MILLIPORE

Rat Mesenchymal Stem Cell Kit

Catalog No. SCR026

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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 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 presents the Rat Mesenchymal Stem Cell Kit (Catalog No. SCR026).

Millipore's Rat Mesenchymal Stem Cell Kit provides ready-to-use primary mesenchymal stem cells isolated from the bone marrow of adult Fisher 344 rats along with a panel of positive and negative selection markers for the characterization of the mesenchymal stem cell population. Positive cell markers include antibodies directed against two cell-surface molecules (integrin β1 and CD54) (2, 4) that are present on mesenchymal stem cells. Negative cell markers include antibodies directed against two specific hematopoietic cell surface markers, (CD14, present on leukocytes and CD45, present on monocytes and macrophages) that are not expressed by mesenchymal stem cells (2, 3, 4). Mouse and rabbit immunoglobulins for the assessment of background staining are also included.

All of the antibodies provided in the kit have been tested and optimized for use in immunocytochemistry on rat mesenchymal stem cells. We recommend that Millipore's Rat Mesenchymal Stem Cell Kit (Catalog No. SCR026) 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.

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Kit Components

1. $1x10^6$ viable Rat Mesenchymal Stem Cells: (Part No. 2004005) derived from adult female Fisher 344 rats, cryopreserved. Store in liquid nitrogen.
2. Rabbit anti-Integrin β1: (Part No. AB1952-20) One vial containing 20 µL polyclonal rabbit serum. Store at -20 °C.
3. Mouse anti-CD54 (ICAM-1): (Part No. MAB2130) One vial containing 100 µL ascites monoclonal antibody. Store at -20 °C.
4. Mouse anti-CD45: (Part No. 2003607) One vial containing 100 µL of 1 mg/mL monoclonal antibody. Store at -20 °C.
5. Mouse anti CD14: (Part No. 2003608) One vial containing 10 µg of 0.1 mg/mL monoclonal antibody. Store at -20 °C.
6. Mouse IgG: (Part No. PP54-100UG) One vial containing 100 µg of 1 mg/mL purified mouse IgG control antibody. Store at -20°C.
7. Rabbit IgG: (Part No. PP64-100UG) One vial containing 100 µg of 1 mg/mL purified rabbit IgG antibody. Store at –20 °C

Characterization of Cells

Each lot of primary rat mesenchymal stem cells has been validated for high level of expression of integrin β1 and CD54 and for their self-renewal and multi-lineage differentiation capacities (please refer to insert figures). Cells also display normal karyotype as assessed by chromosome spread and tested negative for mycoplasma.

Materials Required But Not Supplied

1. Mesenchymal Stem Cell Expansion Media (DMEM-low glucose, without glutamine, 10% heat-inactivated fetal bovine serum (Millipore Catalog No. ES-009-D), 2 mM L-Glutamine and (Millipore Catalog No. TMS-002-C), 1X solution of penicillin and streptomycin (Millipore Catalog No. TMS-AB2-C).
2. Accutase™ Cell Detachment Solution (Millipore Catalog No. SCR005)
3. Chamber slides
4. Glass coverslips
5. Phosphate-Buffered Saline (1X PBS) (Millipore Catalog No. BSS-1005-B)
6. Fixative (e.g. 4% Paraformaldehyde in 1X PBS)
7. Blocking Solution (5% normal donkey serum, 0.3% Triton X-100 in 1X PBS).

8. Non-Permeable Blocking Solution (5% normal donkey serum in 1X PBS)
9. Fluorescent-labeled secondary antibodies. Donkey anti-mouse IgG, Cy3 conjugated (Millipore Catalog No. AP192C), donkey anti-rabbit IgG, Cy3 conjugated (Millipore ® Catalog No. AP182C), donkey anti-mouse IgG, FITC conjugated (Millipore Catalog No. AP192F) and donkey anti-rabbit IgG, FITC conjugated (Millipore Catalog No. AP182F) are recommended
10. 4'-6-Diamidino-2-phenylindole (DAPI) / PBS solution
11. Anti-fading mounting solution (DABCO/PVA)
12. Hemacytometer
13. 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. We recommend that the cells be used within ten passages.

Thawing of Cells

1. Do not thaw the cells until the recommended medium and appropriate plasticware and/or glassware are on hand.
2. Remove the vial of rat 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 to not introduce any bubbles during the transfer process.
5. Using a 10 mL pipette, slowly add dropwise 9 mL of Mesenchymal Stem Cell Expansion Medium (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 4-8 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in a total volume of 10 mL of Mesenchymal Stem Cell Expansion Medium (pre-warmed to 37^{o}C).
10. Plate the cell mixture onto a 10-cm tissue culture plate.
11. Incubate the cells at 37^{o}C in a 5% CO_2 humidified incubator.
12. The next day, exchange the medium with fresh Mesenchymal Stem Cell Expansion Medium (pre-warmed to 37^{o}C). Exchange with fresh medium every two to three days thereafter.
13. When the cells are approximately 80% confluent, they can be dissociated with Accutase™ 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 rat mesenchymal stem cells.
2. Apply 3-5 mL of Accutase™ and incubate in a 37^{o}C incubator for 3 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. Apply 5 mL of Mesenchymal Stem Cell Expansion Medium (pre-warmed to 37^{o}C) to the plate.
5. Transfer the dissociated cells to a 15 mL conical tube.
6. Centrifuge the tube at 300 x g for 2-3 minutes to pellet the cells.
7. Discard the supernatant.
8. Apply 2 mL of Mesenchymal Stem Cell Expansion Medium to the conical tube and resuspend the cells thoroughly. IMPORTANT: Do not vortex.
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. We typically plated the cells at ~2 million cells per 10-cm plate or T75 flask.

Staining Protocol (for 8-well chamber slides)

1. Culture the bone marrow-derived rat 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 medium and wash the wells two times with 1X PBS. Be careful to not aspirate the cells.
3. Fix the cells by incubation in 4% paraformaldehyde for 30-40 minutes at room temperature.
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 integrin β1. Use the Non-Permeable Blocking Solution (5% Normal donkey serum in 1X PBS) with cellular differentiation antibodies (CD54, CD45 and CD14).
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):

   Rabbit anti-integrin β1: 1/500 dilution of rabbit serum

   Mouse anti-CD54: 1/100 dilution of ascites monoclonal antibody

   Mouse anti-CD14: negative staining at 1/1000 dilution based on 1mg/mL, final 1ng/µL

   Mouse anti-CD45: negative staining at 1/100 dilution based on 1mg/mL, final 10ng/µL
7. 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 appropriate blocking solution. 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 appropriate blocking solution. In an adjacent control well, add 5 µL mouse IgG (1 mg/mL) to 0.5 mL of the appropriate blocking solution.
8. 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 the 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 blocking solution just before use. The following secondary antibodies can be used: donkey anti-mouse IgG Cy3 conjugated (Millipore Cat. No. AP192C), donkey anti-mouse IgG FITC conjugated (Millipore Cat. No. AP192F), donkey anti-rabbit IgG Cy3 conjugated (Millipore Cat. No. AP182C), and donkey anti-rabbit IgG FITC conjugated (Millipore Cat. No. AP182F) antibodies at a 1:250 or 1:500 dilution.
12. Overlay the cells with the appropriate donkey anti-mouse and anti-rabbit 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 antifading 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 Rat Mesenchymal Stem Cells

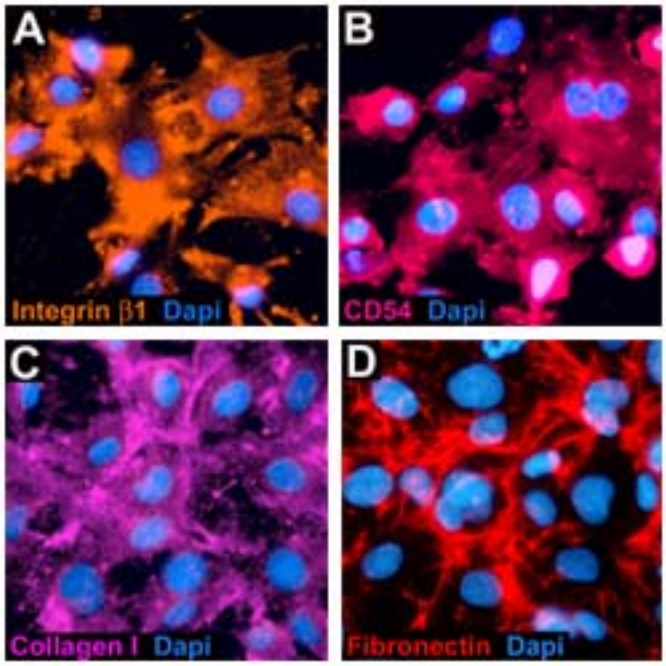

Figure 1. Rat Mesenchymal Stem Cells (Millipore Catalog No. SCR026) express mesenchymal stem cell markers, integrin β1 **(A)**, CD54 **(B)**, collagen Type I **(C)**, and fibronectin **(D)**. Nuclei of the cells were visualized with DAPI (blue).

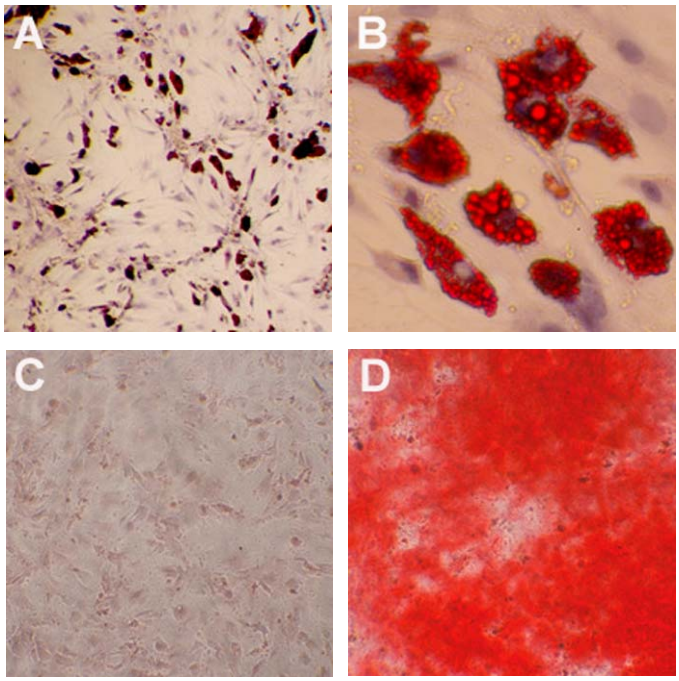

Figure 2. Rat Mesenchymal Stem Cells (Millipore Catalog No. SCR026, SCR027) are multipotent. Rat mesenchymal stem cells were differentiated in adipogenic **(A, B)** and osteogenic **(D)** differentiation medium. Using Millipore's Mesenchymal Stem Cell Adipogenesis Kit (Millipore Catalog No. SCR020), rat mesenchymal stem cells differentiated after 21 days to mature adipocytes as indicated by the accumulation of lipid vacuoles that stain with Oil Red O **(A**, 10X magnification; **B**, 40X magnification). Cell nuclei (purple) were stained with Hematoxylin Solution. Control rat skin fibroblast cells did not contain any lipid droplets (data not shown). Using Millipore's Mesenchymal Stem Cell Osteogenesis Kit (Millipore Catalog No. SCR028), rat mesenchymal stem cells readily differentiated to an osteocyte lineage as indicated by Alizarin Red S (ARS) staining **(D)**. ARS staining was not observed in control rat skin fibroblasts that were treated in the same manner **(C)**. Alizarin red S staining demonstrates mineral deposition throughout the culture.

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

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.

5. Salasznyk, R. M., Williams W. A., Boskey, A., Batorsky, A., and Plopper, G. E. (2004). Adhesion to vitronectin and collagen I promotes osteogenic differentiation of human mesenchymal stem cells. *J. Biomed. Biotechnol.* **2004 (1)**: 24-34.

Related Products

The following stem cell products are available from Millipore as separate items:

1. Cryopreserved Rat Mesenchymal Stem Cells: (Catalog No. SCR027)
2. Mesenchymal Stem Cell Characterization Kit: (Catalog No. SCR018)
3. Mesenchymal Stem Cell Adipogenesis Kit: (Catalog No. SCR020)
4. Mesenchymal Stem Cell Osteogenesis Kit: (Catalog No. SCR028)
5. Rabbit anti Human Integrin β1, 100 µL: (Catalog No. AB1952)
6. Rabbit anti Rat Collagen Type I, purified 100 µg: (Catalog No. AB755P)
7. Rabbit anti Rat Fibronectin, purified 100 µg: (Catalog No. AB1954)
8. Mouse anti Human CD54, 100 µL: (Catalog No.MAB2130)
9. Mouse anti Rat 45, purified 500 µg: (Catalog No. CBL1502)
10. Mouse anti Human CD14, purified 100 µg: (Catalog No. CBL453)
11. Mouse-IgG, purified 10 mg: (Catalog No. PP54)
12. Rabbit-IgG, purified 25 mg: (Catalog No. PP64)

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