



Human Mesenchymal Stem Cell Kit (Derived from Bone-Marrow)

Cat. No. SCR108

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Not for use in diagnostic procedures**

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Introduction

Mesenchymal stem cells, also known as marrow stromal cells (1), are defined as a self-renewing population of adherent, bone-marrow-derived 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 also provide support and maintenance for the other major stem cell population in the bone marrow, the hematopoietic stem cells (2).

Mesenchymal stem cells (MSC's) have historically been isolated based on the ability of these cells to form adherent cell layers in culture and the concomitant lack of adherence of other cells in the bone marrow stroma such as hematopoietic stem cells, adipocytes, and macrophage (1, 3). While this procedure results in enriched populations of mesenchymal stem cells, the resulting bone-marrow derived cell populations are, nonetheless, heterogeneous – comprised not only of mesenchymal stem cells, but also of committed lineage-restricted progenitors (1, 3). To aid researchers in the accurate identification and characterization of mesenchymal stem cells, Millipore provides cryopreserved Human Mesenchymal Stem Cells (Catalog No. SCC034).

Millipore's cryopreserved Human Mesenchymal Stem Cells are ready-to-use primary mesenchymal stem cells isolated from the iliac crest of normal human bone marrow (18-30 years old).

It is recommended that Millipore's cryopreserved Human Mesenchymal Stem Cells (Catalog No. SCC034) be used in conjunction with the Mesenchymal Stem Cell Expansion Medium (Catalog No. SCM015) and the Human Mesenchymal Stem Cell Characterization Kit (SCR067). In addition, Millipore's Mesenchymal Stem Cell Adipogenesis Kit (Catalog No. SCR020) and Mesenchymal Stem Cell Osteogenesis Kit (Catalog No. SCR028) are two differentiation assays that can be used to differentiate these cells into adipocytes and osteocytes respectively.

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Kit Components

>1 x 10⁶ viable Human Mesenchymal Stem Cells: (Catalog No. SCC034) derived from adult human bone marrow, cryopreserved. Store in liquid nitrogen.

500 mL Mesenchymal Stem Cell Expansion Medium: (Catalog No. SCM015)

Characterization of Cells

Millipore's Human Mesenchymal Stem Cells are derived from human bone marrow and have been validated for high expression level of cell surface molecules that are present on mesenchymal stem cells: H-CAM (CD44), THY-1 (CD90) and STRO-1 and for their absence of hematopoietic cell surface markers, CD14 (present on leukocytes) and CD19 (present on B-lymphocytes). Millipore's Human Mesenchymal Stem Cells are also negative for the endothelial marker, M-CAM (CD146). 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. Human Mesenchymal Stem Cell Characterization Kit (Catalog No. SCR067)
2. Basic fibroblast growth factor (bFGF; FGF-2, Catalog No. GF003)
3. Trypsin-EDTA in Hank's Balanced Salt Solution, 0.25% Trypsin & 1 mM EDTA, without Ca²⁺, & Mg²⁺, 100 mL (Catalog No. SM-2003-C)
4. Tissue culture-ware
5. Phosphate-Buffered Saline (1X PBS) (Catalog No. BSS-1005-B)
6. EmbryoMax ES Cell Qualified Ultra Pure Water, sterile H₂O, 500 mL (Catalog No. TMS-006-B)
7. EmbryoMax ES Cell Qualified 0.1% Gelatin Solution, 500 mL (Catalog 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 Catalog 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: (Catalog No. SCC034) should be stored in liquid nitrogen. It is recommended that the cells be used within ten passages.

Mesenchymal Stem Cell Expansion Medium: (Catalog No. SCM015) should be stored at -20°C until ready to use. Upon thawing, the basal medium should be stored at 2-8°C for up to one month.

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 (Catalog 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 Mesenchymal Stem Cell Expansion Medium (Catalog No. SCM015) (pre-warmed to 37°C) 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 of Mesenchymal Stem Cell Expansion Medium (Catalog No. SCM015) (pre-warmed to 37°C) containing freshly added 8 ng/mL FGF-2.

***Note:** FGF-2 should always be added fresh to the Mesenchymal Stem Cell Expansion Medium.*
10. Plate the cell mixture onto a 10-cm tissue culture plate or a T75 tissue culture flask.
11. Incubate the cells at 37°C in a 5% CO₂ humidified incubator.
12. The next day, exchange the medium with fresh Mesenchymal Stem Cell Expansion Medium (pre-warmed to 37°C) containing 8 ng/mL FGF-2. Exchange with fresh medium containing FGF-2 every two to three days thereafter.
13. When the cells are approximately 80% confluent, they can be dissociated with Trypsin-EDTA (Catalog No. SM-2003-C) 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 of Trypsin-EDTA Solution 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 Mesenchymal Stem Cell Expansion Medium (SCM015, 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 Mesenchymal Stem Cell Expansion Medium (SCM015, pre-warmed to 37°C) containing 8 ng/mL FGF-2 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 Mesenchymal Stem Cell Expansion Medium containing 8 ng/mL FGF-2. Plating ~2 million cells per 10-cm plate or T75 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 chamber slides in Mesenchymal Stem Cell Expansion Medium (Catalog No. SCM015 or equivalent) containing 8 ng/mL FGF-2 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. For optimal results, use the Non-Permeable Blocking Solution (5% normal donkey serum in 1X PBS) with all the antibodies provided in the Human Mesenchymal Stem Cell Characterization Kit (SCR067).

- Dilute the primary antibodies in the Human Mesenchymal Stem Cell Characterization Kit (Catalog No. SCR067) to working concentrations in the appropriate blocking solutions. For optimal results, the following antibody dilutions are recommended for immunocytochemistry (see images):

Mouse anti-H-CAM:	1/500 dilution
Mouse anti-THY-1 (CD90):	1/500 dilution
Mouse anti-STRO-1:	1/500 dilution of ascites fluid
Mouse anti-MCAM (CD146) **:	1/500 dilution
Mouse anti-CD19*:	1/500 dilution
Mouse anti-CD14*:	1/500 dilution

* these antibodies serve as negative markers and will not stain mesenchymal stem cells.

** While MCAM (CD146) has been known to stain some preparations of bone marrow-derived mesenchymal stem cells, it does not stain Millipore's Human Mesenchymal Stem Cells.

- 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-CD146 (1 mg/mL), 1 μ L of the antibody is added to 0.5 mL volume of the appropriate blocking solution. In an adjacent control well, add 1 μ L mouse IgG (1 mg/mL) to 0.5 mL of the appropriate blocking solution.
- 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.**
- The next day, wash the cells twice with 1X PBS (5-10 minutes each wash) and twice with blocking solution.
- At the completion of the last wash, leave the cells in blocking solution for at least 30 minutes.
- Dilute secondary antibodies in the appropriate blocking solution just before use. Donkey anti-Mouse IgG Cy3 conjugated (Catalog No. AP192C) or Donkey anti-Mouse IgG FITC conjugated (Catalog No. AP192F) 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
- Carefully aspirate the blocking solution from the slide chambers and overlay the cells with the appropriate Donkey anti-Mouse secondary antibodies that are conjugated to fluorescent molecules for 2 hours at room temperature.
- Wash 3-5 times (5-10 minutes each) with 1X PBS.
- Counterstain the cell nuclei with DAPI / 1X PBS solution.
- Mount a glass coverslip over the chamber slides using anti-fading mounting solution (e.g. DABCO/PVA).
- 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. SCC034)

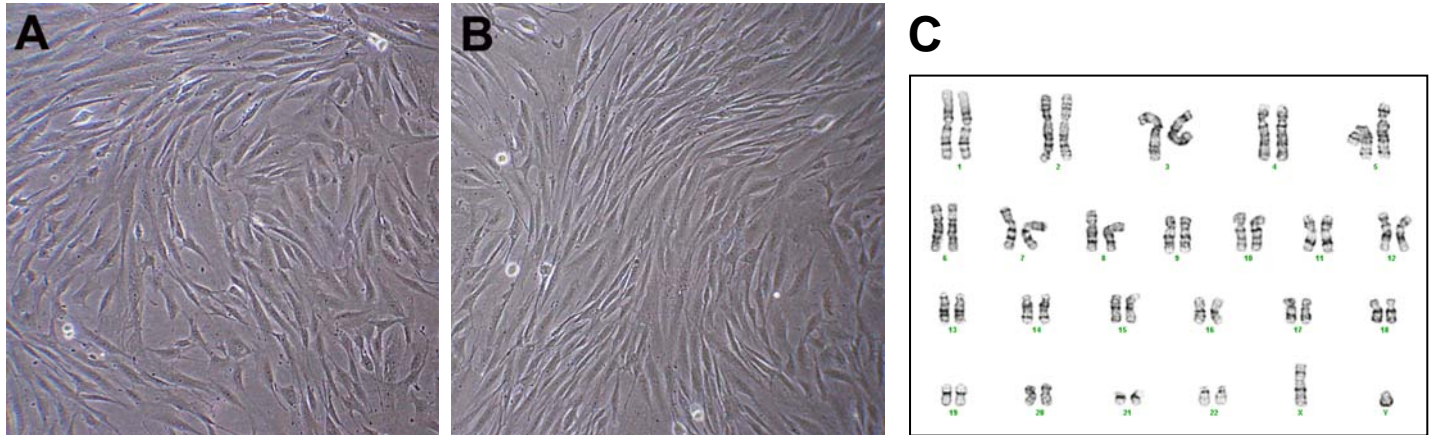


Figure 1. Phase contrast images of Human Mesenchymal Stem Cells (Catalog No. SCC034) one (A) and two (B) days after thawing. Right before passaging, cells should be >80% confluent (B). Cells possess an apparently normal karyotype (C). Cytogenetic analysis was performed by Cell Line Genetics on twenty G-banded metaphase cells. All twenty cells demonstrated an apparently normal male karyotype (46, XY). No abnormal cells were detected.

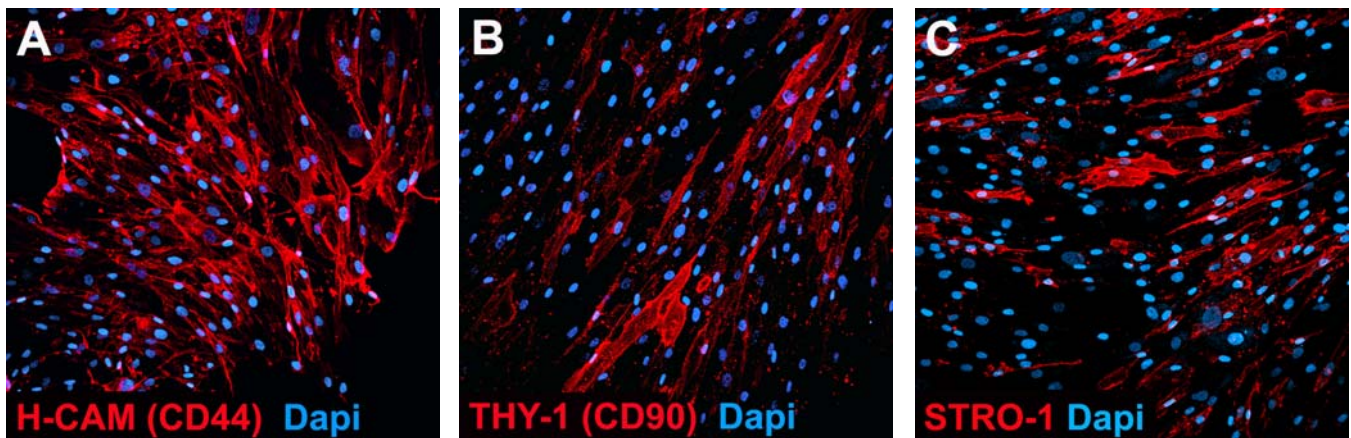


Figure 2. Immunocytochemical staining of cultured human bone marrow-derived mesenchymal stem cells with antibodies provided in the Human Mesenchymal Stem Cell Characterization Kit (Catalog No. SCR067, not provided). Human Mesenchymal Stem Cell express H-CAM (CD44) (A, CBL154;1/500 dilution), THY-1 (CD90) (B, CBL415: 1/500 dilution) and STRO-1 (C, MAB4315: 1/500 dilution). Nuclei of the cells were visualized with DAPI (blue). Expression of hematopoietic stem cell markers, CD19 (MAB1794) and CD14 (MAB1219) and endothelial marker, CD146 (MAB16985) were not observed in human mesenchymal stem cells (data not shown).

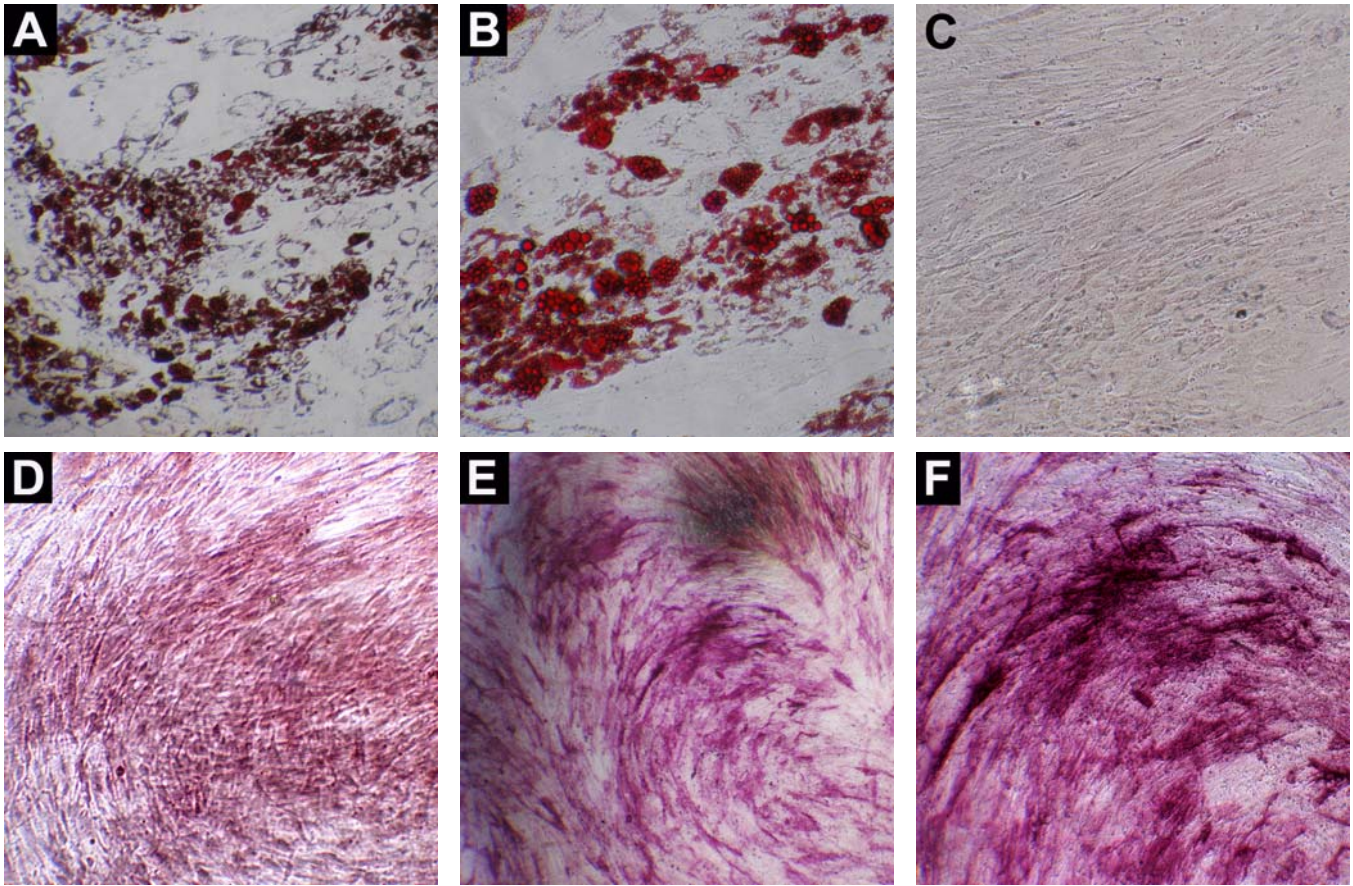


Figure 3. Human Mesenchymal Stem Cells (Catalog No. SCC034) are multipotent. Human Mesenchymal Stem Cells were differentiated in adipogenic (**A, B**) and osteogenic (**D-F**) differentiation medium. Using Millipore's Mesenchymal Stem Cell Adipogenesis Kit (Catalog No. SCR020), human mesenchymal stem cells differentiated after 21 days to mature adipocytes as indicated by the accumulation of lipod vacuoles that stain with Oil Red O (**A**, 10X magnification; **B**, 20X magnification). Control untreated human mesenchymal stem cells did not contain any lipid droplets (**C**). Using Millipore's Mesenchymal Stem Cell Osteogenesis Kit (Catalog No. SCR028), human mesenchymal stem cells readily differentiated to an osteocyte lineage as indicated by Alizarin Red S (ARS) (**D**), and alkaline phosphatase (**E**, 4X magnification; **F**, 20X magnification) staining. Alizarin Red S staining demonstrates mineral deposition throughout the culture.

*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).

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. The presence of positive staining with either one of these negative selection markers in the mesenchymal stem cell population indicates contamination of the particular cell lineage in question.

References

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Related Products

The following products are available from Millipore as separate items:

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

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