Rat Mesenchymal Stem Cells (Bone Marrow)

Cell Line

Cat. # SCR027

Lot # xxxxxxx

Pack size: ≥1X10^6 cells/vial

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES
NOT FOR HUMAN OR ANIMAL CONSUMPTION

Store in Liquid Nitrogen

Certificate of Analysis

Background

Mesenchymal stem cells, also known as marrow stromal cells, 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. Mesenchymal stem cells also provide support and maintenance for the other major stem cell population in the bone marrow, the hematopoietic stem cells

These cryopreserved Rat Mesenchymal Stem Cells are a ready-to-use source of multipotent mesenchymal stem cells derived from the bone marrow of adult Fisher 344 rats.

Source

Isolated from the iliac crest of adult Fisher 344 rats.

Quality Control Testing

- Each vial contains ≥ $1X10^6$ viable cells at passage 2.
- Cell viability is ≥75% at thaw and cells can be passaged 3 times after thaw.
- Derived from certified pathogen-free Fisher 344 rats.
- Cells tested negative for mycoplasma contamination.
- Cells tested positive for CD90 expression; negative for CD45 and CD31 expression.

Storage and Handling

Rat Mesenchymal Stem Cells (Bone Marrow) should be stored in liquid nitrogen. The cells can be passaged for at least 3-5 additional passages.

Materials Required But Not Supplied

1. Mesenchymal Stem Cell Medium (Cat. No. SCM015), contains FBS
2. Accutase™ (Cat. No. SCR005) or Trypsin-EDTA in Hank's Balanced Salt Solution, 0.25% Trypsin & 1 mM EDTA, w/o Ca2+ & Mg2+ (Cat. No. SM-2003-C)
3. Tissue culture-ware
4. EmbryoMax ES Cell Qualified 0.1% Gelatin Solution, 500 mL (Cat. No. ES-006-B)
5. Hemocytometer
6. Microscope with appropriate fluorescent filters

SPECIES LEGEND: **H** Human **Ca** Canine **M** Mouse **R** Rat **Rb** Rabbit **B** Bovine **P** Porcine **WR** Most Common Vertebrates

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Protocols

A. 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.

B. 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 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 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 (Cat. 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 pipetting 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 (Cat. No. SCM015), pre-warmed to 37°C.
10. Plate the cell mixture onto a 10-cm tissue culture plate or a T75 tissue culture flask.
11. Maintain the cells at 37°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°C). Replace with fresh medium every two to three days thereafter.
13. When the cells are approximately 80% confluent, they can be dissociated with Accutase (Cat. No. SCR005) or Trypsin-EDTA (Cat. No. SM-2003-C) and passaged further or frozen for later use.

    NOTE: Depending on seeding density and passage number (i.e. later passages), cells may take longer to reach 80% confluence.

C. Subculturing of Cells

1. Subculture cells once they have reached approximately 80% confluence and are actively proliferating.

   IMPORTANT: Subculture cells before reaching 100% confluence.
2. Carefully remove the medium from the 10-cm tissue culture plate containing the confluent layer of human mesenchymal stem cells. Apply 3-5 mL of Accutase or 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 (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 (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 hemocytometer.
10. Plate the cells at a density of 10,000 cells per cm^2 into the appropriate flasks, plates, or wells in Mesenchymal Stem Cell Expansion Medium.

Representative Lot Data

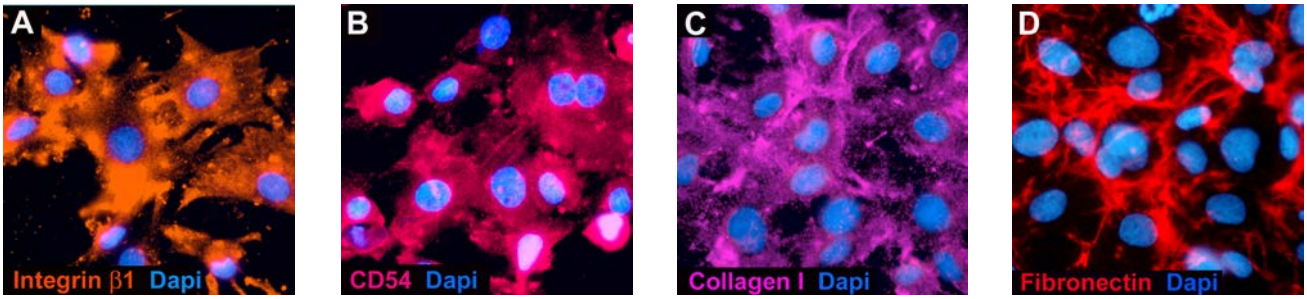

Figure 1. Immunocytochemical staining of cultured rat bone marrow-derived mesenchymal stem cells with integrin β1 (**A**), CD54 (**B**), collagen type 1 (**C**) and fibronectin (**D**). Nuclei of the cells were visualized with DAPI (blue).

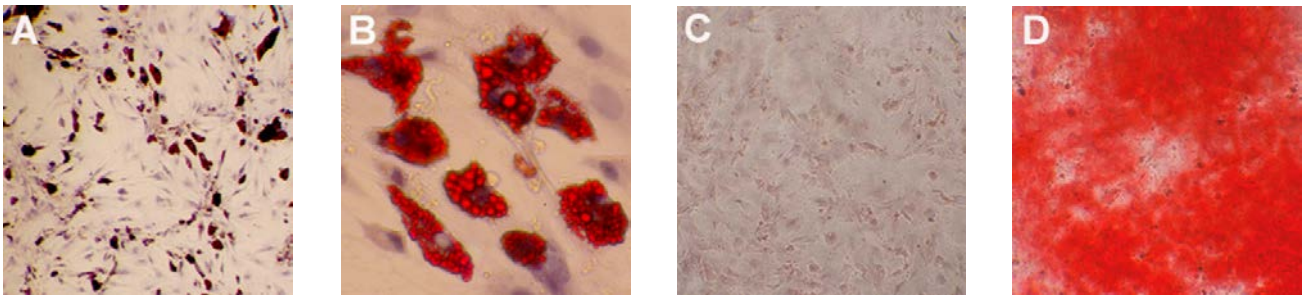

Figure 2. Rat Mesenchymal Stem Cells (Cat. No. SCR027) are multipotent. Rat mesenchymal stem cells were differentiated in adipogenic (**A, B**) and osteogenic (**D**) differentiation medium. Using Mesenchymal Stem Cell Adipogenesis Kit (Cat. No. SCR020), rat mesenchymal stem cells differentiated after 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 Mesenchymal Stem Cell Osteogenesis Kit (Cat. 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.

Related Products

The following products are available as separate items:

1. Mesenchymal Stem Cell Characterization Kit (Cat. No. SCR018)
2. Mesenchymal Stem Cell Adipogenesis Kit (Cat. No. SCR020)
3. Mesenchymal Stem Cell Osteogenesis Kit (Cat. No. SCR028)
4. Rabbit anti-Human Integrin β1, 100 µL (Cat. No. AB1952)
5. Mouse anti-Human CD54, 100 µL (Cat. No. MAB2130)
6. Rabbit anti-Rat Collagen Type I, purified 100 µg (Cat. No. AB755P)
7. Rabbit anti-Rat Fibronectin, purified 100 µg (Cat. No. AB1954)