Every 2-3 days remove and discard spent media and replace with 20 mL of fresh, pre-warmed StemXVivo Xeno-Free Human MSC Expansion Media.

   Note: *Dispense media down the side of the flask so as not to disrupt cells.*

6. Subculture when cells become 80-90% confluent. Do not let the cultures become 100% confluent.

PROCEDURE *CONTINUED*

Subculture of Mesenchymal Stem Cells

1. Pre-coat the plates with 5 µg/mL of Recombinant Human Fibronectin in PBS for 3 hours at room temperature or overnight at 2-8 °C.
2. Pre-warm 30 mL of StemXVivo Xeno-Free Human MSC Expansion Media and 2 mL of TrypLE Express to room temperature for each T75 flask used.
3. Remove and discard the media from the flask(s).
4. Wash the cells once with 10 mL of PBS for each T75 flask.

   Note: *Do not dispense the PBS directly onto the cells during washing so as not to disrupt the cells.*
5. Add enough TrypLE Express to just cover the cells. Gently rock the flasks to disperse the TrypLE Express evenly over the cells; 2 mL for each T75 flask.
6. Incubate the flasks at 37 °C, monitoring periodically for cell detachment by observing the cells under the microscope. Cells will start to round and detach. Tap the side of the flask to aid the detachment of the cells.
7. Add 5 mL of StemXVivo Xeno-Free Human MSC Expansion Media to each flask. Disperse the cells by pipetting the media over the entire growing surface of the flask.
8. Transfer the cells to a 15 mL conical tube and centrifuge at 400 x g for 5 minutes. Aspirate off the liquid.
9. Resuspend the cell pellet in a small amount of pre-warmed StemXVivo Xeno-Free Human MSC Expansion Media and count the cells with a hemocytometer.
10. Resuspend 4.5-5.0 x 10^5 cells into 20 mL of the pre-warmed StemXVivo Xeno-Free Human MSC Expansion Media for each Fibronectin-coated T75 flask.
11. Gently pipette and remove the Fibronectin solution from the flask, and slowly add the cell suspension to a T75 flask. Be careful to avoid scraping the coated surface. Incubate the cells at 37 °C and 5% CO_2 in a humidified incubator.

DATA EXAMPLES

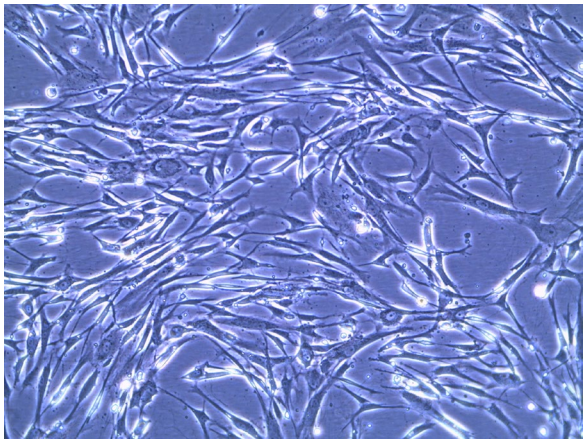

Figure 1: Morphology of MSCs Cultured in StemXVivo Xeno-Free Human MSC Expansion Media. Bone marrow-derived human MSCs were cultured in StemXVivo Xeno-Free Human MSC Expansion Media for 4 passages and then imaged using brightfield microscopy.

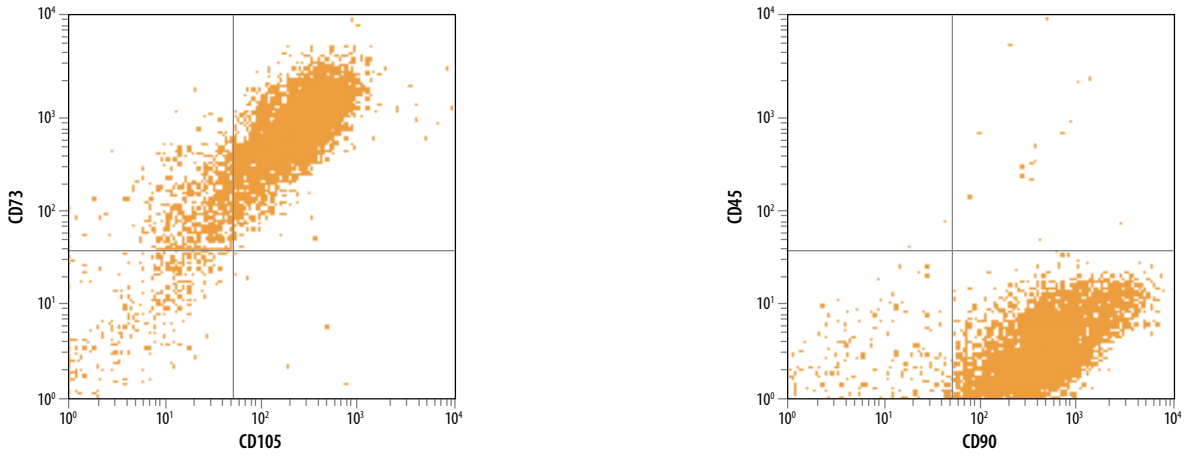

Figure 2: Expression of Mesenchymal Stem Cell (MSC) Markers Expanded in StemXVivo Xeno-Free Human MSC Expansion Media. Human bone marrow-derived MSCs were expanded for 4 passages in StemXVivo Xeno-Free Human MSC Expansion Media. The cells were harvested and stained for expression of positive and negative MSC markers. Positive MSC markers were detected with APC-conjugated Mouse Anti-Human Endoglin/CD105 Monoclonal Antibody (Clone 166707; R&D Systems, Catalog # FAB10971A), PE-conjugated Mouse Anti-Human 5′-Nucleotidase/CD73 Monoclonal Antibody (Clone 606112; R&D Systems, Catalog # FAB5795P), and APC-conjugated Mouse Anti-Human CD90/Thy1 Monoclonal Antibody (Clone Thy-1A1; R&D Systems, Catalog # FAB2067A). Expression of the negative MSC marker, CD45 was detected with PE-conjugated Mouse Anti-Human CD45 Monoclonal Antibody (Clone 2D1; R&D Systems, Catalog # FAB1430P). Quadrants were set based on isotype controls.

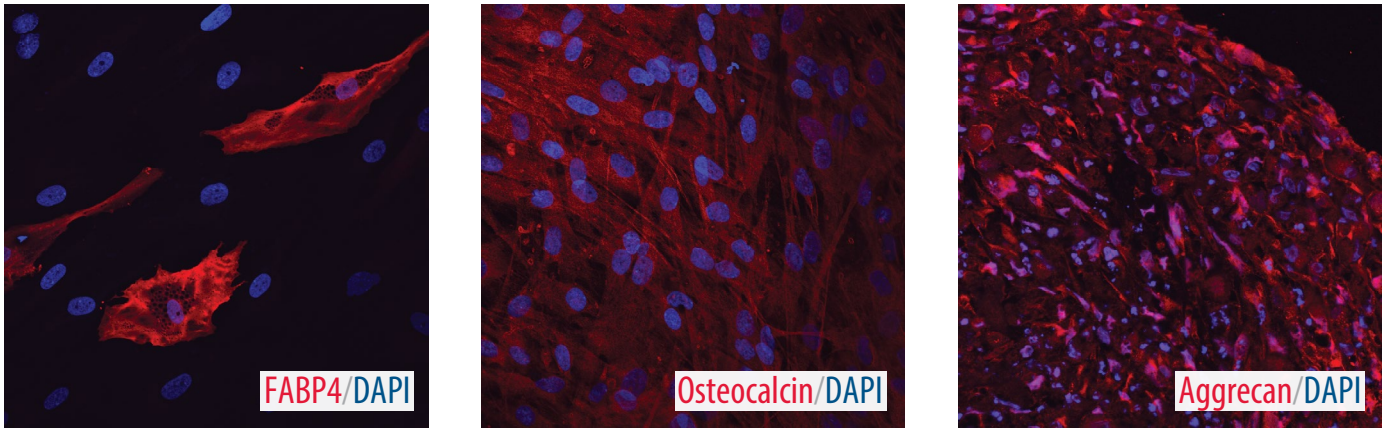

Figure 3: Differentiation of Human MSCs Expanded in StemXVivo Xeno-Free Human MSC Expansion Media. MSCs grown in StemXVivo Xeno-Free Human MSC Expansion Media were differentiated using the Human Mesenchymal Stem Cell Functional Identification Kit (R&D Systems, Catalog # SC006). Adipocytes were stained with Goat Anti-Mouse FABP4 Affinity Purified Polyclonal Antibody (R&D Systems, Catalog # AF1443), osteocytes were stained with Mouse Anti-Human Osteocalcin Monoclonal Antibody (R&D Systems, Catalog # MAB1419), and chondrocytes were stained with Goat Anti-Human Aggrecan Affinity-purified Polyclonal Antibody (R&D Systems, Catalog # AF1220). The cells were then stained with NorthernLights™ 557-conjugated secondary antibodies (R&D Systems, Catalog # NL001 and NL007; red), and the nuclei were counterstained with DAPI (blue).