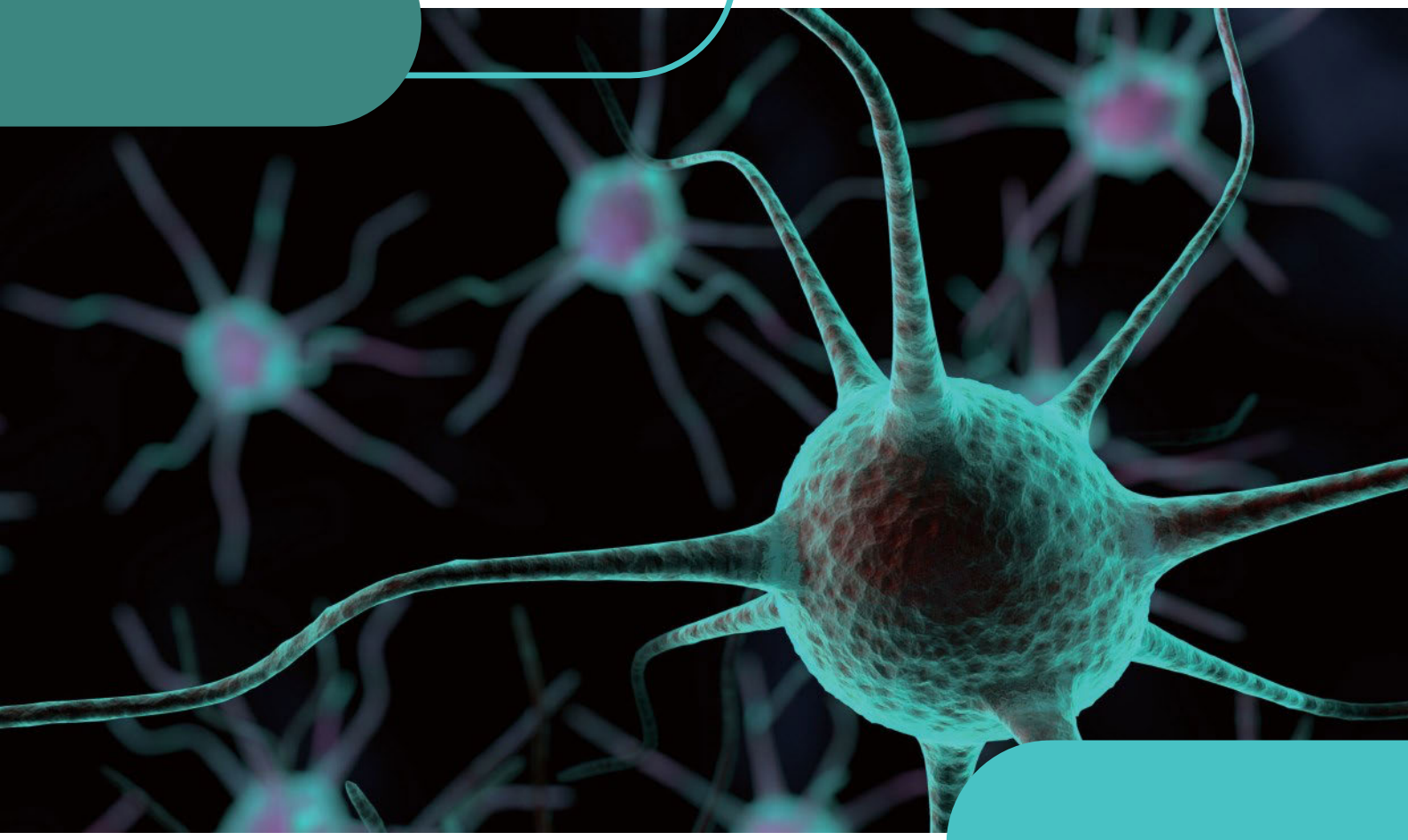

Creative Bioarray

Mesenchymal Stem Cell Characterization Services

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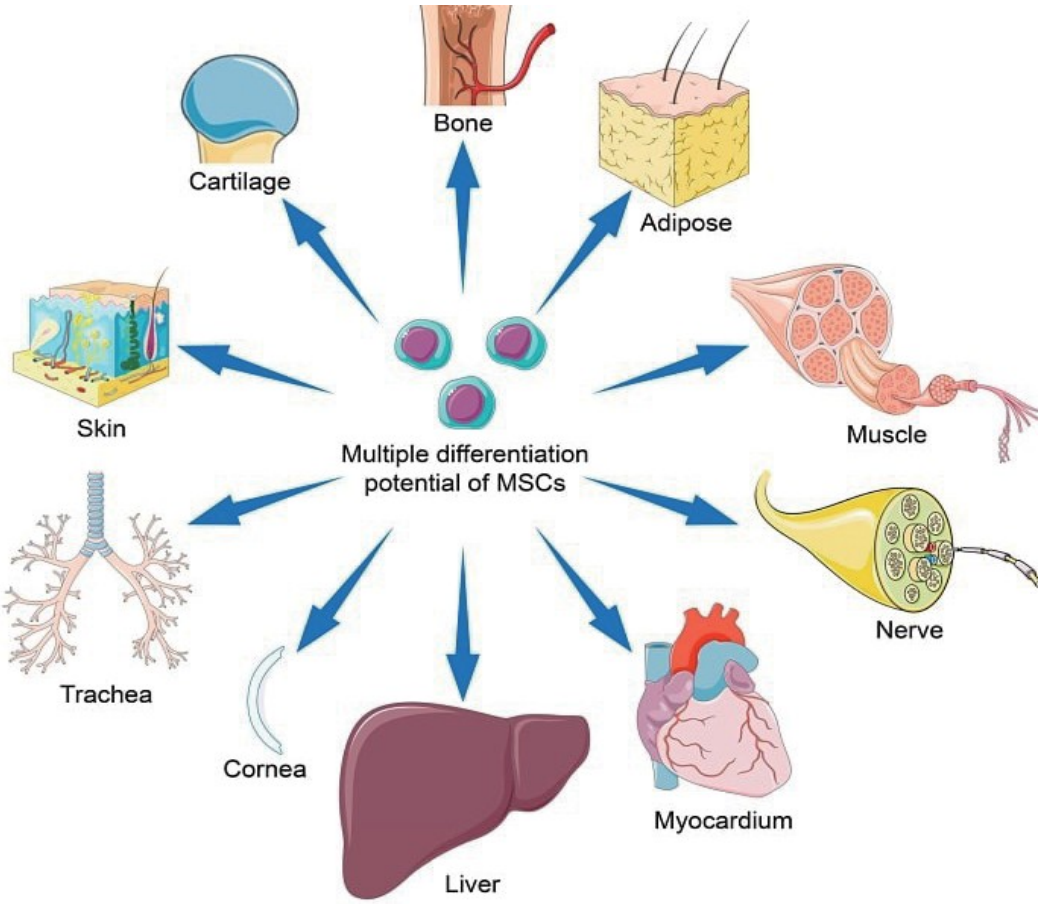
About Mesenchymal Stem Cell

Human multipotent mesenchymal stromal cells (MSCs, also termed mesenchymal stem cells) are a heterogeneous population of plastic-adherent, fibroblast-like cells that can self-renew and differentiate into bone, adipose, and cartilage tissue in culture. In recent years there has been increased interest in MSCs and their potential therapeutic applications in both tissue engineering and repair.

The MSC phenotype can vary with tissue source and any differences in cell isolation and culture procedures used. This phenotypic variability underscores the importance of characterizing MSCs, such as by assessing their ability to expand and differentiate, as well as their immunomodulatory capacity. Characterization is particularly important when considering MSCs for therapeutic applications, such as cell therapy.



Mesenchymal Stem Cell Self-Renewal

Quantitatively assess your MSC samples for their proliferation and doubling rates using our proliferation assay. In this assay, MSCs are expanded for 8 passages in an animal component-free media formulation and then assessed for growth or doubling time.

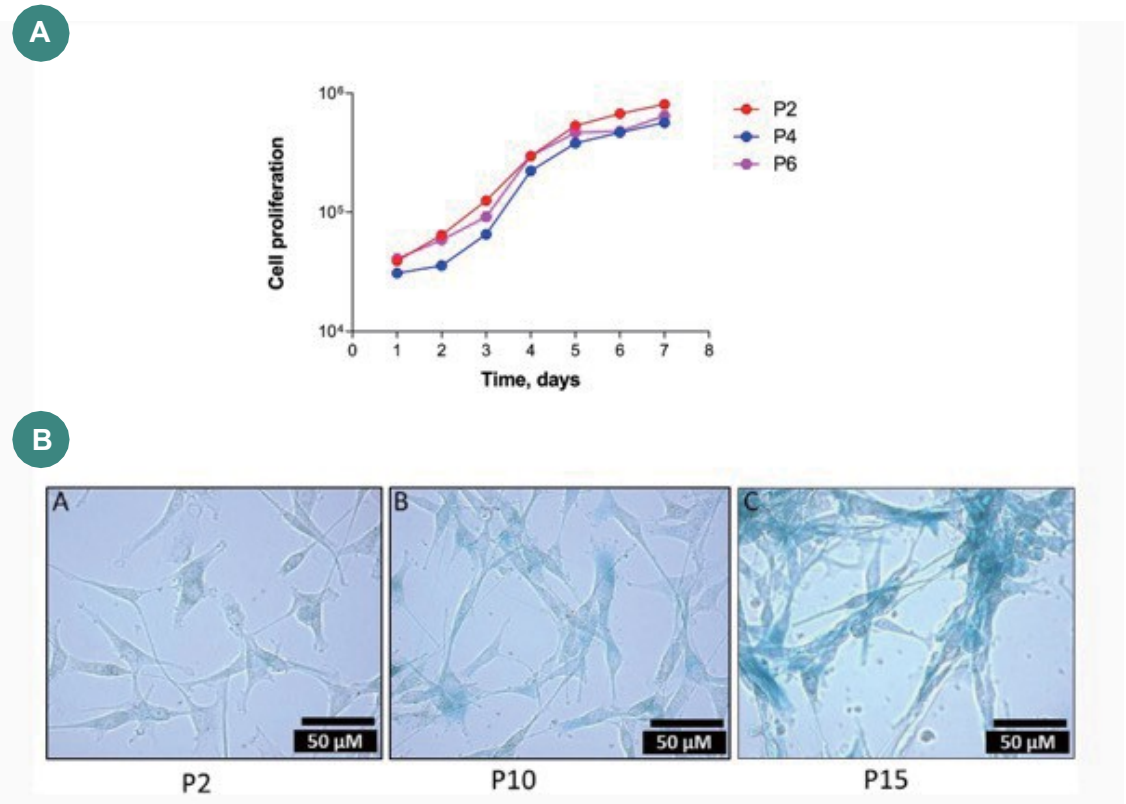


Figure 1. Growth and the proliferation doubling time of the isolated MSCs. A Growth curve of cell in P2, P4, and P6; X, axis culture time in days; y, axis mean ± SEM of the cell count. B MSCs senescence. à-Galactosidase staining (blue) of MSCs at P2, P10, and P15. P2 cell line was used as a negative control of the à-galactosidase staining.

Surface Marker Characterization

Human MSCs must express the following positive and negative surface markers analyzed by flow cytometry or immunocytochemistry: ≥ 95% Positive: CD105, CD73, CD90 and ≤ 2% Negative: CD45, CD34, CD14, CD19, HLA-DR.

Table 1. List of cell surface antigens recommended by the ISCT for identifying MSCs.

	Surface Antigen	Cells Expressing
Positive Markers	CD105	Vascular homeostasis; modulates TGFβ functions via interaction with TGFβ RI and TGFβ RII
	CD73	Catalyzes production of extracellular adenosine from AMP
	CD90	Wound repair, cell-cell matrix interactions
Negative Markers	CD45	Pan-leukocyte marker
	CD34	Primitive hematopoietic progenitors and endothelial cells
	CD14 /CD11b	Monocytes and macrophages
	CD79α/CD19	B cells
	HLA-DR	Antigen-presenting cells and lymphocytes

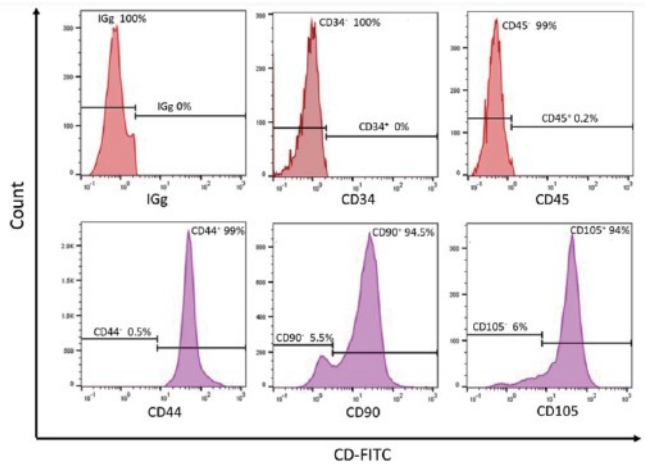


Figure 2. Flow cytometry analysis of the surface molecule markers. Positive expression of mesenchymal stem cell markers (CD44, CD90, and CD105) and negative hematopoietic markers (CD34 and CD45) in MSCs at passage 4.

In Vitro MSC Differentiation Assays

Evaluate the differentiation potential of your MSCs by using our suite of in vitro differentiation assays. Whether differentiating your MSCs into adipocytes, osteocytes, or chondrocytes, you can create efficiencies in your workflow by partnering with our in-house experts for your assay needs.

Adipogenic Differentiation Assay:

Using Adipogenic Differentiation Medium (Human) to culture and differentiate your MSC samples into the adipogenic lineage. The differentiation is visualized by Oil Red O staining, which detects the presence of lipids in adipogenic cells. The dye is extracted from the cultures to quantify the level of adipogenic differentiation.

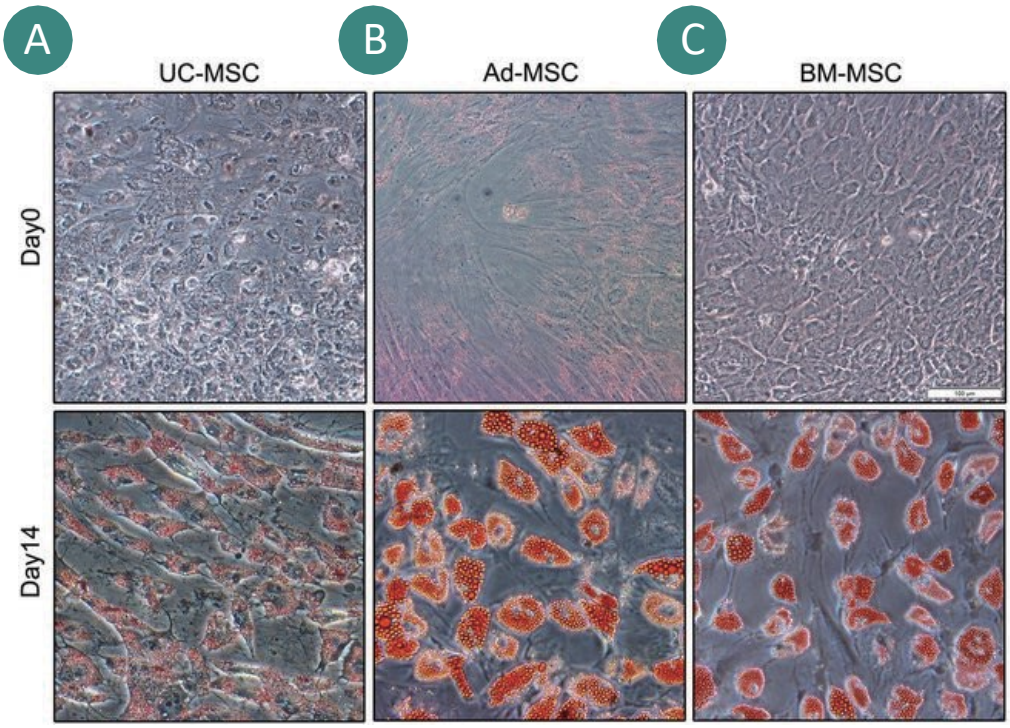


Figure 3. Comparison of adipogenic differentiation in the three tissues-derived MSCs by oil red O staining. UC-MSC, Ad-MSC and BM-MSC were incubated with or without adipogenic medium for 14 days. Then the cells were stained with oil red O and captured using the IX71 Olympus microscope.

Osteogenic Differentiation Assay:

Using Osteogenic Differentiation Kit (Human) to culture and differentiate your MSC samples into the osteogenic lineage. The differentiation is visualized by Alizarin Red staining, which detects calcium deposits during mineralization of the osteogenic cells. The dye is extracted from the cultures to quantify the level of osteogenic differentiation. Osteogenic differentiation is achieved in 14 - 21 days.

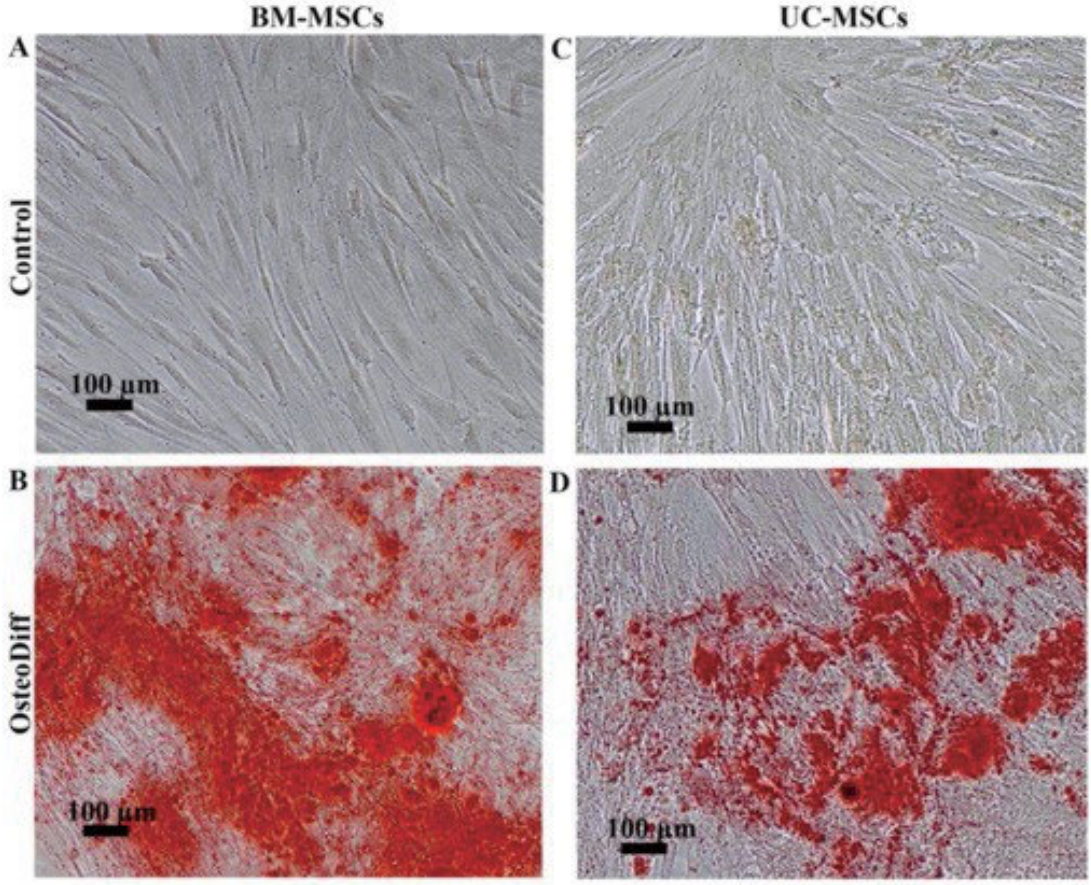


Figure 4. Osteogenic differentiation potential of umbilical cord-derived mesenchymal stromal cells (UC-MSCs) in comparison to bone marrow-derived mesenchymal stromal cells (BM-MSCs).

Chondrogenic Differentiation Assay:

Using animal component-free Chondrogenic Differentiation Medium to culture and differentiate your MSC samples into the chondrogenic lineage. The differentiation and the formation of bone cartilage is visualized by Alcian Blue, which detects aggrecan within the cartilage matrix.

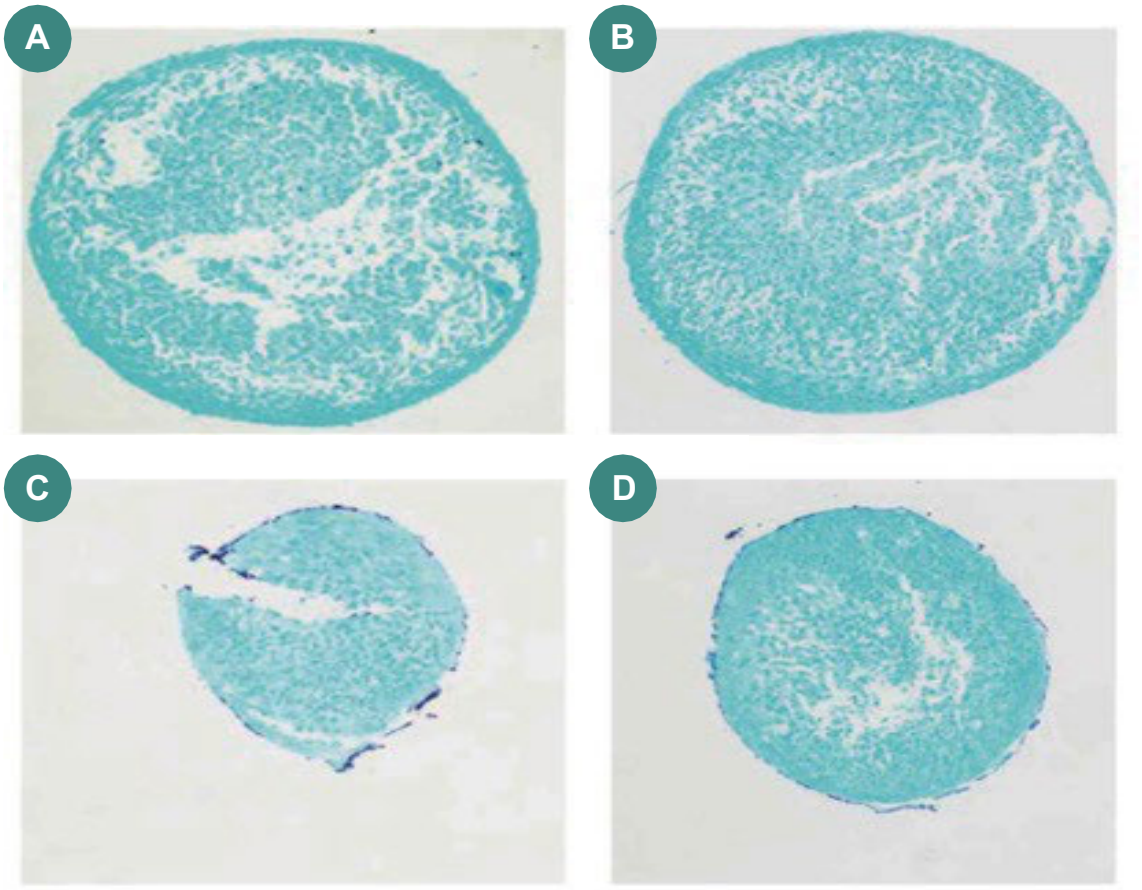


Figure 5. Alcian Blue staining in cell pellets generated by chondrogenic differentiation following mesenchymal stem cells for 7 days.

MSC T Cell Suppression Assay

MSCs are endowed with remarkable immunoregulatory properties, which make them ideal candidates for cellular therapies. The ability of MSCs to modulate the responses of various immune cell types can be observed in vitro, in co-cultures.

Evaluate the immunosuppressive function of your MSC sample using our suppression assay, where CD4+ T cells are stimulated to proliferate in the presence of MSCs for 4 - 5 days. After the stimulation, the responder cell proliferation is assessed by flow cytometry.

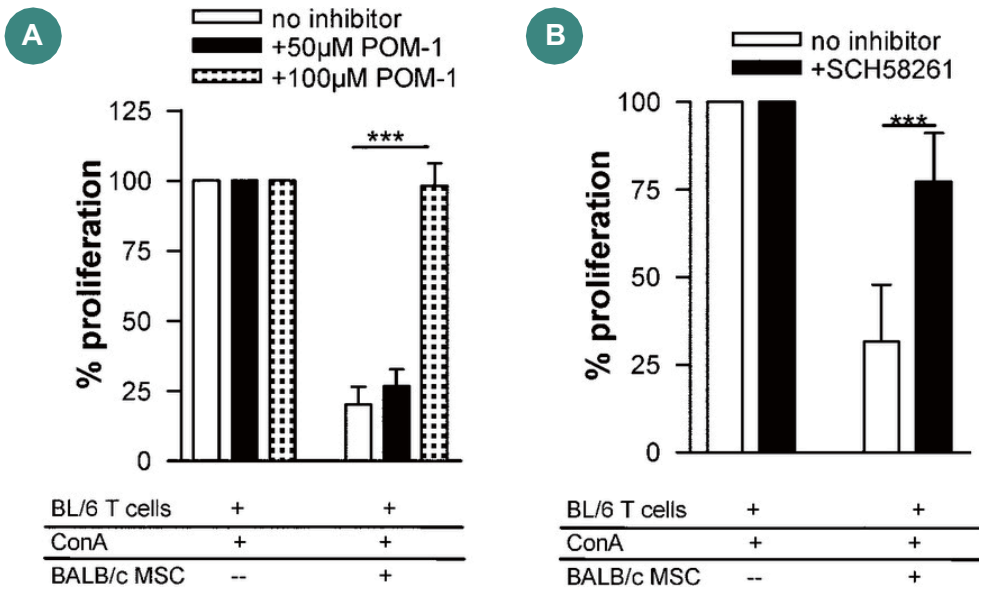


Figure 6. MSC suppress T-cell proliferation by generation of adenosine.