Human Mesenchymal Stem Cells (Derived from MEL-1 Human ESC)

Cat. No. SCC036

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

See Use Restrictions contained herein

USA & Canada • Phone: +1(800) 437-7500 • Fax: +1 (951) 676-9209
Europe +44 (0) 23 8026 2233 Australia +61 3 9839 2000
www.millipore.com

Introduction

Mesenchymal stem cells (MSCs) are defined as a self-renewing population of adherent, multipotent progenitor cells with the capacity to differentiate into several mesenchymal cell lineages. In defined *in vitro* assays, mesenchymal stem cells have been shown to readily differentiate into lineage-specific cells that form bone, cartilage, fat, tendon, and muscle tissues (1, 2). Mesenchymal stem cells have been isolated from adult bone marrow, adipose tissue dermis, and other connective tissues. Isolation of MSCs from these sources typically requires invasive procedures and availability of a suitable donor. The number of MSCs that can be isolated from a single donor and the capacity for long term proliferation is limited. Mesenchymal stem cells derived from human embryonic stem cells provide an attractive alternative source to a potentially unlimited supply of cells with multilineage differentiation capacity.

EMD Millipore's Human Mesenchymal Stem Cells (MSC) are derived from MEL-1 human embryonic stem cells (hESC). The cells proliferate as an adherent cell monolayer and can be expanded for up to 10 passages. Human ESC-derived MSC express the appropriate MSC markers including H-CAM (CD44), STRO-1, Thy-1 (CD90), and M-CAM (CD146) and do not express hematopoietic cell surface markers, CD14 (present on leukocytes) and CD19 (present on B-lymphocytes) and pluripotent markers, Oct-4 and SSEA-4. Cells can readily differentiate into adipocytes and osteoctyes.

EMD Millipore's cryopreserved Human Mesenchymal Stem Cells derived from human ESC (Cat. No. SCC036) can be optimally expanded in low-serum media (FibroGRO™ LS Complete Media Kit for Culture of Human Fibroblasts, Cat. No. SCMF002). For serum-free applications, cells can also be expanded in serum-free media (FibroGRO™ Complete Media Kit for Culture of Human Fibroblasts, Cat. No. SCMF001).

For Research Use Only; not for use in diagnostic procedure.

Kit Components

>1 x 10^6 viable Human Mesenchymal Stem Cells: (Catalog No. SCC036) derived from MEL-1 human embryonic stem cells, cryopreserved. Store in liquid nitrogen.

Characterization of Cells

EMD Millipore's Human Mesenchymal Stem Cells are derived from MEL-1 human embryonic stem cells and have been validated for high expression level of cell surface molecules that are present on mesenchymal stem cells: H-CAM (CD44), M-CAM (CD146), THY-1 (CD90), and STRO-1 and for their absence of hematopoietic and pluripotent cell surface markers, CD14 (present on leukocytes), CD19 (present on B-lymphocytes) and Oct-4 and SSEA-4 (present on undifferentiated human ES cells), respectively. The cells have also been validated for their self-renewal and multi-lineage differentiation capacities (please refer to product manual figures for representative data). Cells display normal karyotype as assessed by G-banding of 20 metaphase cells and tested negative for mycoplasma.

Materials Required But Not Provided

1. Mesenchymal Stem Cell Expansion Medium: the following media are recommended.
   - FibroGRO™ LS (Low Serum) Complete Media Kit for Culture of Human Fibroblasts (Cat. No. SCMF002)
   - FibroGRO™ (Serum-Free) Complete Media Kit for Culture of Human Fibroblasts (Cat. No. SCMF001)
2. Human Mesenchymal Stem Cell Characterization Kit (Cat. No. SCR067)
3. Accumax (Cat. No. SCR006)
4. Tissue culture-ware
5. Phosphate-Buffered Saline (1X PBS) (Cat. No. BSS-1005-B)
6. EmbryoMax ES Cell Qualified Ultra Pure Water, sterile H_2O, 500 mL (Cat. No. TMS-006-B)
7. EmbryoMax ES Cell Qualified 0.1% Gelatin Solution, 500 mL (Cat. No. ES-006-B)
8. Fixative (e.g. 4% Paraformaldehyde in 1X PBS)
9. Non-Permeable Blocking Solution (5% normal donkey serum in 1X PBS)
10. 4'-6-Diamidino-2-phenylindole (DAPI) / PBS solution
11. Tryphan Blue
12. Nunc Lab-Tek II 8 well chamber slides (Fisher Cat. No. 12-565-8)
13. Anti-fading mounting solution (DABCO/PVA)
14. Hemacytometer
15. Microscope with appropriate fluorescent filters

Storage/Handling

Human Mesenchymal Stem Cells (derived from MEL-1 Human ESC): (Cat. No. SCC036) should be stored in liquid nitrogen. It is recommended that the cells be used within ten passages.

Preparation of Coated Plates

Tissue culture plastic- or glassware plates should be coated with 0.1% gelatin as follows:

1. Add sufficient 0.1% gelatin solution (Cat. No. ES-006-B) to cover the entire surface of the cultureware plate. Use 10 mL volume for 10-cm plates and T75 flasks. Incubate for at least 30 minutes at room temperature.
2. Just before use, aspirate the gelatin solution from the coated plate or flask.

Thawing of Cells

1. Do not thaw the cells until the recommended medium and appropriately coated 0.1% gelatin plasticware and/or glassware are on hand.

2. Remove the vial of Human Mesenchymal Stem Cells from liquid nitrogen and incubate in a 37°C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells. **IMPORTANT: Do not vortex the cells.**

3. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.

4. In a laminar flow hood, use a 1 or 2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.

5. Using a 10 mL pipette, slowly add dropwise 9 mL FibroGRO™ LS (Low Serum) Complete Media (not provided) (pre-warmed to room temperature) to the 15 mL conical tube. **IMPORTANT: Do not add the whole volume of media at once to the cells. This may result in decreased cell viability due to osmotic shock.**

6. Gently mix the cell suspension by slow pipeting up and down twice. Be careful to not introduce any bubbles. **IMPORTANT: Do not vortex the cells**.

7. Centrifuge the tube at 300 x g for 2-3 minutes to pellet the cells.

8. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).

9. Resuspend the cells in a total volume of 10 mL FibroGRO™ LS (Low Serum) Complete Media (pre-warmed to 37°C).

10. Plate the cell mixture onto a 10-cm tissue culture plate or a T-75 tissue culture flask.

11. Incubate the cells at 37°C in a 5% CO_2 humidified incubator.

12. The next day, exchange the medium with fresh FibroGRO™ LS (Low Serum) Complete Media (pre-warmed to 37°C). Exchange with fresh medium every two to three days thereafter.

13. When the cells are approximately 80% confluent, they can be dissociated with Accumax (Cat. No. SCR006) and passaged or alternatively frozen for later use.

Subculturing

1. Carefully remove the medium from the 10-cm tissue culture plate containing the confluent layer of human mesenchymal stem cells.

2. Apply 3-5 mL Accumax and incubate in a 37°C incubator for 3-5 minutes.

3. Inspect the plate and ensure the complete detachment of cells by gently tapping the side of the plate with the palm of your hand.

4. Add 5 mL FibroGRO™ LS (Low Serum) Complete Media (pre-warmed to 37°C) to the plate.

5. Gently rotate the plate to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.

6. Centrifuge the tube at 300 x g for 3-5 minutes to pellet the cells.

7. Discard the supernatant

8. Apply 2 mL FibroGRO™ LS (Low Serum) Complete Media (pre-warmed to 37°C) to the conical tube and resuspend the cells thoroughly. **IMPORTANT: Do not vortex the cells.**

9. Count the number of cells using a hemacytometer.

10. Plate the cells to the desired density into the appropriate flasks, plates, or wells in FibroGRO™ LS (Low Serum) Complete Media. Plating ~1 million cells per 10-cm plate or T-75 flask is recommended.

Staining Protocol (for 8-well chamber slides)

Note: *For use with the Human Mesenchymal Stem Cell Characterization Kit (Catalog No. SCR067, available separately).*

1. Culture the human mesenchymal stem cells on gelatin-coated chamber slides in FibroGRO™ LS (Low Serum) Complete Media until the cells are 80-90% confluent.

2. Carefully aspirate the media, using caution not to aspirate the cells.

3. Fix cells by incubating with a fixative (i.e. 4% paraformaldehyde in 1X PBS) for 30-40 minutes at room temperature.

4. Carefully aspirate the fixative and rinse cells three times (5-10 minutes each) with 1X PBS.

5. Apply a blocking solution for at least 2 hours at room temperature or overnight at 4°C.
IMPORTANT: Do not shake the cells.

6. Dilute primary antibodies to working concentrations in the appropriate blocking solutions.

7. In a separate control well, depending upon the specific antibody used, add equivalent concentrations of mouse IgG or mouse IgM in 0.5 mL of the appropriate blocking solution. For example, to obtain a 1/500 dilution of mouse anti-H-CAM (1 mg/mL), 1 μL of the antibody is added to 0.5 mL volume of the appropriate blocking solution. In an adjacent control well, add 1 μL mouse IgG (1 mg/mL) to 0.5 mL of the appropriate blocking solution.

8. Aspirate the blocking solution and then add the diluted primary antibodies from step 6 and 7. Incubate the cells in primary antibodies overnight at 4°C. **IMPORTANT: Do not shake.**

9. The next day, wash the cells twice with 1X PBS (5-10 minutes each wash) and twice with blocking solution.

10. At the completion of the last wash, leave the cells in blocking solution for at least 30 minutes.

11. Dilute secondary antibodies in the appropriate blocking solution just before use. Donkey anti-mouse IgG Cy3 conjugated (Catalog No. AP192C), donkey anti-mouse IgG FITC conjugated (Catalog No. AP192F), donkey anti-rabbit IgG Cy3 conjugated (Catalog No. AP182C), donkey

anti-rabbit IgG FITC conjugated (Catalog No. AP182F) and donkey anti-mouse IgM Cy3 conjugated (Jackson Laboratories Catalog No. 715-165-140) antibodies at a 1:250 or 1:500 dilution are recommended

12. Carefully aspirate the blocking solution from the slide chambers and overlay the cells with the appropriate secondary antibodies that are conjugated to fluorescent molecules for 2 hours at room temperature.

13. Wash 3-5 times (5-10 minutes each) with 1X PBS.

14. Counterstain the cell nuclei with DAPI / 1X PBS solution.

15. Mount a glass coverslip over the chamber slides using anti-fading mounting solution (e.g. DABCO/PVA).

16. Visualize the cell staining with a fluorescent microscope.

Note: *Be sure to use the correct filter to visualize fluorescent-labeled cells.*

Characterization of Human Mesenchymal Stem Cells (Catalog No. SCC036)

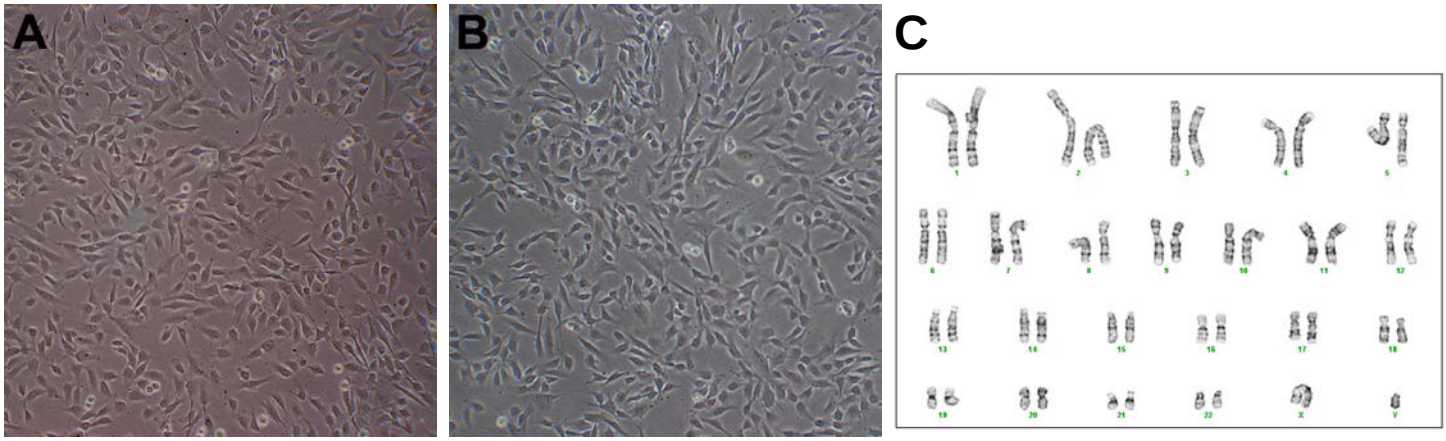

Figure 1. Phase contrast images of Human Mesenchymal Stem Cells (Catalog No. SCC036) one (**A**) and two (**B**) days after thawing. Right before passaging, cells should be ~80% confluent (**B**). Cells possess an apparently normal karyotype (**C**). Cytogenetic analysis was performed by Cell Line Genetics on seventeen G-banded metaphase cells. Sixteen cells demonstrated an apparently normal male karyotype (46, XY), while one cell demonstrated a non-clonal chromosome aberration (45, XY, -21), which is most likely a technical artifact.

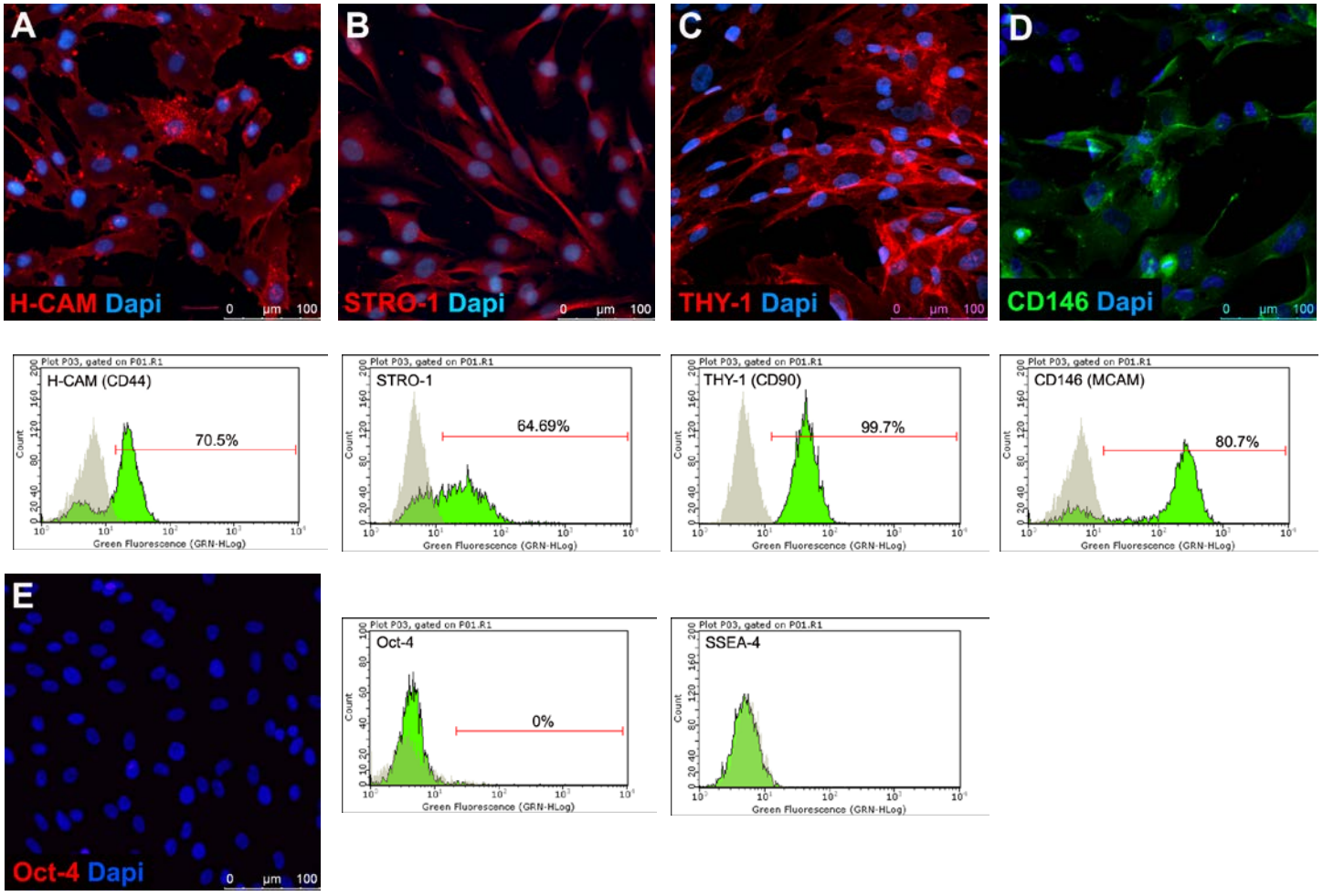

Figure 2. Immunocytochemical and Guava flow analysis of cultured human mesenchymal stem cells derived from MEL-1 human ESC. Human Mesenchymal Stem Cell express H-CAM (CD44) (**A,** Catalog No. CBL154: 1/500 dilution), STRO-1 (**B**, Catalog No. MAB4315: 1/25 dilution), THY-1 (CD90) (**C**, Catalog No. CBL415: 1/100 dilution), and CD146 (**D**, Catalog No. 04-1147: 1/100 dilution). Nuclei of the cells were visualized with DAPI (blue). Expression of hematopoietic stem cell markers, CD19 (Catalog No. MAB1794) and CD14 (Catalog No. MAB1219) (data not shown) and human ESC markers, Oct-4 (**E,** Catalog No. MAB4401) and SSEA-4 (Catalog No. MAB4304) were not detected.

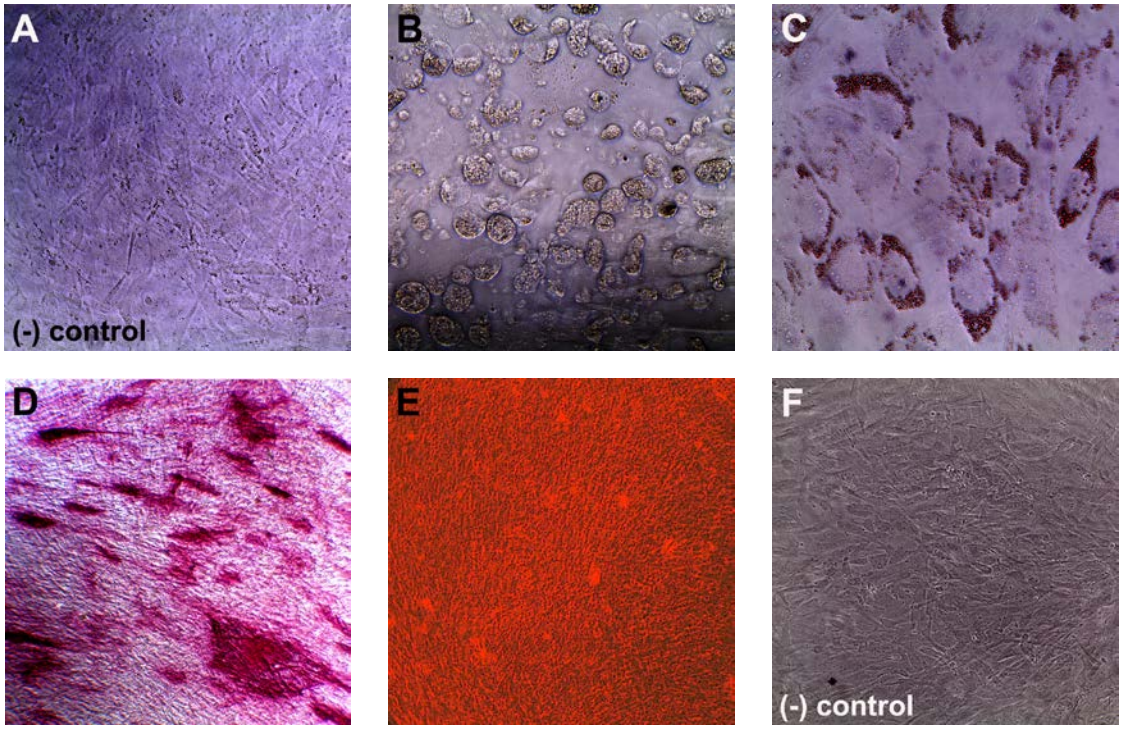

Figure 3. Human Mesenchymal Stem Cells (Catalog No. SCC036) are multipotent. Human Mesenchymal Stem Cells were differentiated in adipogenic (**B, C**) and osteogenic (**D, E**) differentiation medium. Using EMD Millipore's Mesenchymal Stem Cell Adipogenesis Kit (Catalog No. SCR020), human mesenchymal stem cells differentiated after 21 days to mature adipocytes as indicated by the accumulation of lipid vacuoles that stain with Oil Red O (**B**, unstained; **C**, 40X magnification). Control untreated human mesenchymal stem cells did not contain any lipid droplets (**A**). Using EMD Millipore's Mesenchymal Stem Cell Osteogenesis Kit (Catalog No. SCR028), human mesenchymal stem cells readily differentiated to an osteocyte lineage as indicated by alkaline phosphatase (**D**, 10X magnification) and Alizarin Red S (ARS) (**E**) staining. Alizarin Red S staining staining demonstrates mineral deposition throughout the culture. Alizarin Red S staining was not observed in control untreated cells (**F**).

*For color images, please go to www.millipore.com

Interpretation of Results

The determination that a cell is or is not a mesenchymal stem cell is based on the differential expression of a panel of markers and cannot be determined based on the expression (or lack thereof) of a single marker. While certain markers may be expressed by many cell types, it is the concomitant expression of multiple markers by a single cell and the non-expression of others that ultimately identify the cell as a particular cell type. It is generally accepted that cells that express CD44, CD90, and STRO-1 but do not express CD14 and CD19 represent a mesenchymal stem cell population (2-7). CD146 (MCAM) may or may not be expressed in particular MSC populations.

Antibodies directed against CD44, CD90, and STRO-1 are provided as Mesenchymal Stem Cell positive selection markers. Mesenchymal Stem Cells will express each of these antigens and identification of a population of cells as Mesenchymal Stem Cells requires that the cells stain with each of the positive selection antibodies. In addition, antibodies to CD14 and CD19 are two surface

markers that are present on leukocytes and B lymphocytes, respectively and are not expressed on mesenchymal stem cells. Oct-4 and SSEA-4 are human pluripotent markers and should be significantly downregulated in human ESC-derived MSC differentiated cell populations. The presence of positive staining with any one of these negative selection markers in the mesenchymal stem cell population indicates contamination of the particular cell lineage in question.

References

1. Prockop, D. J. (1997). Marrow stromal cells as stem cells for non-hematopoietic tissues. *Science* **276**: 71-74.
2. Pittenger, M. F., and Marshak, D. R. in *Stem Cell Biology* (Eds Marshak, D., R., Gardner, R. L., & Gottlieb, D.) (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001).
3, Alhadlaq, A., and Mao, J. J. (2004). Mesenchymal stem cells: isolation and therapeutics. *Stem Cells and Development* **13**: 436-448.
4. Pittenger, M. F., Mackay, A. M., Beck, S. C., Jaiswal, R. K., Douglas, R. (1999). Multilineage potential of adult human mesenchymal stem cells. *Science* **284**: 143-147.
5. Kolf, C. M., Cho, E., Tuan, R. S. (2007). Mesenchymal stromal cells. Biology of adult mesenchymal stem cells: regulation of niche, self-renewal and differentiation. *Arthritis Res. Ther*. **9 (1)**: 204.
6. Simmons P. J. Torok-Storb, B. (1991). Identification of stromal cell precursors in human bone marrow by a novel monoclonal antibody, STRO-1. *Blood* **78**: 55-62.
7. Delorme, B., Ringe, J., Gallay, N., Le Vern, Y., Kerboeuf, D., Jorgensen, C., Rosset, P., Sensebe, L., Layrolle, P., Haupl, T., Charbord, P. (2008). Specific plasma membrane protein phenotype of culture-amplified and native human bone marrow mesenchymal stem cells. *Blood* **111**: 2630-2635.

Related Products

The following products are available from Millipore as separate items:

1. Mouse anti-Human Oct-4, clone 10H11.2 (Catalog No. MAB4401)
2. Mouse anti-SSEA-4, clone MC-813-70 (Catalog No. MAB4304)
3. Mouse anti-Human CD44, 100 μg (Catalog No. CBL154)
4. Mouse anti-Human THY-1, 100 μg (Catalog No. CBL415)
5. Mouse anti-STRO-1, 100 μL (Catalog No. MAB4315)
6. Mouse anti-Endothelial Cells (CD146), 100 μg (Catalog No. MAB16985)
7. Mouse anti-Human CD14, 100 μg (Catalog No. MAB1219)
8. Mouse anti Human B Cells (CD19), 100 μg (Catalog No. MAB1794)
9. Mesenchymal Stem Cell Expansion Medium, 500 mL (Catalog No. SCM015).
10. Human Mesenchymal Stem Cell Characterization Kit (Catalog No. SCR067)
11. Mesenchymal Stem Cell Adipogenesis Kit (Catalog No. SCR020)
12. Mesenchymal Stem Cell Osteogenesis Kit (Catalog No. SCR028)

Warranty

EMD Millipore Corporation ("EMD Millipore") warrants its products will meet their applicable published specifications when used in accordance with their applicable instructions for a period of one year from shipment of the products. **EMD MILLIPORE MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE.** The warranty provided herein and the data, specifications and descriptions of EMD Millipore products appearing in EMD Millipore's published catalogues and product literature may not be altered except by express written agreement signed by an officer of EMD Millipore. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized and if given, should not be relied upon.

In the event of a breach of the foregoing warranty, EMD Millipore Corporation's sole obligation shall be to repair or replace, at its option, the applicable product or part thereof, provided the customer notifies EMD Millipore Corporation promptly of any such breach. If after exercising reasonable efforts, EMD Millipore Corporation is unable to repair or replace the product or part, then EDM Millipore shall refund to the Company all monies paid for such applicable Product. **EMD MILLIPORE CORPORATION SHALL NOT BE LIABLE FOR CONSEQUENTIAL, INCIDENTAL, SPECIAL OR ANY OTHER DAMAGES RESULTING FROM ECONOMIC LOSS OR PROPERTY DAMAGE SUSTAINED BY ANY COMPANY CUSTOMER FROM THE USE OF ITS PRODUCTS.**

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.