Mouse Mesenchymal Stem Cells-bone marrow (MMSC-bm)

Catalog Number: M7500

Cell Specification

Mesenchymal stem cells (MSC) are a well-characterized population of adult stem cells. They have the potential to develop into mature cells that produce fat, cartilage, bone, tendons, and muscle [1, 2]. These properties in combination with their developmental plasticity have generated tremendous interest in the potential use of mesenchymal stem cells to replace damaged tissues. MSC cultured without serum in the presence of transformation growth factor will differentiate into chondrocytes, whereas MSC cultured in serum with ascorbic acid and dexamethasone will differentiate into osteoblasts. MSC has the capability for renewal and differentiation into various lineages of mesenchymal tissues. In essence MSC could be cultured to expand their numbers then transplanted to the injured site or after seeding in/on shaped biomimetic scaffold to generate appropriate tissue constructs.

MMSC-bm from ScienCell Research Laboratories are isolated from adult mouse bone marrow. MMSC-bm are cryopreserved at passage one culture and delivered frozen. Each vial contains >5 x 10^5 cells in 1 ml volume. MMSC-bm are characterized by immunofluorescent method with antibodies to CD73, CD90, CD105 and lipid staining after differentiation. MMSC-bm are negative for mycoplasma, bacteria, yeast and fungi. MMSC-bm are guaranteed to further culture at the conditions provided by ScienCell Research Laboratories.

Recommended Medium

It is recommended to use Mesenchymal Stem Cell Medium (MSCM, Cat. No. 7501) for the culturing of MMSC-bm *in vitro*.

Product Use

MMSC-bm are for research use only. It is not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

Storage

Directly and immediately transfer cells from dry ice to liquid nitrogen upon receiving and keep the cells in liquid nitrogen until cell culture is needed for experiments.

Shipping

Dry ice.

Reference

1. Kassem, M. Mesenchymal stem cells: biological characteristics and potential clinical applications. 2004. Cloning Stem Cells. 6(4):369-74.
2. Barry, F. P., and J. M. Murphy. Mesenchymal stem cells: clinical applications and biological characterization. 2004. Int J Biochem Cell Biol. 36(4):568-84.

Instruction for culturing cells

Caution: Cryopreserved cells are very delicate. Thaw the vial in a 37°C waterbath and return them to culture as quickly as possible with minimal handling!

Set up culture after receiving the order:

1. Prepare a poly-lysine coated flask (2 μg/cm^2, T-75 flask is recommended). Add 10 ml of sterile cell culture-grade water to a T-75 flask and then add 15 μl of ply-lysine stock solution (10 mg/ml, ScienCell cat. no. 0413). Leave the flask in incubator overnight (minimum one hour at 37°C incubator).
2. Prepare complete medium: decontaminate the external surfaces of medium and medium supplements with 70% ethanol and transfer them to sterile field. Aseptically open each supplement tube and add them to the basal medium with a pipette. Rinse each tube with medium to recover the entire volume.
3. Aspirate poly-lysine solution and rinse the flask with sterile cell culture-grade water twice. Add 20 ml of complete medium to the flask. Leave the flask in the hood and go to thaw the cells.
4. Place the vial in a 37°C waterbath, hold and rotate the vial gently until the contents are completely thawed. Remove the vial from the waterbath immediately, wipe it dry, rinse the vial with 70% ethanol and transfer it to a sterile field. Remove the cap, being careful not to touch the interior threads with fingers. Using 1 ml eppendorf pipette gently resuspend the contents of the vial.
5. Dispense the contents of the vial into the equilibrated, poly-lysine coated culture vessels. A seeding density of 5,000 cells/cm^2 is recommended.
   Note: Dilution and centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of DMSO residue in the culture. It is also important that cells are plated in poly-lysine coated culture vessels that promote mesenchymal stem cell attachment.
6. Replace the cap or cover of flask, and gently rock the vessel to distribute the cells evenly. Loosen caps if necessary to permit gas exchange.
7. Return the culture vessels to the incubator.
8. For best result, do not disturb the culture for at least 16 hours after the culture has been initiated. Change the growth medium the next day to remove the residual DMSO and unattached cells, then every other day thereafter. A healthy culture will display fibroblast/smooth muscle cell-like morphology, usually in a scattered single cells rather than a homogeneous bundle or sheet of cells; and the cell number will be doubled after two to three days in culture.

Maintenance of Culture:

1. Change the medium to fresh supplemented medium the next morning after establishing a culture from cryopreserved cells. For subsequent subcultures, change medium 48 hours after establishing the subculture.
2. Change the medium every other day thereafter, until the culture is approximately 50% confluent.
3. Once the culture reaches 50% confluence, change medium every day until the culture is ready for subculture.

Subculture:

1. Subculture the cells when they are over 90% confluent.
2. Prepare Poly-L-Lysine coated flasks (2 μg/cm^2) one day before subculture.
3. Warm medium, trypsin/EDTA solution (T/E, cat. no. 0103), trypsin neutralization solution (TNS, cat. no. 0113), and DPBS to **room temperature**. We do not recommend warming the reagents and medium at 37^{o}C waterbath prior to use.
4. Rinse the cells with DPBS.
5. Add 10 ml of DPBS first and then 2 ml of trypsin/EDTA solution into flask (in the case of T-75 flask); gently rock the flask to make sure cells are covered by trypsin/EDTA solution; incubate the flask at 37^{o}C incubator for 1 to 2 minutes or until cells are completely rounded up (monitored with inverted microscope). During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine serum (FBS, cat. no. 0500); transfer trypsin/EDTA solution from the flask to the 50 ml centrifuge tube (a few percent of cells may detached); continue incubate the flask at 37^{o}C for 1 minutes (no solution in the flask at this moment); at the end of trypsinization, one hand hold one side of flask and the other hand gently tap the other side of the flask to detach cells from attachment; check the flask under inverted microscope to make sure all cells are detached, add 5 ml of trypsin neutralization solution to the flask and transfer detached cells to the 50 ml centrifuge tube; add another 5 ml of TNS to harvest the residue cells and transfer it to the 50 ml centrifuge tube. Examine the flask under inverted microscope to make sure the cell harvesting is successful by looking at the number of cells left behind. There should be less than 5%.
   Note: Use ScienCell Research Laboratories' trypsin/EDTA solution that is optimized to minimize the killing of the cells by over trypsinization.
6. Centrifuge the 50 ml centrifuge tube (harvested cell suspension) at 1000 rpm (*Beckman Coulter* Allegra 6R centrifuge or similar) for 5 min; re-suspend cells in growth medium.

7. Count cells and plate cells in a new, Poly-L-Lysine coated flask with cell density as recommended.

Caution: Handling animal derived products is potentially biohazardous. Proper precautions must be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working these materials. Never mouth pipette. We recommend following the universal procedures for handling products of animal origin as the minimum precaution against contamination [1].

[1]. Grizzle, W. E., and Polt, S. S. (1988) Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues. J Tissue Culture Methods. 11(4).