Mesenchymal Stem Cell Characterization Kit (Rat)

Catalog No. SCR018

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 Mesenchymal Stem Cell Characterization Kit (Catalog Number SCR018) contains a panel of positive and negative selection markers for the characterization of the mesenchymal stem cell population in rat. Positive cell markers include antibodies directed against cell-surface molecules (integrin β1) and CD54 (2, 4) present on mesenchymal stem cells along with two extracellular matrix molecules (fibronectin and type I collagen) that are synthesized by cultured mesenchymal stem cells (1). Along with the positive selection markers, two specific hematopoietic cell surface markers, (CD14 – present on leukocytes and CD45 - present on monocytes and macrophages) are provided whose expressions should not be present on mesenchymal stem cells (2, 3, 4). Mouse and rabbit immunoglobulins for the assessment of background staining are also included.

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Kit Components

1. Rabbit anti-Integrin β1 (Catalog No. AB1952-20): Known species reactivity: Hu, Ms, Rt, other species not tested. One vial containing 20 µL polyclonal rabbit serum. Store at -20 °C. Catalog number AB1952-20 is not available separately; see AB1952
2. Mouse anti-CD54 (ICAM-1)(Catalog No. MAB2130, clone W-CAM-1): Known species reactivity: Hu, Rt, other species not tested. One vial containing 100 µL ascites monoclonal antibody. Store at -20 °C.
3. Rabbit anti-Collagen Type I (Catalog No. 2003605): Known species reactivity: Rt, other species not tested. One vial containing 20 µL purified polyclonal antibody. Store at -20 °C. Catalog number 2003605 is not available separately; see AB755P
4. Rabbit anti-Fibronectin (Catalog No. 2003606): Known species reactivity: Rt, other species not tested. One vial containing 10 µg purified polyclonal antibody. Store at -20°C. Catalog number 2003606 is not available separately; see AB1954.
5. Mouse anti-CD45 (Catalog No. 2003607, clone OX-1): One vial containing 100 µL monoclonal antibody. Store at -20 °C. Known species reactivity: Rt, other species not tested. Catalog number 2003607 is not available separately; see CBL1502.
6. Mouse anti CD14 (Catalog No. 2003608), clone UCHM-1: One vial containing 10 µg monoclonal antibody. Store at -20 °C. Known species reactivity: Hu, Mky, other species not tested. Catalog number 2003608 is not available separately; see CBL453.
7. Mouse IgG (Catalog No. PP54-100UG): One vial containing 100 µg purified mouse IgG control antibody. Store at -20 °C. Catalog number PP54-100UG is not available separately; see PP54.
8. Rabbit IgG (Catalog No. PP64-100UG): One vial containing 100 µg purified rabbit IgG antibody. Store at -20 °C. Catalog number PP64-100UG is not available separately; see PP64.

Materials Not Supplied

1. Mesenchymal stem cells and culture reagents (i.e. Millipore® SCR027).
2. Mesenchymal Stem Cell Expansion Media (DMEM-low glucose, without glutamine, 10% heat-inactivated fetal bovine serum, 2 mM L-Glutamine and 1X solution of penicillin, streptomycin and fungizone (PSF).
3. Chamber slides
4. Glass coverslips
5. Phosphate-Buffered Saline (1X PBS)
6. Fixative (e.g. freshly made 4% Paraformaldehyde in 1X PBS, pH 7.4)
7. Blocking Solution (5% normal donkey serum, with 0.3% Triton X-100 in 1X PBS filtered through a 0.45µm filter)
8. Non-Permeable Blocking Solution (5% normal donkey serum in 1X PBS, filtered through a 0.45µm filter)
9. Fluorescent-labeled secondary antibodies. Donkey anti-mouse IgG, Cy3 conjugated (Cat. No. AP192C) and donkey anti-rabbit IgG, Cy3 conjugated (Cat. No. AP182C) are recommended
10. Antibody dilution buffer (1X PBS without blocking or detergents).
11. 4'-6-Diamidino-2-phenylindole (DAPI) / PBS solution (200ng/mL in PBS). *Note keep solution out of light.*
12. Anti-fading mounting solution, Millipore® 5013.
13. Fluorescent Microscope

Storage

When stored at the recommended storage conditions (refer to Kit Components), components are stable up to the expiration date. Do not expose to elevated temperatures. Discard any remaining reagents after the expiration date.

Staining Protocol (for 8-well chamber slides)

1. Culture the bone marrow-derived mesenchymal stem cells on chamber slides in Mesenchymal Stem Cell Expansion Medium until the cells are 80-90% confluent (3, 4).
2. Carefully aspirate the media and fix the cells with a fixative (i.e. 4% paraformaldehyde in 1X PBS). Be careful not to aspirate the cells.

3. Incubate in 4% paraformaldehyde for 5-10 minutes at room temperature. Over fixing can lead to poor staining results.
4. Carefully aspirate the fixative and rinse 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 Blocking Solution (5% Normal donkey serum, 0.3% Triton X-100 in 1X PBS) with antibodies directed against fibronectin, collagen and integrin β1. Use the Non-Permeable Blocking Solution (5% Normal donkey serum in 1X PBS) with cellular differentiation antibodies (CD54, CD45 and CD14).
6. Carefully aspirate the blocking solutions and add the primary antibodies diluted as directed below in antibody dilution buffer (1X PBS only).
7. Dilute the primary antibodies included in this kit to working concentrations in the antibody dilution buffer. The following antibody dilutions are suggested as guidelines for immuno-cytochemistry. Some dilution optimization may be required depending upon the model system and fixation conditions used.

Rabbit anti-integrin β1:	1/500 dilution of rabbit serum
Mouse anti-CD54:	1/100 dilution of ascites monoclonal antibody
Rabbit anti-collagen Type I:	1/500 dilution based on 1mg/mL, final 2ng/µL
Rabbit anti-fibronectin:	1/1000 dilution based on 0.5mg/mL, final 0.5ng/µL
Mouse anti-CD14:	negative staining at 1/100 dilution based on 0.1 mg/mL, final 1ng/µL
Mouse anti-CD45:	negative staining at 1/100 dilution based on 1mg/mL, final 10ng/µL

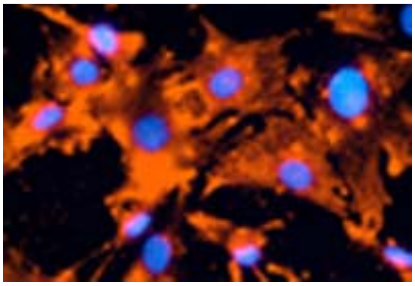
8. In a separate control well, depending upon the specific antibody used, add equivalent concentrations of mouse IgG (1 mg/mL) and rabbit IgG (1 mg/mL) to 0.5 mL of the antibody dilution buffer. For example, to obtain a 1/100 dilution of mouse anti-Rat-CD45 (1 mg/mL), 5 µL of the antibody is added to 0.5 mL volume of the antibody dilution buffer. In an adjacent control well, add 5 µL mouse IgG (1 mg/mL) to 0.5 mL of the antibody dilution buffer.
9. Incubate the cells in primary antibodies overnight at 4°C. **IMPORTANT: Do not shake because cells can come off during the long incubation.**

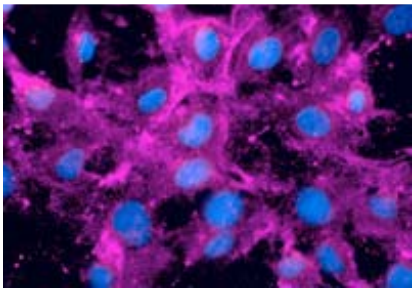
10. The next day, wash the cells four times with 1X PBS (5-10 minutes each wash) , 0.5mL per well. Shake or aspirate the wash buffer carefully.
11. At the completion of the last wash,, block with the Non-permeable blocking solution 15-30 minutes at room temperature (optional).
12. Dilute secondary antibodies in antibody dilution buffer just before use. We typically use donkey anti-mouse IgG Cy3 conjugated (Cat. No. AP192C) and donkey anti-rabbit IgG Cy3 conjugated (Cat. No. AP182C) antibodies at a 1:250 or 1:500 dilution. Keep in the dark.
13. Overlay the cells with the appropriate donkey anti-mouse and anti-rabbit secondary antibodies that are conjugated to fluorescent molecules for 1-2 hours at room temperature in the dark.
14. Wash 3-5 times (5-10 minutes each, 0.5ml per well) with 1X PBS. Keep fluorescent lights low or slides shielded to prevent fading. For the final washes, it is sometimes easier to remove the 8-well chamber unit for maximum washing ability.
15. Counterstain the cell nuclei with DAPI / 1X PBS solution by incubating the wells/slides for 2-5 minutes in DAPI solution. Rinse 2-3X with PBS to remove excess dye.
16. Mount a glass coverslip over the chamber slides using antifading mounting solution (e.g. Millipore® 5013).
17. Visualize the cell staining with a fluorescent microscope. Staining is stable refrigerated in the dark & damp for 12-24 hours, typically.

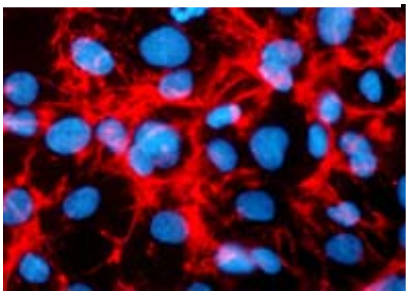
    Note: *Be sure to use the correct filter to visualize fluorescent-labeled cells.*

Staining Results Obtained With Cultured Rat Mesenchymal Stem Cells

Integrin β1 DAPI







Collagen Type I DAPI **Fibronectin DAPI**

Immunofluorescent images of cultured rat mesenchymal stem cells stained with rabbit anti integrin β1 (orange), rabbit anti collagen Type I (purple), and rabbit anti fibronectin (red). Nuclei of the cells were visualized with DAPI (blue).

*For colored image, 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 bone marrow derived cells that express beta 1 Integrin, CD54, Type I Collagen and Fibronectin but do not express CD14 and CD54 represent a mesenchymal stem cell population (2,3,4).

Antibodies directed against beta 1 Integrin, CD54, Type I Collagen and Fibronectin 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 CD45 are two negative selection markers that are provided with the kit. CD14 and CD45 are two surface markers that are present on leukocytes and on monocytes and macrophages, 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

1. Prockop, D. J. (1997). Marrow stromal cells as stem cells for nonhematopoietic 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.

Related Products

The following products are available from MILLIPORE® as separate items:

1. Rabbit anti-Human Integrin β1, 100 µL (Catalog No. AB1952)
2. Rabbit anti-Rat Collagen Type I, purified 100 µg (Catalog No. AB755P)
3. Rabbit anti-Rat Fibronectin, purified 100 µg (Catalog No. AB1954)
4. Mouse anti-Rat CD45, purified 500 µg (Catalog No. CBL1502)
5. Mouse anti-Human CD14, purified 100 µg (Catalog No. CBL453)
6. Mouse anti-Human ICAM-1/CD54, 100uL ascites (Catalog No. MAB2130)
7. Mouse IgG, purified 10 mg (Catalog No. PP54)
8. Rabbit IgG, purified 25 mg (Catalog No. PP64)

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