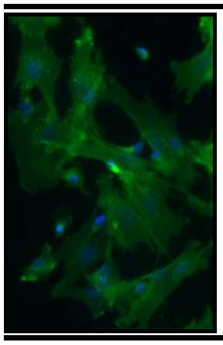
REF: P10536

MESENCHYMAL STEM CELL SYSTEM

RAT MESENCHYMAL STEM CELLS – BONE MARROW



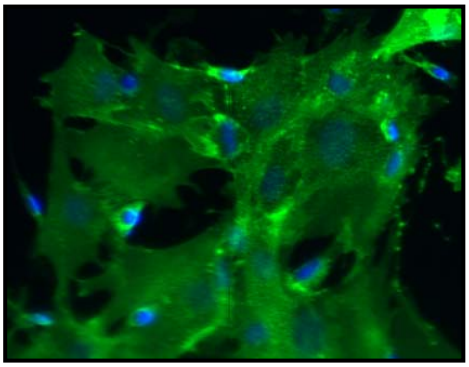
Product Type:	Cryo-preserved Mesenchymal Stem Cells
Catalog Number:	P10536
Source:	Rat Bone Marrow
Number of Cells:	5 x 10^5 Cells / vial (1ml)
Storage:	Liquid Nitrogen

Rat mesenchymal stem cells provided by Innoprot are isolated from bone marrow aspirates from femur of male Sprague-Dawley rats. The cells are cultured during 5 days prior to cryopreservation. RMSC-bm are guaranteed to further culture in the conditions described in the technical sheet.

Mesenchymal stem cells (MSC) are well-characterized population of adult stem cells. They have the potential to develop into mature cells that produce fat, cartilage, bone, tendons, and muscle. 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.

Recommended Medium

- Mesenchymal Stem Cell Medium (Reference: P60115)



Product Characterization

Immunofluorescent method

- CD90
- CD105

The cells test negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi

Product Use

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in vitro diagnostic or clinical procedures.

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INSTRUCTIONS FOR CULTURING CELLS

IMPORTANT: 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 fibronectin-coated culture vessel (2 µg/cm², T-75 flask is recommended). Add 5 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. #P8248). Leave the vessel in a 37°C incubator overnight.

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 fibronectin solution and add 15 ml of complete medium to the culture vessel. The fibronectin solution can be used twice. Leave the vessel in the sterile field and proceed to thaw the cryopreserved cells.

4. Place the vial in a 37°C waterbath. Hold and rotate the vial gently until the contents are completely thaw. Remove the vial from the waterbath immediately, wipe it dry, rinse the vial with 70% ethanol and transfer it to a sterile field.

5. Remove the cap, being careful not to touch the interior threads with fingers. Gently resuspend and dispense the contents of the vial into the equilibrated, fibronectin-coated culture vessel. A seeding density higher than 5,000 cells/cm² 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 fibronectin-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 fibroblasl-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. Refresh supplemented culture medium the next morning after establishing a culture from cryopreserved cells.

2. Change the medium every 48 hours thereafter, until the culture is approximately 50% confluent.

3. Once the culture reaches 50% confluence, change medium every day until the culture is approximately 90% confluent.

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Subculture:

1. Subculture the cells when they are over 90% confluent.

2. Prepare fibronectin-coated culture vessels (2µg/cm²) one day before subculture.

3. Warm medium, trypsin/EDTA solution, trypsin neutralization solution, and DPBS to room temperature. We do not recommend warming the reagents and medium at 37°C waterbath prior to use.

4. Rinse the cells with DPBS.

5. Add 10 ml of DPBS and then 2 ml of T/E solution into flask (in the case of a T-75 flask). Gently rock the flask to ensure complete coverage of cells by T/E solution. Incubate the flask in a 37°C incubator for 1 to 2 minutes or until cells completely round up. Use a microscope to monitor the change in cell morphology.

6.During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine serum.

7. Transfer T/E solution from the flask to the 50 ml centrifuge tube (a small percent of cells may detach) and continue to incubate the flask at 37°C for another 1 minute (no solution in the flask at this moment).

8. At the end of incubation, gently tap the side of the flask to dislodge cells from the surface. Check under a microscope to make sure that all cells detach.

9. Add 5 ml of TNS solution to the flask and transfer detached cells to the 50 ml centrifuge tube. Rinse the flask with another 5 ml of TNS to collect the residual cells.

10. Examine the flask under a microscope for a successful cell harvest by looking at the number of cells being left behind; there should be less than 5%.

11. Centrifuge the 50 ml centrifuge tube at 1000 rpm for 5 minutes. Resuspend cells in culture medium.

12. Count and plate cells in a new fibronectin-coated culture vessel with the recommended cell density.

Caution: *Handling human derived products is potentially bioharzadous. Although each cell strain testes negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions mush 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 human 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).

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