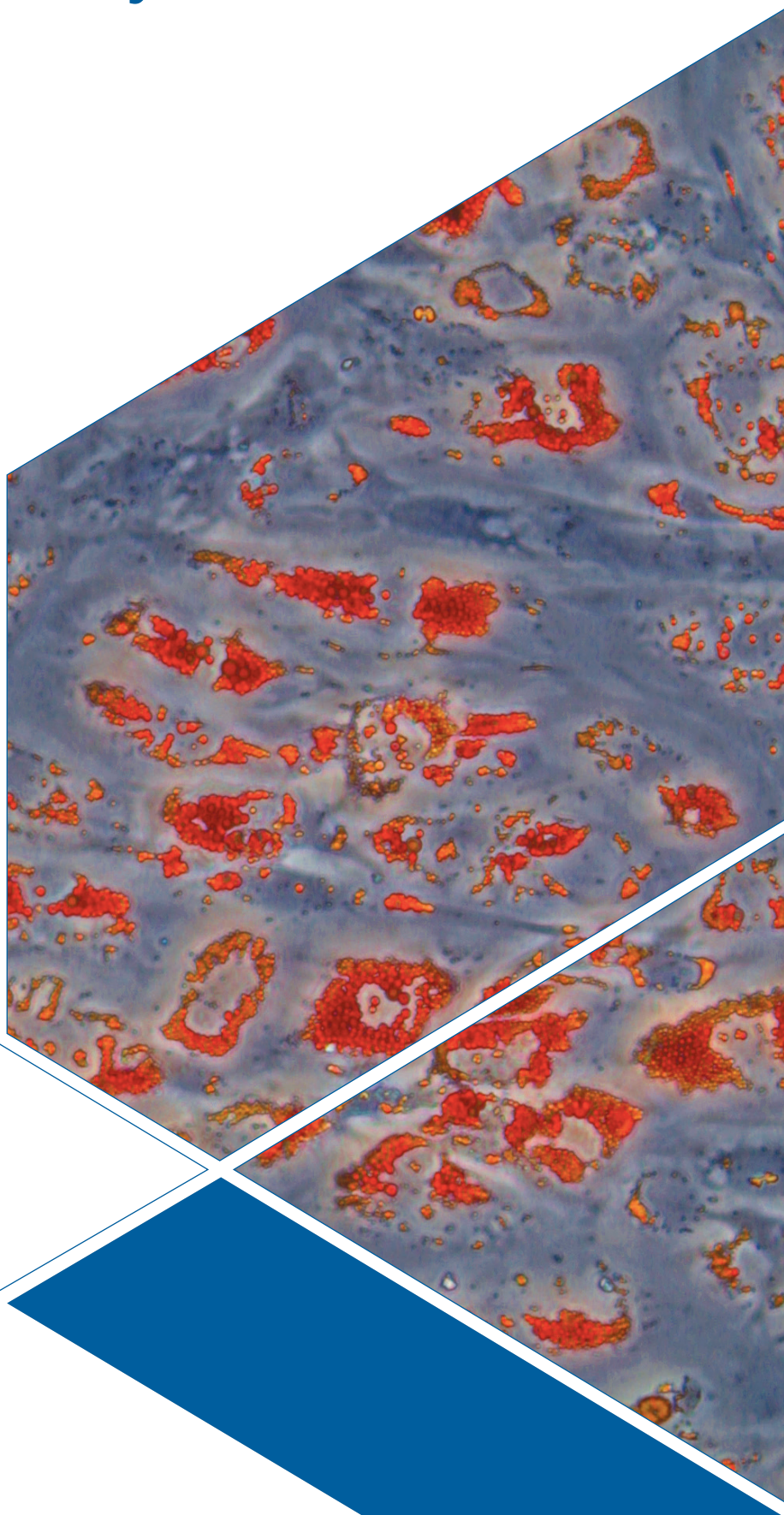
R&D SYSTEMS
a bio-techne brand

Mesenchymal Stem Cells



ISOLATE & CULTURE

VERIFY

DIFFERENTIATE

INVESTIGATE

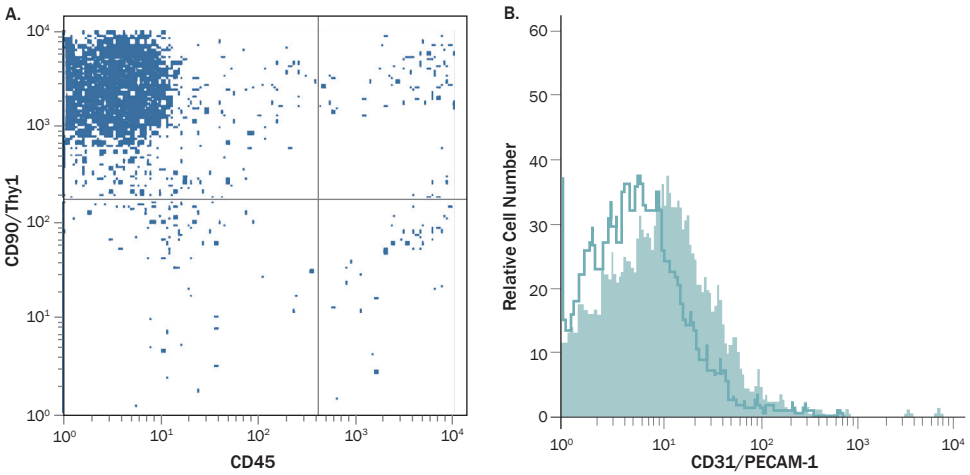
ISOLATE AND CULTURE

A number of protocols are available to isolate MSCs, however each can vary in the yield, purity, quality, and the ability of the cells to proliferate. It is important to begin experimentation with a verified, homogeneous, multipotent MSC starting population to ensure confidence in differentiation and subsequent data interpretation. With this necessity in mind, R&D Systems offers a variety of products to establish and maintain a homogenous starting MSC population, including purified MSCs, MSC isolation kits, and a range of media for MSC expansion.

Product	Description	Catalog #
Rat Mesenchymal Stem Cells	$CD90^+$, $CD45^-$, $CD31^-$	PSC003
StemXVivo® Xeno-Free Human MSC Expansion Media	Free of non-human animal-derived components	CCM021
StemXVivo® Serum-Free Human MSC Expansion Media	Serum free expansion media	CCM014
StemXVivo® Mesenchymal Stem Cell Expansion Media	Media for human, mouse, and rat MSCs	CCM004
CryoDefend®-Stem Cells Media	For defined MSC cryopreservation	CCM018

Rat Mesenchymal Stem Cells (Catalog # PSC003)

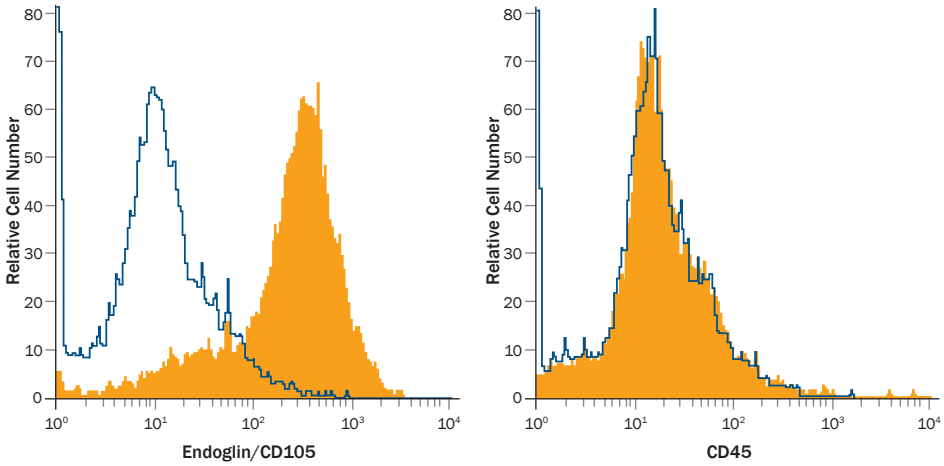
- **Verified** – confirmed phenotype of $CD90^+$, $CD45^-$, $CD31^-$
- **Multipotent** – confirmed differentiation to adipocytes, chondrocytes, and osteocytes
- **High purity** – homogeneous population reduces experimental variation
- **Ready to use** – free of mycoplasma and microbial contamination



Rat MSCs Show Positive Expression of CD90/Thy1 and Negative Expression of CD45 and CD31. (**A**) Rat MSCs (Catalog # PSC003) were stained for the indicated markers after passage 5. CD90/Thy1 was detected using an Anti-Rat CD90/Thy1-PE Antibody, CD45 was detected using an Alexa Fluor® 647-conjugated Anti-Rat CD45 Antibody. (**B**) CD31 was detected using a Goat Anti-Mouse CD31/PECAM-1 Affinity Purified Polyclonal Antibody (Catalog # AF3628) (filled histogram) or a Normal Goat IgG isotype control (Catalog # AB-108-C, open histogram) followed by a PE-conjugated Donkey Anti-Goat Secondary Antibody (Catalog # F0107).

StemXVivo® MSC Expansion Media

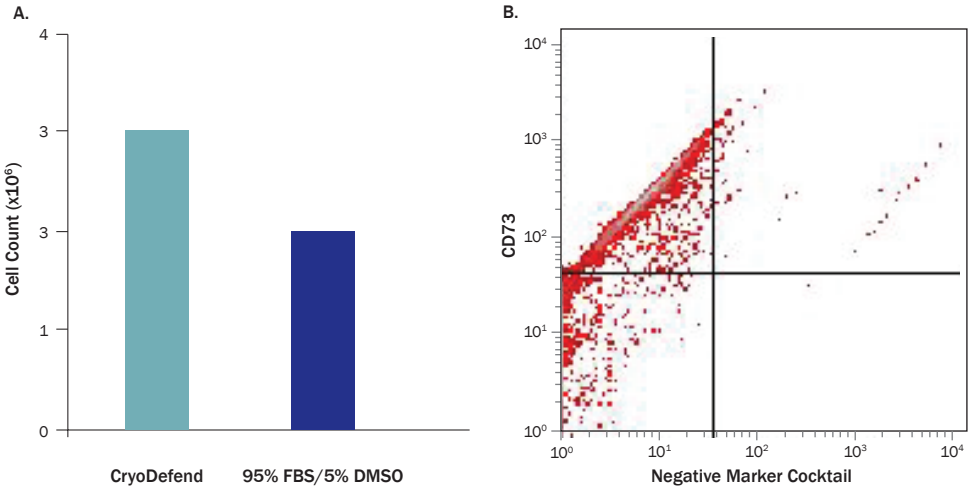
- **Reliable** – StemXVivo media products are specifically designed and validated for stem cell culture
- **Consistent** – tested for lot-to-lot consistency
- **Flexible** – available as xeno-free, serum-free, or serum-containing media



Phenotypic Analysis of Human MSCs Expanded in StemXVivo® MSC Expansion Media. Human MSCs were expanded using StemXVivo® Mesenchymal Stem Cell Expansion Media (Catalog # CCM004). Filled histograms indicate cells stained with markers of undifferentiated MSCs including PE-conjugated Mouse Anti-Human Endoglin/CD105 Monoclonal Antibody (Catalog # FAB10971P) or PE-conjugated Mouse Anti-Human CD45 Monoclonal Antibody (Catalog # FAB1430). The open histograms show isotype-matched control staining. MSCs appropriately lack expression of CD45.

CryoDefend®-Stem Cells Media

- **Robust** – greater recovery of viable MSCs compared to conventional media
- **Flexible** – available as serum-free or fully defined, protein free cryopreservation media
- **Validated** – specifically designed and validated for stem cell culture



Recovery and Marker Expression of Rat MSCs Cryopreserved in CryoDefend®-Stem Cells Media. Rat MSCs (Catalog # PSC003) were cryopreserved in CryoDefend®-Stem Cells Media (Catalog # CCM018) or conventional freezing media (95% FBS/5% DMSO) at 0.7×10^6 cells/vial. The cryopreserved cells were thawed, cultured for four days, and then assessed for recovery and marker expression. **A**) The number of cryopreserved cells recovered after four days in culture. **B**) CryoDefend®-Stem Cells preserved MSCs stain positive for APC-conjugated Mouse Anti-Human CD73 Monoclonal Antibody and negative for the PE-conjugated antibodies contained in the Mesenchymal Stem Cell Verification Flow Kit's (Catalog # FMC020) negative marker cocktail.

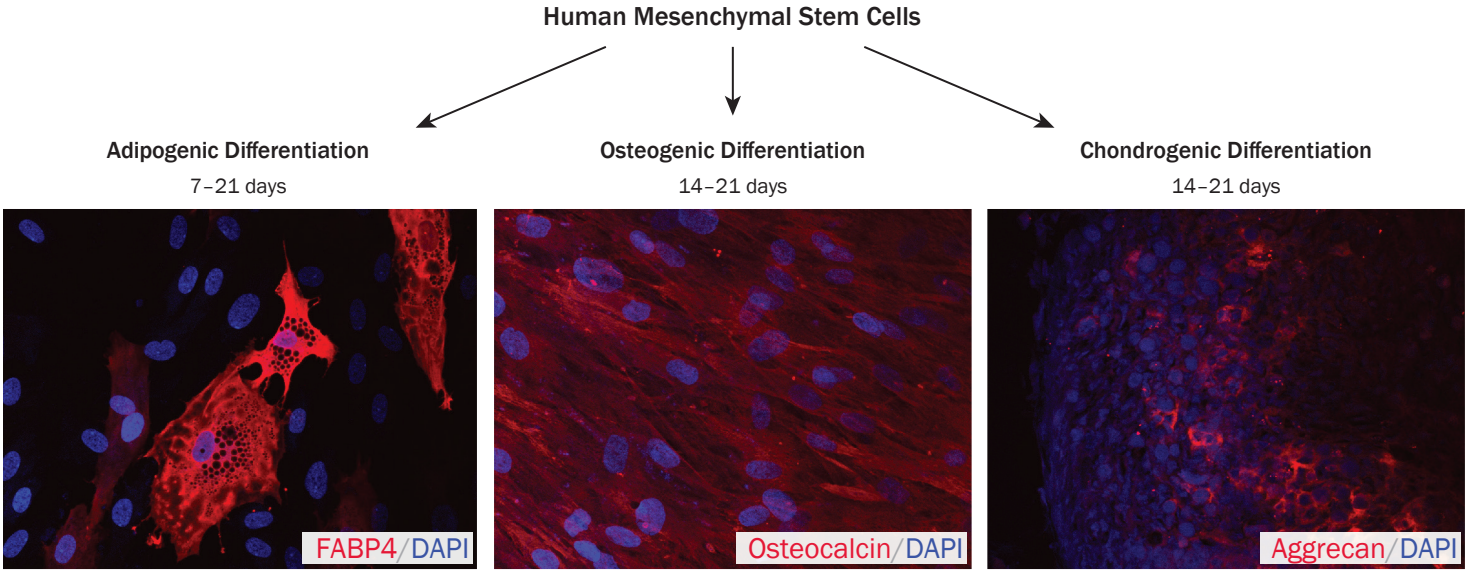
VERIFY

Throughout the expansion and differentiation of MSCs it is important to be confident in the starting populations multipotency. Beginning an experiment with suboptimal, unverified populations will put the investigator at risk for inconsistent results in their downstream experiments, thus wasting time and reagents. R&D Systems offers a series of all-in-one kits that verify MSC mulitpotency through functional differentiation or cell-specific marker expression.

Product	Catalog #
Human Mesenchymal Stem Cell Functional Identification Kit	SC006
Mouse Mesenchymal Stem Cell Functional Identification Kit	SC010
Rat Mesenchymal Stem Cell Functional Identification Kit	SC020
Human Mesenchymal Stem Cell 4-Color Flow Kit	FMC002
Human Mesenchymal Stem Cell Verification Flow Kit	FMC020
Mouse Mesenchymal Stem Cell 4-Color Flow Kit	FMC003

Mesenchymal Stem Cell Functional Identification Kits

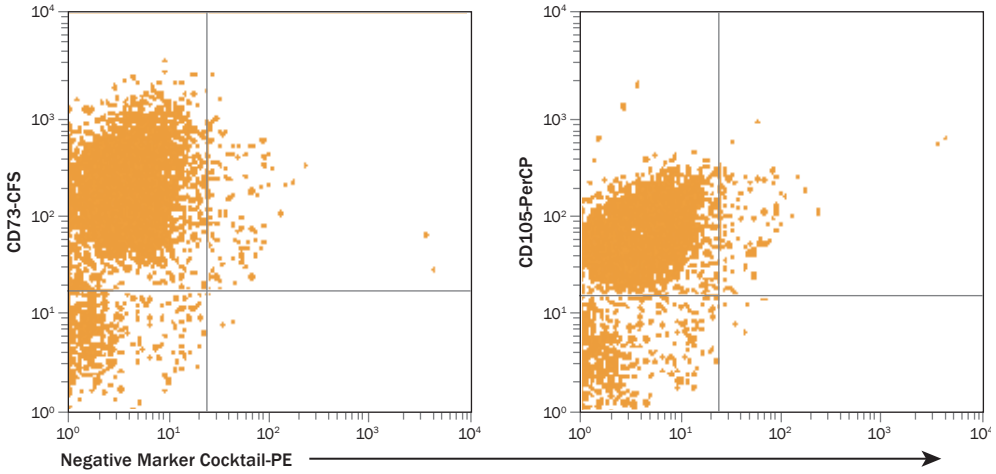
- **Reliable** – induces MSC trilineage differentiation with kit-provided supplements
- **Complete** – contains antibodies to confirm successful differentiation
- **Compliant** – defines human MSCs according to International Society for Cellular Therapy (ISCT) recommendations
- **Flexible** – available for verification of human, mouse, and rat MSCs



Verification of Multipotency using the Human Mesenchymal Stem Cell Functional Identification Kit. Human MSCs were cultured in StemXVivo® Mesenchymal Stem Cell Expansion Media (Catalog # CCM004) and differentiation was induced as indicated using the media supplements included in the Human Mesenchymal Stem Cell Functional Identification Kit (Catalog # SC006). The kit also contains a Goat Anti-Mouse FABP-4 Antigen Affinity-purified Polyclonal Antibody (adipocytes), a Mouse Anti-Human Osteocalcin Monoclonal Antibody (osteocytes) and a Goat Anti-Human Aggrecan Antigen Affinity-purified Polyclonal Antibody (chondrocytes) for the confirmation of differentiation status. The cells were stained using the NorthernLights™ 557-conjugated Donkey Anti-Goat (Catalog # NL001; red) or Anti-Mouse (Catalog # NL007; red) IgG Secondary Antibodies, and the nuclei were counterstained with DAPI (blue).

MSC Multi-Color Flow Cytometry Kits

- **Efficient** – simultaneously detect established positive and negative mulitpotency markers
- **Flexible** – available for verification of human and mouse MSCs
- **Compliant** – defines human MSCs according to International Society for Cellular Therapy (ISCT) recommendations

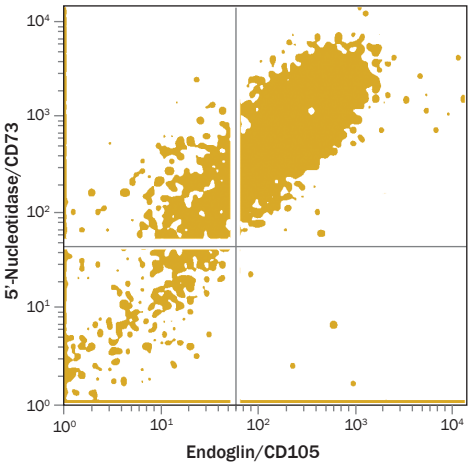


Human Bone-Marrow Derived Cells Fulfill the ISCT's Definition of Human MSCs Based on Marker Expression. Human bone marrow-derived MSCs were stained using the antibodies and reagents provided in the Human Mesenchymal Stem Cell Marker Verification Flow Kit (Catalog # FMC020). The data shows positive expression of MSC-associated surface antigens CD73 and CD105. In contrast, minimal expression of antigens recognized by the Negative Marker Cocktail was detected.

Individual Antibodies for MSC Characterization

Customize your MSC characterization by choosing your preferred R&D Systems® Antibodies.

- **Reliable** – specifically bind to lineage-committed bone marrow-derived cells
- **Flexible** – can be used with magnetic separation systems or with flow cytometry cell sorting for enrichment of uncommitted MSCs



Detection of Endoglin/CD105 and 5'-Nucleotidase/CD73 on Human MSCs. Human bone marrow-derived MSCs were stained for positive MSC markers using a APC-conjugated Mouse Anti-Human Endoglin/CD105 Monoclonal Antibody (Catalog # FAB10971A) and a PE-conjugated Mouse Anti-Human 5'-Nucleotidase/CD73 Monoclonal Antibody (Catalog # FAB5795P). Quadrants were set based on isotype controls.

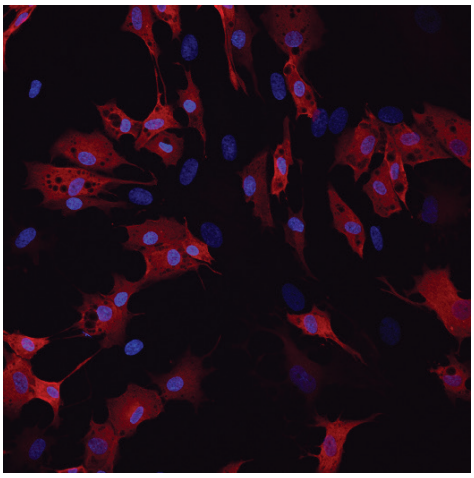
DIFFERENTIATE

Efficiently and consistently driving MSCs into adipocytes, chondrocytes, or osteocytes is essential for experimental productivity as well as for reducing costs and labor associated with the lengthy differentiation process. These challenges are remedied by our StemXVivo® line of base media and supplements, which contain defined, premium quality recombinant proteins to effectively drive MSC differentiation while limiting experimental variation.

StemXVivo® Media and Supplements

- **Flexible** – available for induction of adipocytes, chondrocytes, or osteocytes
- **User-defined** – combine specific base media and StemXVivo supplements
- **Optimized** – media and supplements developed to support MSC differentiation
- **Diverse** – differentiates MSCs from human, mouse, and rat

Product	Catalog #
Osteogenic/Adipogenic Base Media	CCM007
Chondrogenic Base Media	CCM005
Adipogenic Supplement	CCM011
Chondrogenic Supplements	CCM006, CCM020
Osteogenic Supplements	CCM008, CCM009



Rat MSCs were Differentiated into Adipocytes using StemXVivo® Adipogenic Media and Supplements. Rat MSCs were differentiated with StemXVivo® Osteogenic/Adipogenic Base Media and StemXVivo® Adipogenic Supplement. Adipocyte differentiation was confirmed by staining with a Goat Anti-Mouse FABP4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1443) followed by a NorthernLights 557-conjugated Donkey Anti-Goat Secondary Antibody (Catalog # NL001; red). Nuclei were counterstained with DAPI (blue).

Small Molecules for Differentiation

- **Precise** – Gain temporal control of differentiation pathways
- **Targeted** – Modulate cell fate by targeting specific signaling pathways
- **Defined** – Minimize the use of animal-derived factors

Compound	Use in Stem Cell Research	Catalog #
Indomethacin	Induces differentiation of MSCs into adipocytes	1708
Dexamethasone	Induces differentiation of human MSCs into adipocytes	1126
IBMX	Induces differentiation of MSCs into adipocytes	2845
Insulin (human) recombinant	Promotes adipogenesis	3435
Kartogenin	Potently induces chondrogenesis in MSCs	4513
AICAR	Induces differentiation of bone marrow-derived MSCs into osteoblasts	2840
GSA 10	Induces differentiation of MSCs into osteoblasts	4918
Purmorphamine	Induces differentiation of MSCs into osteoblasts	4551
SP 600125	Prevents differentiation of MSCs into osteocytes	1496
Zebularine	Induces cardiomyogenesis in MSCs	2293
5-Azacytidine	Induces cardiomyogenesis in MSCs	3842
Pioglitazone hydrochloride	Improves cardiomygenesis of MSCs	4124

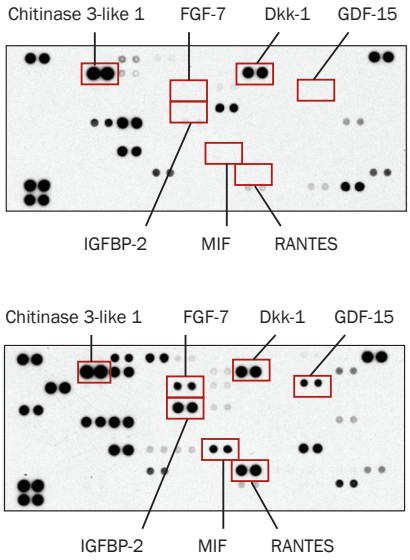
INVESTIGATE

After carefully validating MSC multipotency, efficiently expanding the cell population, and driving differentiation toward a desired cell lineage it is time to investigate the function of the terminally differentiated cells. Our Proteome Profiler™ Array Kits expedite protein analysis by eliminating the time-consuming gel electrophoresis and protein transfer steps required for a Western blot. Adipogenic, osteogenic, and chondrogenic analytes can be easily quantified using R&D Systems ELISAs, including the Quantikine® and DuoSet Development Kits®.

Proteome Profiler™ Antibody Array Kits

- **Rapid** – analyze the expression level of dozens of cytokines simultaneously
- **Economical** – contains 4 membranes – each cytokine is spotted in duplicate
- **Convenient** – no specialized equipment is required

Product	Catalog #
Proteome Profiler Human Adipokine Array Kit	ARY024
Proteome Profiler Mouse Adipokine Array Kit	ARY013
Proteome Profiler Rat Adipokine Array Kit	ARY016
Proteome Profiler XL Human Cytokine Array for Osteogenesis	ARY022



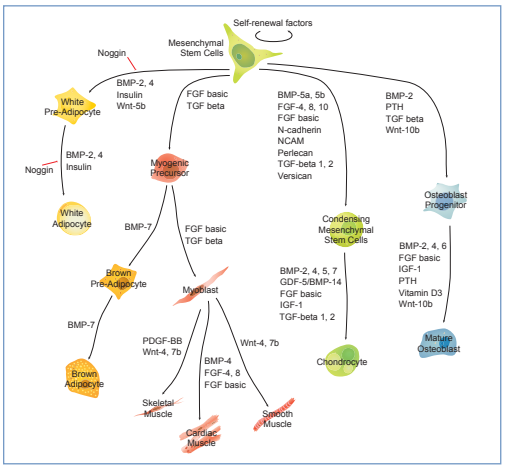
Expression of Osteoblast-Associated Proteins during Osteogenesis. Human bone marrow-derived MSCs were differentiated in culture using StemXVivo® Osteogenic/Adipogenic Base Media (Catalog # CCM007) and StemXVivo® Human Osteogenic Supplement (Catalog # CCM008). Nine days after the induction of osteogenesis, cell culture supernatants were used to assess expression of osteoblast-associated proteins using the Proteome Profiler Human XL Cytokine Array. The data show an expected increase in the expression of KGF/FGF-7, IGFBP-2, GDF-15, CCL5/RANTES, and MIF as cells differentiated into osteoblasts. Additionally, the data demonstrate that Chitinase 3-like 1 and Dkk-1 are strongly expressed in both MSCs (top) and osteoblasts (bottom).

Quantikine® ELISA and DuoSet® Immunoassay Kits

Available for proteins associated with adipogenesis, chondrogenesis, and osteogenesis.

		Catalog #	
	Species	**DuoSet**	**Quantikine**
Adipocytes			
Adiponectin/Acrp30	human	DY1065	DRP300
	mouse	DY1119	MRP300
	rat		RRP300
Leptin	human	DY398	DLP00
	mouse	DY498	MOB00
	rat	DY498	MOB00
Chondrocytes			
Aggrecan	human	DY1220	
SPARC/Osteonectin	human		DSP00
Osteocytes			
Osteocalcin	human		DSTCN0
Osteopontin	human	DY1433	DOST00
	mouse	DY441	MOST00
	rat		MOST00
Pro-Collagen I α 1	human	DY622-05	
SPARC/Osteonectin	human		DSP00

View Pathway



rndsystems.com/pathways_msc

Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are defined as multipotent, self-renewing progenitors that can be differentiated into adipocytes, chondrocytes, and osteocytes. Originally identified in mouse bone marrow, MSCs have now been discovered in a variety of species and isolated from numerous tissues including adipose, placental, dental pulp, and umbilical cord. Despite the classical trilineage differentiation that functionally identifies MSCs, these cells have also been shown to differentiate into non-traditional lineages to produce cardiomyocytes, endothelial cells, hepatocytes, and neural cells. To date, the biological properties of MSC identification, differentiation, and function have yet to be confirmed *in vivo*, raising caution for the extrapolation of *in vitro* generated data. Given their rarity, incompletely defined immunophenotype, and localization in multiple organs, studying MSCs *in situ* is not a trivial task. With these challenges in mind, R&D Systems and Tocris Bioscience present tools to assist in the reliable isolation, differentiation, verification, and investigation of MSCs.