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Mouse Mesenchymal Stem Cells-Bone Marrow (MMSC-bm)

Catalog Number	10MU-042	**Cell Number**	0.5 million cells/vial
Species	*Mus musculus*	**Storage Temperature**	Liquid Nitrogen

Description

Mesenchymal stem cells (MSC) derived from bone marrow are a well-characterized population of adult stem cells. MSC have the capability for renewal and differentiation into various lineages of mature cells that produce fat, cartilage, bone, tendons, and muscle. These properties, in combination with their developmental plasticity, have generated tremendous interest in regenerative medicine to replace damaged tissues. These findings have spurred the development of MSC-based therapies for treating wide range of non-skeletal diseases [1,2] .

iXCells Biotechnologies offers MMSC-bm isolated from mouse bone marrow. Each vial contains >0.5 million cells. These cells are expanded in Mesenchymal Stem Cell Medium (Cat# MD-0037) and then cryopreserved at passage 2. iXCells MMSC-bm characterized by immunofluorescence and flow cytometry are strongly positive for MSC marker CD29 [Figure 1]. These cells can further be differentiated into adipocytes using Adipocyte Differentiation Medium (Cat# MD-0005) and into osteoblasts using Osteogenic Differentiation Medium (Cat# MD-0006) [Figure 2 and Figure 3]. MMSC-bm are negative for mycoplasma, bacteria, yeast, and fungi and can be expanded for no more than 3 passages in iXCells Mesenchymal Stem Cell Medium.

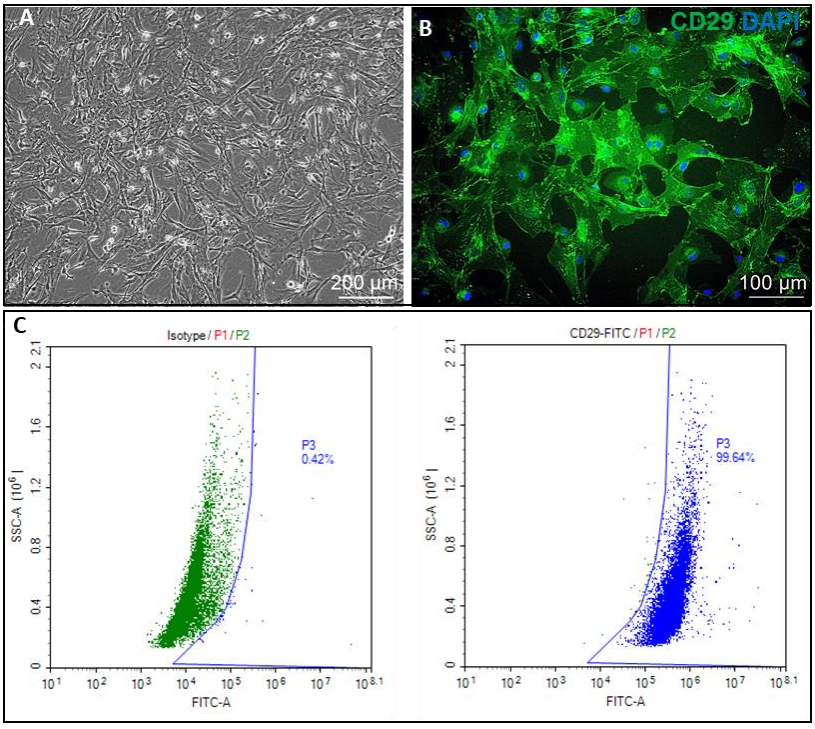

Figure 1. (A) Phase contrast image of MMSC-bm (B) Immunofluorescence staining with antibody against CD29. (C) Flow cytometric analysis shows >90% CD29 positive cells.

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Product Details

Tissue	Mouse bone marrow
Package Size	0.5 million cells/vial
Passage Number	P2
Shipped	Cryopreserved
Storage	Liquid nitrogen
Growth Properties	Adherent
Media	Mesenchymal Stem Cell Medium (Cat# MD-0037) Adipocyte Differentiation Medium (Cat# MD-0005) Osteogenic Differentiation Medium (Cat# MD-0006)

Protocols

Thawing of Frozen Cells

1. Upon receipt of the frozen MMSC-bm, it is recommended to thaw the cells and initiate the culture immediately in order to retain the highest cell viability.
2. To thaw the cells, put the vial in 37°C water bath with gentle agitation for ~2 minutes. Keep the cap out of water to minimize the risk of contamination.
3. Pipette the cells into a 15 mL conical tube with 5 mL fresh **Mesenchymal Stem Cell Medium** (Cat# MD-0037).
4. Centrifuge at 1,000 rpm (~220 g) for 5 minutes under room temperature.
5. Remove the supernatant and re-suspend the cells in fresh Mesenchymal Stem Cell Medium (Cat# MD-0037).
6. Culture the cells in one T75 flask. Change medium every 3~4 days until the cells reach about 80-90% confluence.

   Safety Precaution*: it is highly recommended that protective gloves and clothing should be used when handling frozen vials.*

Standard Culture Procedure

1. Mouse Bone Marrow Mesenchymal Stem Cell (MMSC-bm) can be subcultured in **Mesenchymal Stem Cell Medium** (Cat# MD-0037).
2. When cells reach ~80-90% confluence, aspirate the culture medium and wash the cells with sterile PBS (5 mL/T75 flask).
3. Add ~2 mL of prewarm 0.25% Trypsin-EDTA to the flask and incubate for ~3 minutes at 37°C. When majority of cells have detached from the surface, neutralize the enzyme by adding 2-3 volumes of Mesenchymal Stem Cell Medium (MD-0037).
4. Gently pipette and collect the dissociated cells into a sterile centrifuge tube.

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5. Add another 3 mL of cell culture medium to dislodge the remaining cells and transfer detached cells to the same tube
6. Centrifuge the tube at 1,000 rpm (~220 g) for 5 minutes and re-suspend the cells in desired volume of medium.
7. Count and seed the cells at the recommended cell density (5,000-7000 viable cells/cm^2). Change the medium every 3-4 days until the cells are confluent.

Safety Precaution: *it is highly recommended that protective gloves and clothing should be used when handling frozen vials.*

Adipocytes Differentiation Protocol

1. Culture MMSC-bm in a 6-well plate in **Mesenchymal Stem Cell Medium** (Cat# MD-0037) until cells reach >95% confluence.
2. Aspirate the growth medium and replace with 1.5 mL fresh growth medium/well. Let the cells growth for 2-3 additional days.

   Note*: Cells at this stage may detach from dish easily, so do not use pump to aspirate off the medium at this step. Use pipet and slowly remove the medium instead. Add Adipocyte Differentiation Medium very gently to avoid cell detachment.*
3. Aspirate the medium, apply 1.5 mL **Adipocyte Differentiation Medium** (Cat# MD-0005) per well.
4. Change adipocytes differentiation medium every 3~4 days for up to 2 weeks. The accumulation of lipid droplets in cytoplasm will appear after 1 week which can be analyzed by Oil Red O staining (Figure 2).

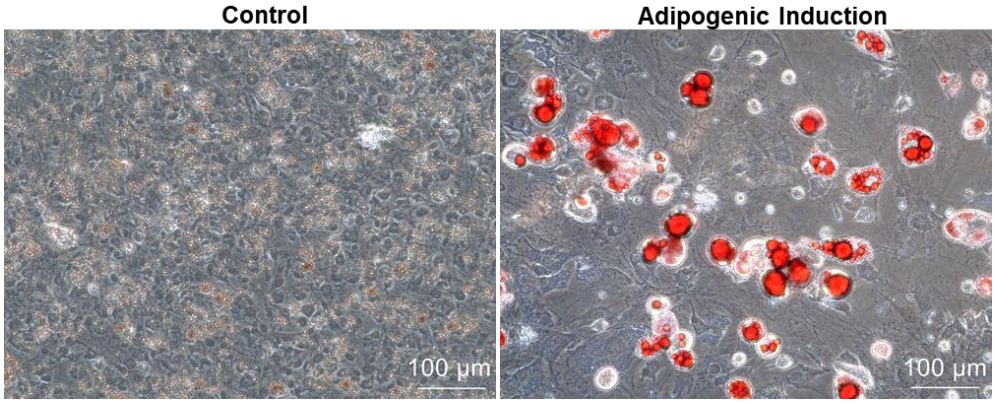

Figure 2. MMSC-bm adipocyte differentiation (Day 14 post adipogenic induction).

Osteogenic Differentiation Protocol

1. Grow MMSC-bm in a 6-well plate in **Mesenchymal Stem Cell Medium** (Cat# MD-0037) to ~80% confluency.
2. Carefully aspirate growth medium, apply 1.5 mL **Osteogenic Differentiation Medium** (Cat# MD-0006) per well.
3. Change fresh Osteogenic Differentiation Medium every 3 days. Be careful not to disturb the cell monolayer.
4. Culture the cells for 3-4 weeks and osteoblasts can be detected by Alizarin Red S staining (Figure 3).

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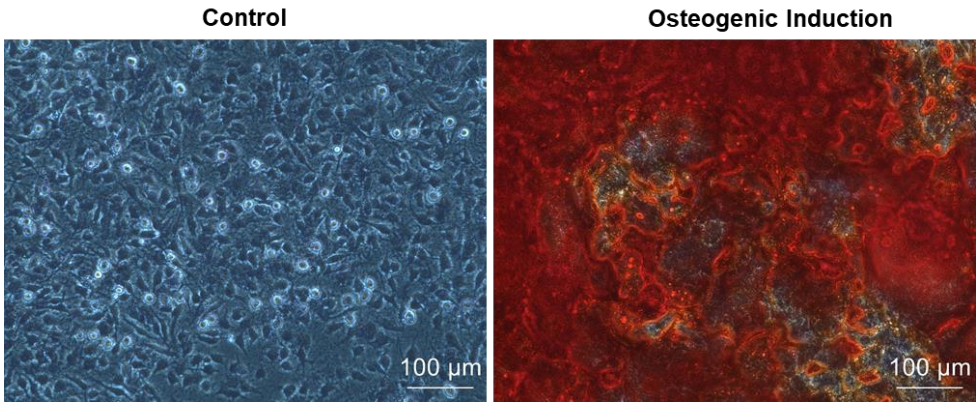

Figure 3. MMSC-bm osteogenic differentiation (Day 27 post osteogenic induction). Alizarin Red S staining of osteoblasts. The extracellular calcium deposit was stained in bright orange-red color.

References

[1] Huang S, Xu L, Sun Y, Wu T, Wang K, Li G. An improved protocol for isolation and culture of mesenchymal stem cells from mouse bone marrow. J Orthop Translat. 2014, 27;3(1):26-33.

[2] García-Gómez I, Elvira G, Zapata AG, Lamana ML, Ramírez M, Castro JG, Arranz MG, Vicente A, Bueren J, García-Olmo D. Mesenchymal stem cells: biological properties and clinical applications. Expert Opin Biol Ther. 2010,10(10):1453-68.

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