

## 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<sup>+</sup>, CD45<sup>-</sup>, CD90<sup>+</sup>, and CD105<sup>+</sup> cells.

## INTENDED USE

The StemXVivo<sup>®</sup> MSC Expansion Media is ready to use or it may be used with cytokine/growth factor supplements for the desired cell culture application. The cytokine/growth factor combinations used to expand 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](http://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.

## PRECAUTION

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.

## PROCEDURE FOR THE EXPANSION OF HUMAN, MOUSE, AND RAT MESENCHYMAL STEM CELLS

The protocol below describes the expansion of human, mouse, or rat MSCs using StemXVivo® 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 cm<sup>2</sup> tissue culture flasks
- 15 mL centrifuge tubes
- Serological pipettes
- Pipette and pipette tips
- 37°C and 5% CO<sub>2</sub> humidified incubator
- Centrifuge
- Hemocytometer
- Inverted Microscope
- Water bath

### REAGENT PREPARATION

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

**Completed StemXVivo® MSC Expansion Media** - Add Penicillin-Streptomycin to the StemXVivo® 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 10X Trypsin-EDTA to 1X using PBS.

### PROCEDURE

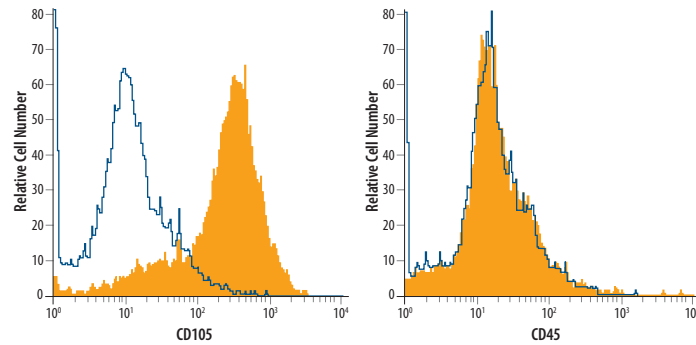
#### Culturing of Mesenchymal Stem Cells

1. Pre-warm the completed StemXVivo® MSC Expansion Media in a 37°C water bath. This procedure uses 20 mL for each T75 flask used.
2. Resuspend 3.5-4.0 x 10<sup>5</sup> 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/cm<sup>2</sup>/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® 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.

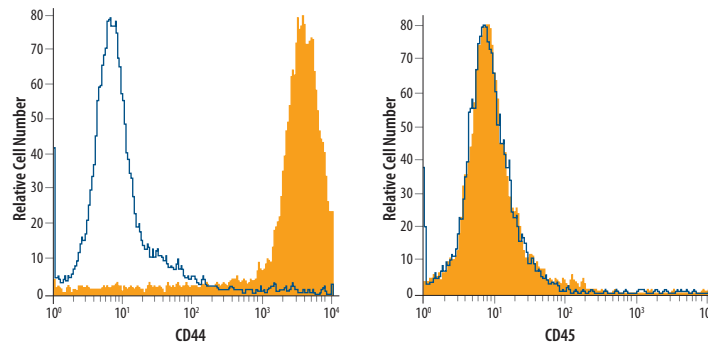
#### Subculturing of MSCs

1. In a 37 °C water bath, pre-warm 30 mL of completed StemXVivo® 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® 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 3.5-4.0 x 10<sup>5</sup> cells into 20 mL of the pre-warmed completed StemXVivo® MSC Expansion Media for each T75 flask.

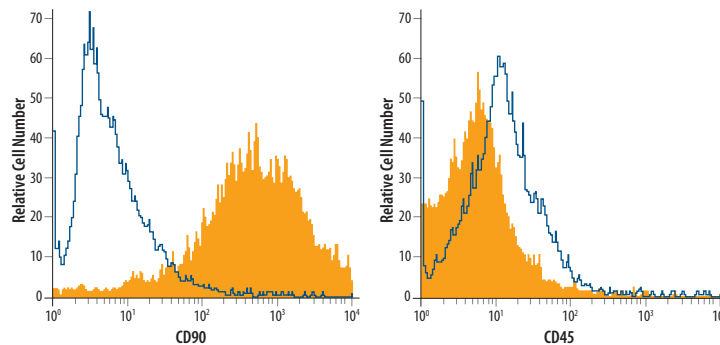
## DATA EXAMPLES



**Figure 1: Phenotypic Analysis of Human MSCs Expanded in MSC Expansion Media.** Human MSCs were expanded using StemXVivo® MSC Expansion Media. Filled histograms indicate cells stained with markers of undifferentiated MSCs including anti-CD105 (R&D Systems, Catalog # FAB10971P) or CD45 (R&D Systems, Catalog # MAB1430). The open histograms show isotype-matched control staining. MSCs appropriately lack expression of CD45.



**Figure 2: Phenotypic Analysis of Mouse MSCs Expanded in MSC Expansion Media.** Mouse MSCs were expanded using StemXVivo® MSC Expansion Media. Filled histograms indicate cells stained with markers of undifferentiated MSCs including anti-CD44 (R&D Systems, Catalog # AF6127) or CD45 (R&D Systems, Catalog # MAB114). The open histograms show isotype-matched control staining. MSCs appropriately lack expression of CD45.



**Figure 3: Phenotypic Analysis of Rat MSCs Expanded in MSC Expansion Media.** Rat MSCs (R&D Systems, Catalog # PSC003) were expanded using StemXVivo® MSC Expansion Media. Filled histograms indicate cells stained with markers of undifferentiated MSCs including anti-CD90 or CD45. The open histograms show isotype-matched control staining. MSCs appropriately lack expression of CD45.

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