Human Mesenchymal Stem Cell Characterization Kit

Cat. No. SCR067

FOR RESEARCH USE ONLY
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 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).

Millipore's Human Mesenchymal Stem Cell Characterization Kit (Catalog Number SCR067) contains a panel of positive and negative selection markers for the characterization of the mesenchymal stem cell population in human samples. Positive cell markers include antibodies directed against cell-surface molecules present on mesenchymal stem cells: CD44, CD90, STRO-1, and CD146 (5, 6, 7). In addition, two specific hematopoietic cell surface markers are provided whose expressions should not be present on mesenchymal stem cells: CD14 (present on leukocytes) and CD19 (present on B-lymphocytes) (2, 3, 4).

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Kit Components

1. Mouse anti-H-CAM: (Catalog No. CBL154-20UL) One vial containing 20 µL of IgG_{2a} monoclonal antibody, clone F10-44-2. Store at -20°C.

2. Mouse anti-THY-1 (CD90): (Catalog No. CBL415-20UL) One vial containing 20 µL of IgG_1 monoclonal antibody, clone F15-42-1. Store at -20°C.

3. Mouse anti-STRO-1: (Catalog No. MAB4315-20UL) One vial containing 20 µL of IgM monoclonal antibody, clone STRO-1. Store at -20°C.

4. Mouse anti-MCAM (CD146): (Catalog No. MAB16985-20UL) One vial containing 20 µL of IgG_1 monoclonal antibody, clone P1H12. Store at -20°C.

5. Mouse anti-CD19: (Catalog No. MAB1794-20UL) One vial containing 20 µL of IgG_{2a} monoclonal antibody, clone FMC63. Store at -20°C.

6. Mouse anti-CD14: (Catalog No. MAB1219-20UL) One vial containing 20 µL of IgG_1 monoclonal antibody, clone 2D-15C. Store at -20°C.

Materials Required But Not Provided

1. Human Mesenchymal stem cells
2. Mesenchymal Stem Cell Expansion Medium (Catalog No. SCM015)
3. Accutase™ Cell Dissociation Solution (Catalog No. SCR005)
4. Tissue culture-ware
5. Phosphate-Buffered Saline (1X PBS) (Catalog No. BSS-1005-B)
6. EmbryoMax ES Cell Qualified Ultra Pure Water, sterile H_20, 500 mL (Catalog No. TMS-006-B)
7. Fixative (e.g. 4% Paraformaldehyde in 1X PBS)
8. Non-Permeable Blocking Solution (5% normal donkey serum in 1X PBS)
9. Mouse IgG, purified 10 mg (Catalog No. PP54)
10. Mouse IgM, purified 1 mg (Catalog No. PP50)
11. Fluorescent-labeled secondary antibodies. Donkey anti-Mouse IgG, Cy3 conjugated (Catalog No. AP192C) and Donkey anti-Mouse IgG, FITC conjugated (Catalog No. AP192F) are recommended
12. 4'-6-Diamidino-2-phenylindole (DAPI) / PBS solution
13. Tryphan Blue
14. Nunc Lab-Tek II 8 well chamber slides (Fisher Catalog No. 12-565-8)
15. Anti-fading mounting solution (DABCO/PVA)
16. Hemacytometer
17. Microscope with appropriate fluorescent filters

Storage

When stored at the recommended storage conditions (refer to Kit Components), components are stable up to 6 months from date of receipt. Do not expose to elevated temperatures.

Staining Protocol (for 8-well chamber slides)

1. Culture the human mesenchymal stem cells on chamber slides in Mesenchymal Stem Cell Expansion Medium (Catalog No. SCM015 or equivalent) until the cells are 80-90% confluent.

2. Carefully aspirate the media, observing 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 this kit.

6. Dilute the primary antibodies included in this kit 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.

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-CD146 (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. We typically use Donkey anti-Mouse IgG Cy3 conjugated (Catalog No. AP192C) or Donkey anti-Mouse IgG FITC conjugated (Catalog No. AP192F) antibodies at a 1:250 or 1:500 dilution.

12. 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.

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

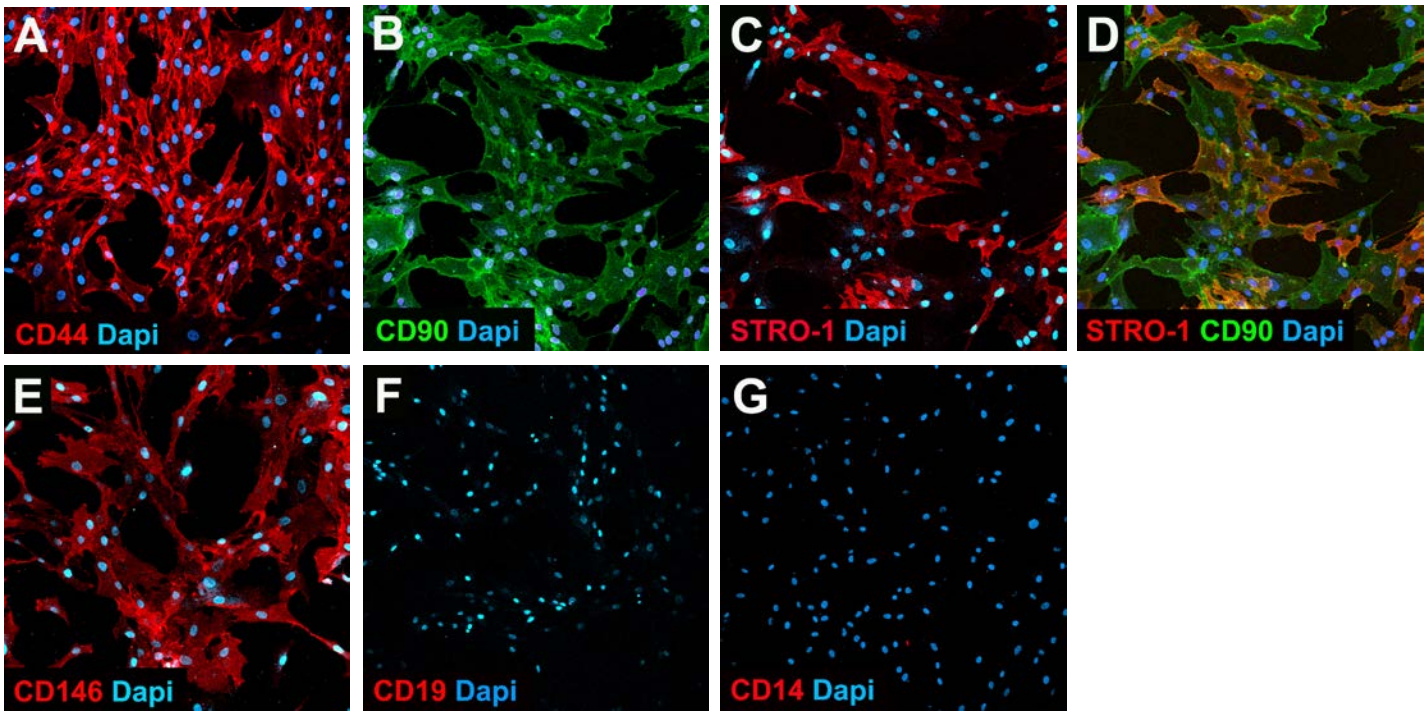
14. Counterstain the cell nuclei with DAPI / 1X PBS solution.

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.*

Results



Immunocytochemical staining of cultured human bone marrow-derived mesenchymal stem cells stained with antibodies provided in the kit. Nuclei of the cells were visualized with DAPI (blue). CD19 and CD14 staining were not present on human mesenchymal stem cells.

*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, STRO-1, and CD146 but do not express CD14 and CD19 represent a mesenchymal stem cell population (2-7).

Antibodies directed against CD44, CD90, STRO-1, and CD146 are provided as Mesenchymal Stem Cell positive selection markers. MSCs will express each of these antigens and identification of a population of cells as MSCs 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.

All of the antibodies provided in the kit have been tested and optimized for use in immunocyto-chemistry on human bone marrow-derived mesenchymal stem cells and also on human ES-cell derived mesenchymal stem cells. We recommend that Millipore's Human Mesenchymal Stem Cell Characterization Kit be used in conjunction with differentiation assays (Catalog No. SCR020, Mesenchymal Stem Cell Adipogenesis Kit, and Catalog No. SCR028, Mesenchymal Stem Cell Osteogenesis Kit) that demonstrate multipotentiality of the starting cell population.

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. S*tem 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 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. Mesenchymal Stem Cell Adipogenesis Kit (Catalog No. SCR020)
9. Mesenchymal Stem Cell Osteogenesis Kit (Catalog No. SCR028)

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Cat No. SCR067MAN

October 2016
Version 3, SCR067MAN