Rat Mesenchymal Stem Cells

ORDERING INFORMATION

Catalog Number: PSC003

Size: 1 vial; 1 x 10^6 cells

Cell Type: Rat Mesenchymal Stem Cells at P2

Storage: Liquid nitrogen

Description

Rat primary mesenchymal stem cells (MSCs), also known as mesenchymal stromal cells, were isolated from the bone marrow of male F344 rats. Cells were cultured in StemXVivo™ Mesenchymal Stem Cell Expansion Media (R&D Systems, Catalog # CCM004). After 10-14 days, cells were harvested. The cells were further passaged for purity and cryopreserved. These cells are designated as passage 2 (P2) cells.

Rat MSCs can be passaged a limited number of times *in vitro* before their multipotency is compromised. P2 cells can be reliably expanded for a minimum of 5 passages using the optimal media and reliably differentiated into adipocytes, osteocytes, and chondrocytes (R&D Systems, Catalog # SC020).

Cells Provided

Rat Mesenchymal Stem Cells (1 x 10^6 cells).

Storage

Store in liquid nitrogen for up to 1 year.

Precautions

This product contains trace amounts of DMSO.

Protocol and Additional Reagents

Refer to the protocol detailing Rat MSCs differentiation into adipocytes, chondrocytes, and osteocytes at www.RnDSystems.com/pdf/SC020.pdf.

Thawing of Cryopreserved Cells

Review the following protocol in detail before thawing the cells. Correct thawing procedures are critical and must be followed.

1. Warm 10 mL of StemXVivo™ Mesenchymal Stem Cell Expansion Media (R&D Systems, Catalog # CCM004) in a 15 mL tube in a 37 °C water bath.
2. Remove the cryovial containing the frozen rat mesenchymal stem cells from the liquid nitrogen. Using a 2 mL pipette, immediately add 1 mL of fresh pre-warmed media to the vial by gently pipetting up and down. As cells begin to thaw, transfer the thawed portion into the pre-warmed media in the 15 mL tube. Repeat this process with the warmed media until all of the cells have thawed.

   Note: *Most of the frozen cells will be at the bottom of the cryovial. Rapid resuspension of frozen cells in warmed media during thawing is critical. Allowing cells to thaw slowly in the DMSO will dramatically reduce viability. Approximately 80% cell viability is expected from the freshly thawed cells when the appropriate thawing procedure is followed.*
3. Centrifuge the tube at 200 x g for 5 minutes to pellet the cells. Carefully remove the supernatant.
4. Resuspend the cells in 10 mL of prewarmed StemXVivo Mesenchymal Stem Cell Expansion Media, and count the cells using a hemocytometer.
5. Seed the cells into two flasks at approximately 5 x 10^5 cells per T75 flask.
6. The cells should be 80% confluent and ready to passage after 2-3 days.

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Cell Passage

1. Passage the cells when they are 80% confluent.
2. For each T75 flask, warm 30 mL of StemXVivo Mesenchymal Stem Cell Expansion Media (R&D Systems, Catalog # CCM004) in a 50 mL tube in a 37 °C water bath.
3. Carefully remove the medium from the T75 tissue culture flask containing the confluent layer of rat MSCs.
4. Wash cells with 10 mL of PBS.
5. Apply 1-2 mL of trypsin, and incubate at room temperature for 3-5 minutes. Inspect the flask, and ensure the complete detachment of MSCs by gently tapping the side of the flask with the palm of your hand.
6. Add 5 mL of StemXVivo Mesenchymal Stem Cell Expansion Media (pre-warmed in 37 °C) to the flask, and transfer the disassociated cells to a 15 mL conical tube.
7. Centrifuge the tube at 200 x g for 5 minutes to pellet the cells. Discard the supernatant.
8. Resuspend the cell pellet with 5 mL of StemXVivo Mesenchymal Stem Cell Expansion Media, and count the number of cells using a hemocytometer.
9. Plate the cells at 2 x 10^5 cells in 20 mL of StemXVivo Mesenchymal Stem Cell Expansion Media in a T75 flask.

Data

Figure 1

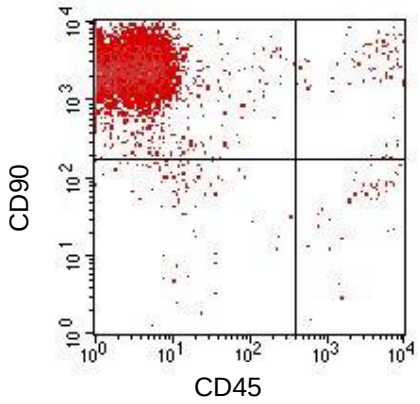

Figure 2

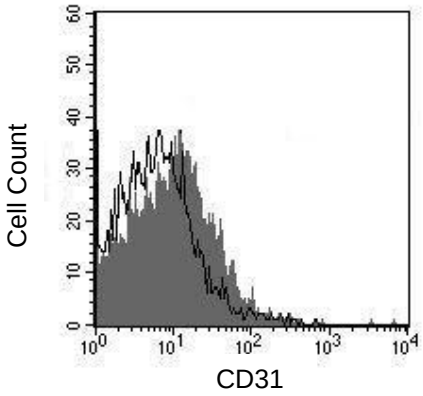

Figure 1: Rat Mesenchymal Stem Cells were stained with anti-rat CD90-PE and anti-rat CD45-Alexa Fluor® 687. Gate based on isotype control antibodies.

Figure 2: Rat Mesenchymal Stem Cells were stained with anti-mouse CD31 (R&D Systems, Catalog # AF3628) or isotype control antibody (R&D Systems, Catalog # AB-108-C; open histogram) followed by PE-conjugated anti-goat antibody (R&D Systems, Catalog # F0107).

Quality Control

Cells from this lot have been thawed and tested for their ability to proliferate for up to 5 passages. Stem and progenitor cells ($CD90^+CD45^-CD31^-$) expanded from the end of passage 5 have been examined for CD90, CD45, and CD31 expression (see figures 1 and 2). They were also tested for their ability to differentiate into adipocytes, chondrocytes, and osteocytes using the Rat Mesenchymal Stem Cell Functional Identification kit (R&D Systems, Catalog # SC020).

The cells tested negative for mycoplasma using the MycoProbe™ Mycoplasma Detection Kit (R&D Systems, Catalog # CUL001B). The cells also tested negative for microbial contamination.

Note: *Testing of the cells was performed using R&D Systems' Mesenchymal Stem Cell Expansion Reagents indicated in the protocols mentioned above. Performance of the cryopreserved cells cannot be guaranteed if reagents from other manufacturers are substituted.*

References

1. Lennon, D.P. *et al.* (2006) Experimental Hematology **34**:1606.
2. Neuhuber, B. *et al*. (2008) Experimental Hematology **36**(9):1176.