

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 phenotypically characterized as CD44⁺, CD45⁻, CD90⁺, and CD105⁺ cells.

INTENDED USE

The StemXVivo GMP MSC Expansion Media is a complete, fetal bovine serum-containing media that supports the expansion of mesenchymal stem cells (MSCs) under GMP-grade culture conditions. This media is ready-to-use out of the bottle, or it may be supplemented with cytokines/growth factors for a desired cell culture application. The cytokine/growth factor combinations used to expand MSCs depend on the experimental design of each researcher.

Note: GMP-grade cytokines and growth factors can be obtained from R&D Systems (www.rndsystems.com/gmp).

STABILITY & STORAGE

Upon receipt, this media should be stored at $\leq -20^{\circ}\text{C}$ in a manual defrost freezer. The media can be thawed at $2-8^{\circ}\text{C}$ or at room temperature. Thawed media can be aliquoted and stored at $\leq -20^{\circ}\text{C}$ in a manual defrost freezer for up to 3 months or used within 1 month when stored in the dark at $2-8^{\circ}\text{C}$. 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 preclinical or clinical *ex vivo* use.
- 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 EXPANSION OF HUMAN MESENCHYMAL STEM CELLS

The protocol below describes the expansion of human MSCs using StemXVivo GMP MSC Expansion Media.

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

OTHER MATERIALS REQUIRED

- Bone marrow-derived MSCs
- Penicillin-Streptomycin (100X)
- Trypsin-EDTA (10X)
- Phosphate-Buffered Saline (PBS)
- 75 cm² tissue culture flasks
- 15 mL centrifuge tubes
- Serological pipettes
- Pipette and pipette tips
- 37 °C and 5% CO₂ humidified incubator
- Centrifuge
- Hemocytometer
- Inverted Microscope
- Water bath

REAGENT PREPARATION

StemXVivo GMP Mesenchymal Stem Cell Expansion Media - Thaw the MSC Expansion Media at 2-8 °C or room temperature.

Completed StemXVivo GMP MSC Expansion Media - Add Penicillin-Streptomycin (100X) to the StemXVivo GMP MSC Expansion Media at a 1:100 dilution. **Note:** *If Penicillin-Streptomycin is not needed for the experiment, it can be omitted.*

Trypsin-EDTA (1X) - Dilute Trypsin-EDTA (10X) to 1X using PBS.

PROCEDURE

Culturing of Mesenchymal Stem Cells

1. Pre-warm the completed StemXVivo GMP MSC Expansion Media in a 37 °C water bath. This procedure uses 20 mL for each T75 flask used.
2. Resuspend $3.5\text{--}4.0 \times 10^5$ cells in 20 mL of the pre-warmed completed media.
Note: *If using a different size tissue culture vessel, seed cells at approximately 5000 cells/cm²/0.2-0.3 mL media.*
3. Add this cell suspension to a T75 flask.
4. Every three days remove and discard spent media and replace with 20 mL of pre-warmed Completed StemXVivo GMP MSC Expansion Media. **Note:** *Dispense media down the side of the flask so as not to disrupt cells.*
5. Subculture when cells become 80-90% confluent. Do not let the cultures become totally confluent.

Subculturing of MSCs

1. In a 37 °C water bath, pre-warm 30 mL of Completed StemXVivo GMP MSC Expansion Media and 2 mL of Trypsin-EDTA (1X) for each T75 flask used.
2. Remove and discard the media from the flasks. Wash the cells twice with 10 mL of PBS.
Note: *Do not dispense the PBS directly onto the cells during washing so as not to disrupt the cells.*
3. Add enough Trypsin-EDTA (1X) to just cover the cells. Gently rock the flask to disperse the Trypsin-EDTA solution evenly over the cells.
4. Incubate the flask 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. This process should take 5-10 minutes.
5. Add 5 mL of pre-warmed Completed StemXVivo GMP MSC Expansion Media to the flask to neutralize the Trypsin-EDTA (1X). Disperse the cells by pipetting the media over the entire growing surface of the flask.
6. Transfer the cells to a 15 mL conical tube and centrifuge at 400 x g for 5 minutes. Aspirate off the liquid.
7. Resuspend the cell pellet in a small amount of pre-warmed Completed StemXVivo MSC Expansion Media and count the cells with a hemocytometer.
8. If further expansion is desired, resuspend $3.5\text{--}4.0 \times 10^5$ cells into 20 mL of the pre-warmed Completed StemXVivo GMP MSC Expansion Media for each T75 flask.

DATA EXAMPLES

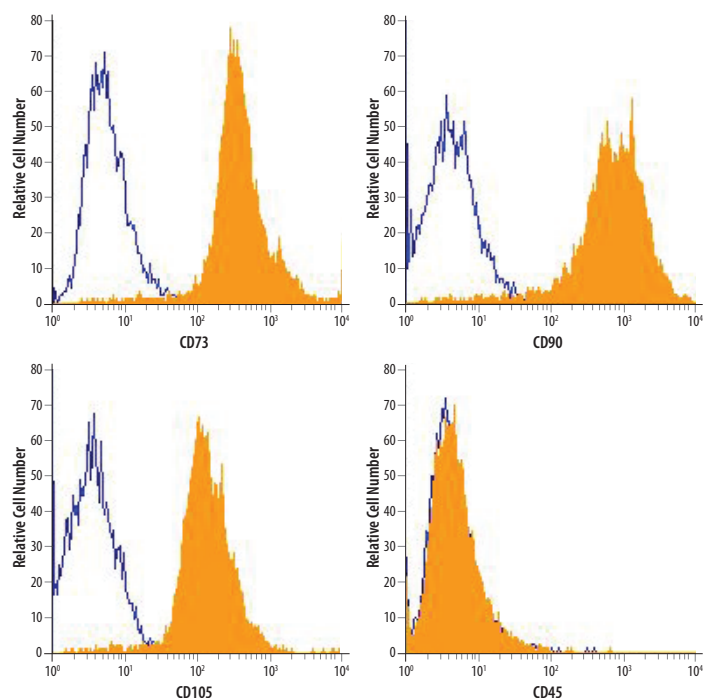


Figure 1: Phenotypic Analysis of Human MSCs Expanded in MSC Expansion Media. Human MSCs were expanded using StemXVivo GMP MSC Expansion Media. Filled histograms indicate cells stained with markers of undifferentiated MSCs including anti-CD105 (R&D Systems®, Catalog # FAB1097A), anti-CD73 (R&D Systems®, Catalog # FAB5795P), anti-CD90 (R&D Systems®, Catalog # FAB2067A), or anti-CD45 (R&D Systems®, Catalog # FAB1430P). The open histograms show isotype-matched control staining. MSCs appropriately express high levels of CD105, CD73, and CD90, and lack expression of CD45.

BACKGROUND

R&D Systems® GMP media is produced according to relevant sections of the following documents: WHO TRS, No. 822, 1992 Annex 1, Good Manufacturing Practices for Biological Products; USP Chapter 1043, Ancillary Materials for Cell, Gene and Tissue Engineered Products and USP Chapter 92, Growth Factors and Cytokines Used in Cell Therapy Manufacturing.

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- Material review process for variances
- Monitoring of stability over product shelf life

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