StemXVivo™ Serum-Free Human MSC Expansion Media

Catalog Number: CCM014

Volume: 500 mL

PRODUCT DESCRIPTION

StemXVivo Serum-Free Human MSC Expansion Media is a complete media formulated and optimized for the maintenance and expansion of purified human mesenchymal stem cells (MSCs). This product does not contain antibiotics.

INTENDED USE

StemXVivo Serum-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. Mesenchymal stem cells 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. Alternatively, 5 µg/mL of Recombinant Human Fibronectin (R&D Systems, Catalog # 4305-FN) can be used.

STABILITY & STORAGE

StemXVivo Serum-Free Human MSC Expansion Media is stable until the expiration date when stored at ≤ -20 °C in a manual defrost freezer. Thawed media can be aliquoted and stored at ≤ -20 °C in a manual defrost freezer for up to 3 months or used within one month when stored in the dark at 2-8 °C. Avoid repeated freeze-thaw cycles.

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 human MSC/progenitor cells derived from different donors.

PRECAUTION

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 handing this media. The acute and chronic effects of over-exposure to this media are unknown.

OTHER SUPPLIES REQUIRED

- Bone marrow-derived human MSCs
- Recombinant Human Fibronectin (R&D Systems, Catalog # 4305-FN)
- Penicillin-Streptomycin (100X), optional
- TrypLE™ Express, or equivalent
- Phosphate-Buffered Saline (PBS)
- 75 cm^2 tissue culture flasks
- 15 mL centrifuge tubes
- Serological pipettes
- Pipettes and pipette tips
- 37° C, 5% CO_2 humidified incubator
- Centrifuge
- Hemocytometer
- Inverted Microscope
- Water bath

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REAGENT & MEDIA PREPARATION

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

StemXVivo Serum-Free Human Mesenchymal Stem Cell (MSC) Expansion Media - Thaw the StemXVivo Serum-Free Human MSC Expansion Media at 2-8 °C or room temperature.

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

PROCEDURE

Culturing 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 T75 flask by adding 6 mL of Fibronectin solution to the flask, and incubate overnight at 2-8 °C or for 3 hours at room temperature.
2. Pre-warm the required amount of StemXVivo Serum-Free Human MSC Expansion Media at 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 off the Fibronectin solution from the flask. Slowly add the cell suspension to a T75 flask. Be careful to avoid scraping the coated surface.
5. Incubate the cells at 37 °C and 5% CO_2 in a humidified atmosphere. Every 2-3 days remove and discard spent media and replace with 20 mL of pre-warmed StemXVivo Serum-Free Human MSC Expansion Media.

   Note: *Dispense media down the side of the flask so as not to disrupt cells.*
6. Subculture when the cells become 80-90% confluent. Do not let the cultures become totally confluent.

Subculturing of Mesenchymal Stem Cells

1. Pre-coat the plates with 5 µg/mL of recombinant human Fibronectin in PBS overnight at 2-8 °C or for 3 hours at room temperature.
2. At room temperature, pre-warm 30 mL of StemXVivo Serum-Free Human MSC Expansion Media and 3 mL of TrypLE Express for each T75 flask used.
3. Remove and discard the media from the flasks. 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.*
4. Add enough TrypLE Express to just cover the cells (3 mL for each T75 flask). Gently rock the flask to disperse the TrypLE Express evenly over the cells.
5. Incubate the flask at 37 °C, monitoring periodically for cell detachment by observing the cells under the microscope. The cells will start to round and detach. Tap the side of the flask to aid the detachment of the cells.
6. Add 5 mL of StemXVivo Serum-Free Human MSC Expansion Media to the flask. Disperse the cells by pipetting the media over the entire growing surface of the flask.
7. Transfer the cells to a 15 mL conical tube and centrifuge at 400 x g for 5 minutes. Aspirate off the liquid.
8. Resuspend the cell pellet in a small amount of pre-warmed StemXVivo Serum-Free Human MSC Expansion Media and count the cells with a hemocytometer.
9. Resuspend 4.5-5.0 x 10^5 cells into 20 mL of the pre-warmed StemXVivo Serum-Free Human MSC Expansion Media for each Fibronectin coated T75 flask.
10. Gently pipette off the Fibronectin solution from each 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 atmosphere.

DATA EXAMPLES

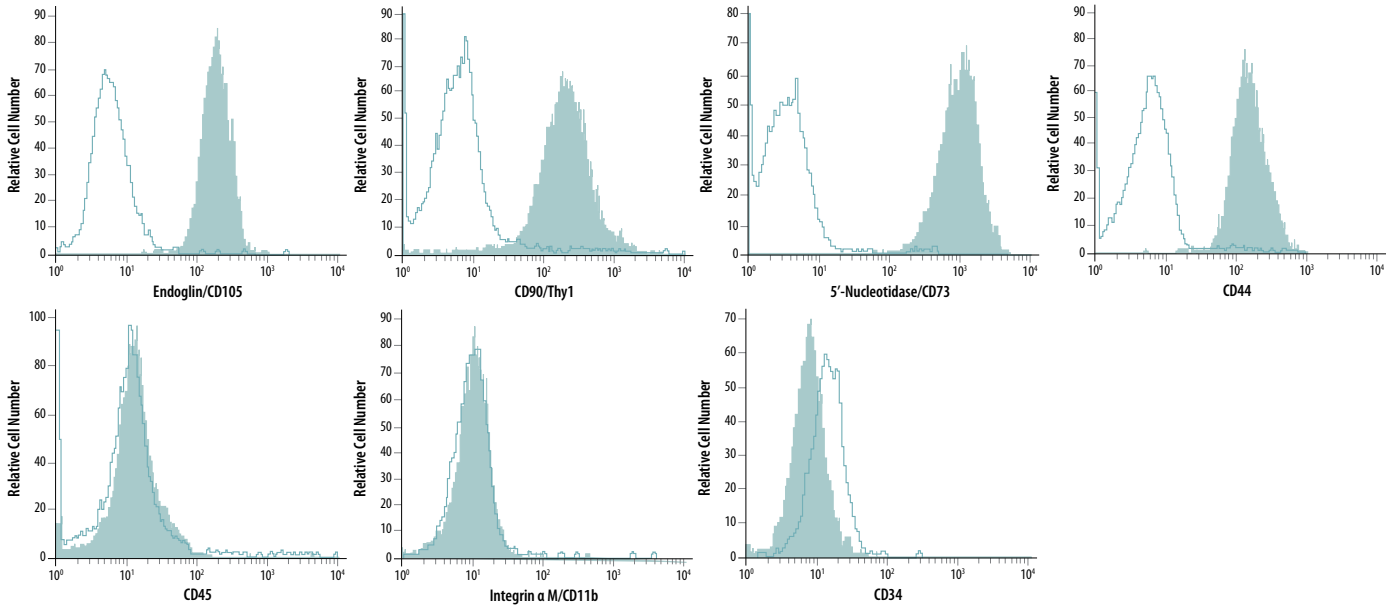

Figure 2: Phenotypic Analysis of Human MSCs Expanded in StemXVivo Serum-Free Human Mesenchymal Stem Cell Expansion Media for 3 Passages. Human MSCs were stained with the following Anti-Human Antibodies to verify MSC identity: CD105 (R&D Systems, Catalog # FAB10971P), CD90 (R&D Systems, Catalog # FAB2067P), CD73 (R&D Systems, Catalog # FAB5795P), CD44 (R&D Systems, Catalog # FAB4948P), CD45 (R&D Systems, Catalog # FAB1430P), CD11b (R&D Systems, Catalog # FAB16991P), and CD34 (filled histograms). Cells were analyzed using a Becton Dickinson FACSCalibur™ flow cytometer. For each antibody, isotype-matched controls are shown with an open histogram.