Rev. 1

Rat Mesenchymal Stem Cells-bone marrow (RMSC-bm)

Catalog #R7500-1

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]. The developmental plasticity of MSC has generated tremendous interest because of the potential use of mesenchymal stem cells in regenerative medicine to replace damaged tissues. MSC cultured without serum in the presence of transformation growth factors will differentiate into chondrocytes. In contrast, MSC cultured in serum with ascorbic acid and dexamethasone will differentiate into osteoblasts. With their renewal capability, MSC have the potential to be transplanted into an injured site or seeded on a biomimetic scaffold to generate appropriate tissue constructs.

RMSC-bm from ScienCell Research Laboratories are isolated from adult CD® IGS rat bone marrow. RMSC-bm are cryopreserved at passage one and delivered frozen. Each vial contains >1 x 10^6 cells in 1 ml volume. RMSC-bm are characterized by immunofluorescence with antibodies to CD73 and/or CD90, Oil Red O staining after adipogenic differentiation, and/or Alizarin Red staining after osteogenic differentiation. RMSC-bm are negative for mycoplasma, bacteria, yeast, and fungi. RMSC-bm are guaranteed to further culture under the conditions provided by ScienCell Research Laboratories.

Recommended Medium

It is recommended to use Mesenchymal Stem Cell Medium (MSCM, Cat. #7501) for culturing RMSC-bm *in vitro*.

Product Use

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

Storage

Upon receiving, directly and immediately transfer the cells from dry ice to liquid nitrogen and keep the cells in liquid nitrogen until they are needed for experiments.

Shipping

Dry ice.

References

[1] Kassem M. (2004) "Mesenchymal stem cells: biological characteristics and potential clinical applications." *Cloning Stem Cells*. 6: 369-74.

[2] Barry FP, Murphy JM. (2004) "Mesenchymal stem cells: clinical applications and biological characterization." *Int J Biochem Cell Biol*. 36: 568-84.

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Instructions for culturing cells

Caution: Cryopreserved primary cells are very delicate. Thaw the vial in a 37ºC water bath and return the cells to culture as quickly as possible with minimal handling! Do not centrifuge the cells after thawing as this can damage the cells.

Initiating the culture:

Note: ScienCell primary cells must be cultured in a 37ºC, 5% CO_2 incubator. Cells are only warranted if ScienCell media and reagents are used and the recommended protocols are followed.

1. Prepare fibronectin-coated culture vessels (2 µg/cm^2, 2 T-75 flasks are recommended). To obtain a 2 µg/cm^2 fibronectin-coated culture vessel, add 10 ml of sterile Dulbecco's phosphate buffered saline, Ca^{++}- and Mg^{++}-free (Cat. #0303) to a T-75 flask and then add 150 µl of fibronectin stock solution (Cat. #8248). Leave vessel in a 37ºC incubator overnight (or for at least 2 hours).
2. Prepare complete medium. Decontaminate the external surfaces of medium bottle and medium supplement tubes with 70% ethanol and transfer them to a sterile field. Aseptically transfer supplement to the basal medium with a pipette. Rinse the supplement tube with medium to recover the entire volume.
3. Aspirate the fibronectin solution and add 20 ml of complete medium to the culture vessel. The fibronectin solution can be reused twice. Leave the vessel in the sterile field and proceed to thaw the cryopreserved cells.
4. Place the frozen vial in a 37ºC water bath. Hold and rotate the vial gently until the contents completely thaw. Promptly remove the vial from the water bath, wipe it down with 70% ethanol, and transfer it to the sterile field.
5. Carefully remove the cap without touching the interior threads. Gently resuspend and equally dispense the contents of the vial into the 2 equilibrated, fibronectin-coated culture vessels.

   Note: Dilution and centrifugation of cells after thawing are not recommended as these actions are more harmful to the cells than the effect of residual DMSO in the culture. It is also important that cells are plated in fibronectin-coated culture vessels to promote cell attachment.
6. Replace the cap or lid of the culture vessels and gently rock the vessels to distribute the cells evenly. Loosen cap, if necessary, to allow gas exchange.
7. Return the culture vessel to the incubator.
8. Do not disturb the culture for at least 16 hours after the culture has been initiated. Refresh culture medium the next day to remove residual DMSO and unattached cells.

Maintaining the culture:

1. Change the medium every 2 to 3 days thereafter, until the culture is approximately 70% confluent.

2. Once the culture reaches 70% confluency, change medium every other day until the culture is approximately 90% confluent.
3. Use directly for experiments.

   Note: We do not recommend cryopreservation of primary cells by the end user. Refreezing cells may damage them and affect cell performance. ScienCell does not guarantee primary cells cryopreserved by the end user.

Caution: Handling animal-derived products is potentially biohazardous. Always wear gloves and safety glasses when working with these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination [1].

[1] Grizzle WE, Polt S. (1988) "Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues." *J Tissue Cult Methods*. 11: 191-9.