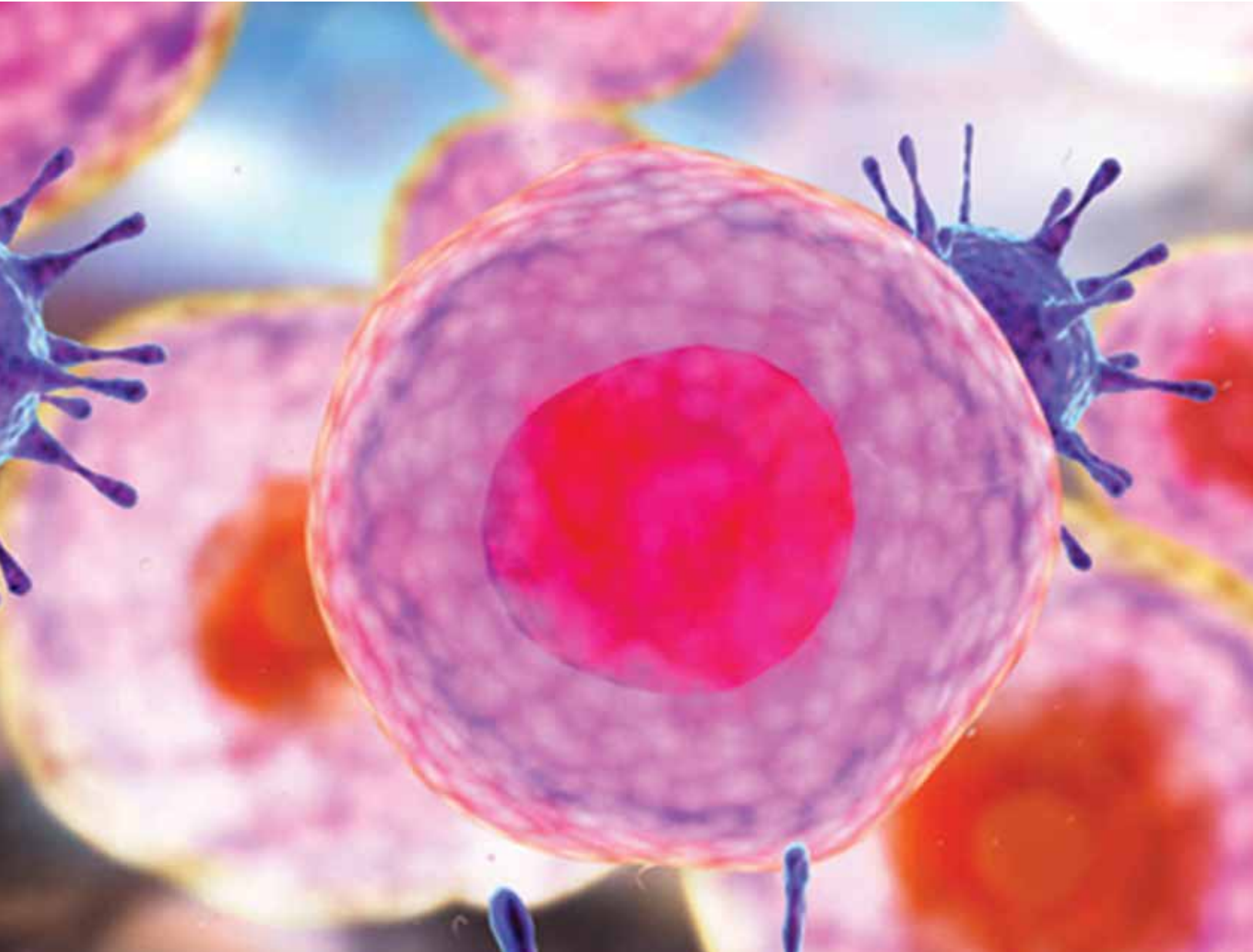
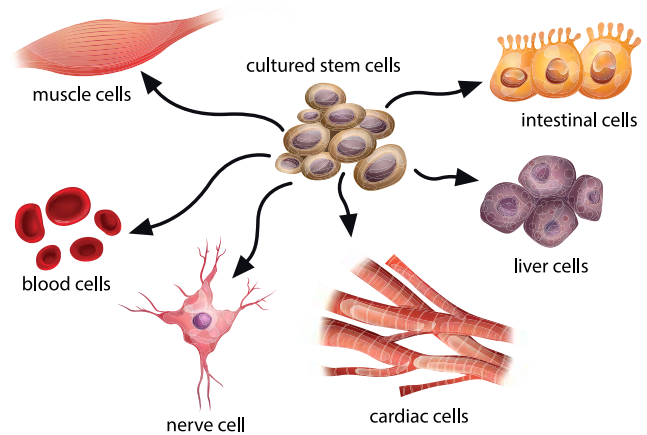


Guide to Culturing Mesenchymal Stem Cells



Because Stem Cells Could Save You Tomorrow.....

Mesenchymal Stem Cells are non-hematopoietic stromal cells that are capable of differentiating into, and contribute to the regeneration of, mesenchymal tissues such as bone, cartilage, muscle, ligament, tendon, and adipose.



Characteristics of MSCs

- **Self-renewal**

A peculiar characteristic exhibited by stem cells, mesenchymal stem cells propagate indefinitely.

- **Multipotent**

Though MSCs can differentiate classically into cells of three mesodermal lineages, osteoblasts, adipocytes and chondrocytes, these cells have also been shown to differentiate into non-traditional lineages to produce cardiomyocytes, endothelial cells, hepatocytes, and neural cell.

Challenges in MSC Research

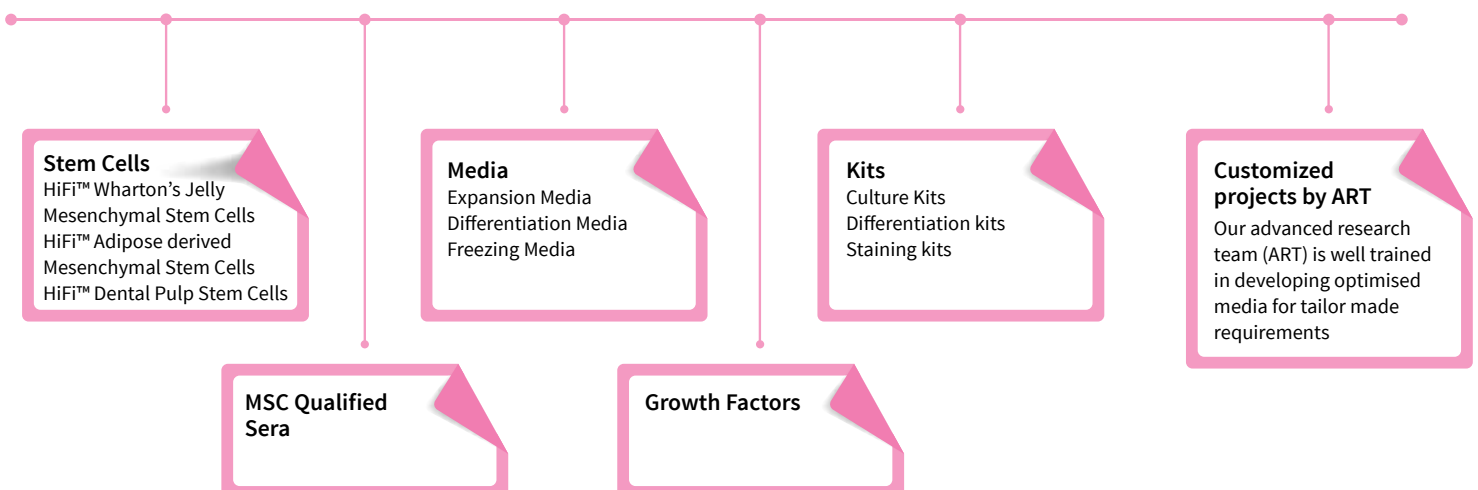
To conduct advanced research in cell therapy using MSCs, MSCs of high quality must be available readily. Isolation of MSCs from various tissues seem to be easy and one may successfully be able to isolate them, there remain enormous number of challenges mentioned below:

- Investment of time to standardize protocol
- Heterogeneity of MSCs isolated
- Cross contamination with cells that are not multipotent
- Generation of non-reproducible data due to variations in cell handling, protocols, media, serum etc.

Why HiFi™ Cells?

- Cells are representative of Indian genetic pool
- Conform to standards recommended by ISCT
- Cryopreserved at early passages (P2)
- Allow use of cells from one donor for entire array of experiments
- Cells pooled from 2-3 different donors available
- Rigorous Quality Control Testing
- Available as flasks as well as Cryovials
- Available with validated media and reagents

All under one Roof!!!



Ethical Regulations

DONOR CONSENT

Cells are isolated from tissues obtained ethically from informed donors signing consent form.

SAFETY

Cells are screened to ensure absence of HIV, Hepatitis B (HBV) and Hepatitis C virus (HCV) and meet the product safety standards.

ETHICAL APPROVAL

All products and procedures related to human stem cells and primary cells have been reviewed and approved by Institutional Ethics Committee (IEC)* and Institutional Committee for Stem Cell Research (IC-SCR)#

* DCG(I) Reg. No. - ECR/165/Indt/MH/2014/RR-17



* FWA Reg. No. 00020203

* IRB Reg. No. 00008839

NAC-SCRT Reg. ID. - NAC-SCRT/29/20131912

Shipping, Handling and Storage

HiMedia assures its users the optimum performance, functionality and viability of cells provided. Therefore, cells are shipped to the destination in the first half of the week to avoid an delay in delivery.

	CRYOPRESERVED CELLS	PROLIFERATING CELLS
Shipping	<p>Cells cryopreserved in cryovials are shipped in dry shipper placed in protective carton at temperature below -130 °C</p> 	<p>Cells are supplied in T25 flask at RT with medium filled upto the neck, sealed with parafilm</p> 
Handling	<p>Place shipper in proximity to LN₂ cylinder ↓ Transfer cryovial into LN₂ cylinder with forceps Note : Do NOT allow cryoshipper lid to fall down</p>	<p>Check for leakage in Biosafety Level II ↓ Intact flask without leakage : Proceed ahead ↓ Leaking/ broken flask : Discard contents</p>
	Storage	<p>Store in LN₂ till thawing and regular use</p> <p>Incubate flask in a CO₂ incubator at 37°C for reattachment of cells to surface for 3-4 hours</p>

Safety Instructions

All the cells are of human origin and should be treated as potentially infectious. Even if the cells provided have been screened for viral and bacterial pathogens, human cells may harbor other known or unknown agents which might pose a health hazard. Universal handling precautions applicable to biological samples must be applied as recommended in the CDC-NIH manual.

Notes

- Flow cytometry data mentioned in this brochure has been generated on Partec CyFlow® Cube 8 Flow Cytometer.
- Please refer individual product information sheet for detailed procedures related to each product.
- For lot-specific results, please refer certificate of analysis.
- All the products enlisted here are intended for research use only. Not for animal, human therapeutic or diagnostic use.

HiFi™ Mesenchymal Stem Cells

Bone marrow (BM) has been extensively investigated as a source of adult stem cells and have been considered the gold standard in mesenchymal stem cell biology. However isolation of stem cells from bone marrow has certain major disadvantages.

- Procedure for obtaining bone marrow is very painful and invasive with possible donor morbidity.
- Stem cells exist in a very small quantity in bone marrow (around 0.01% to 0.001% of nucleated cells) and their number, frequency and differentiation capacity correlate inversely with age of the donor
- Due to low density, the isolated stem cells may require ex vivo expansion to obtain sufficient numbers. This makes the isolation process expensive.
- Donor age variations amongst samples attributes to differences in the initial yield of isolation and the basic proliferative and differentiation capabilities.

In light of these drawbacks, scientists are exploring alternative sources of stem cells that resemble stem cells from bone marrow (BMSC). HiMedia offers,

HiFi™ Wharton’s Jelly Mesenchymal Stem Cells (HWJ-MSC)

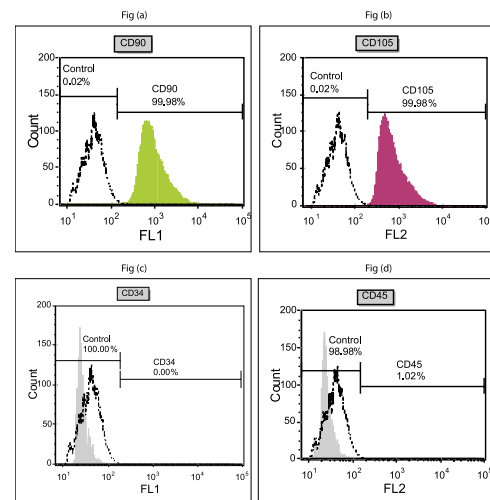
HiFi™ Adipose-derived Mesenchymal Stem Cells (HAD-MSC)

HiFi™ Dental Pulp Stem Cells (HDP-SC)

Marker	BMSCs	WJMSCs	HADMSCs	DPSCs
CD73	+	+	+	+
CD90	+	+	+	+
CD105	+	+	+	+
CD166	+		+	
CD29	+		+	
CD44	+		+	+
CD9	+			+
CD13	+			+
CD106	+			+
CD146	+			+
CD45	-	-		
CD34	-	-		-
CD14	-		-	-
CD45	-		-	
CD31	-		-	

Rely on us for Quality

Quality Control Parameters for MSCs	
Growth Performance	Viability testing Microscopic examination of morphology Growth rate Number of population doublings
Cell Surface Marker Analysis	Immunocytochemistry Flow cytometry (as recommended by ISCT)
Ability to Differentiate	Adipogenic lineage Osteogenic lineage Chondrogenic lineage
Sterility Assay	Bacteria Fungi Yeast Mycoplasma
Virus Screening	HIV HCV HBV

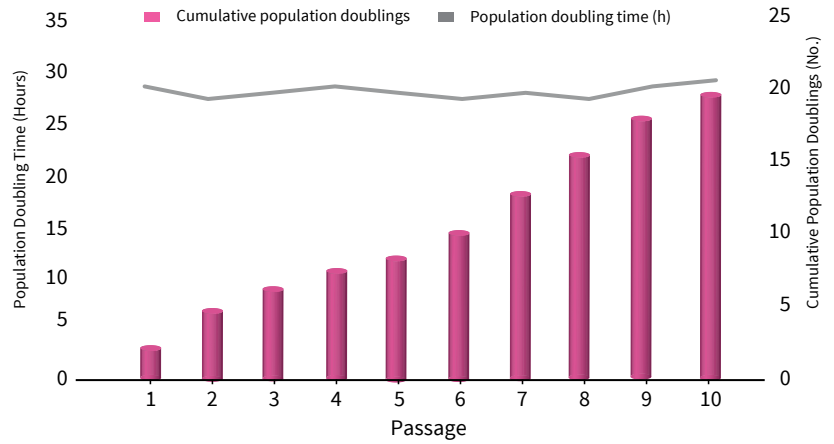


Representative flow cytometry analysis of cell-surface makers in HWJ-MSC at passage 2

Fig (a) & (b): >95% positive expression of classical mesenchymal stem cell markers CD90, CD105 in HWJ-MSC (coloured peaks). Fig (c) & (d): <2% negative expression of hematopoietic cell markers CD34, CD45 in HWJ-MSC (grey peaks). Control cells (non-labeled) denoted by dotted line.

HiMesoXL™ Mesenchymal Stem Cell Expansion Medium

Adult stem cells exist at a very low frequency in adult tissue. They have a limited in vitro expansion capacity after which they exhibit reduced differentiation potential and proliferation. Hence effective expansion of these stem cells is very critical to obtain adequate number of cells for research.



Adipose derived mesenchymal stem cells can be successfully subcultured in HiMesoXL™ Mesenchymal Stem Cell Reduced Serum Medium, up to 20 population doublings over 10 passages, with a stable growth rate of less than 30 hours per population doubling.



CryoXL™ Stem Cell Freezing Medium

CryoXL™ Stem Cell Freezing Medium is a fully supplemented proprietary formulation designed for cryopreservation of human stem cells.

Ensures optimal cell viability and recovery upon revival while maintaining their multipotency and normal morphology

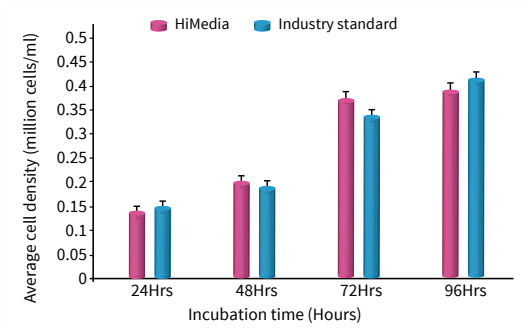
MSC Qualified Sera

Mesenchymal stem cells (MSC) are undifferentiated cells that have ability to differentiate into multiple lineages. A major challenge in culturing MSC is the difficulty to maintain them in undifferentiated state while avoiding their differentiation into unwanted lineage. Serum contains many unknown factors, nutrients and molecules in variable concentration that may lead to unwanted differentiation of stem cells.

MSC qualified sera are suitable for culturing MSC. Prescreening saves time and money by eliminating the need to test multiple serum lots to identify the suitable one for MSC culture. They are prescreened for

Expansion

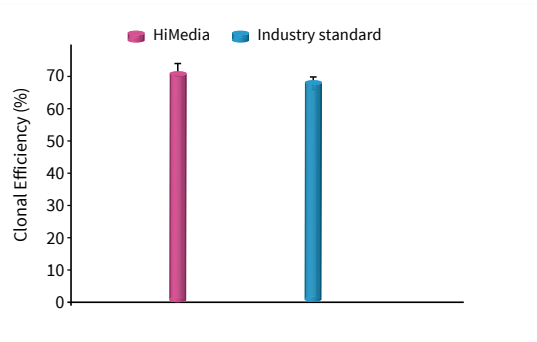
Ability to proliferate and generate high cell densities without getting differentiated



Comparative performance of MSC tested FBS on expansion of Adipose derived MSC's

Clonal efficiency

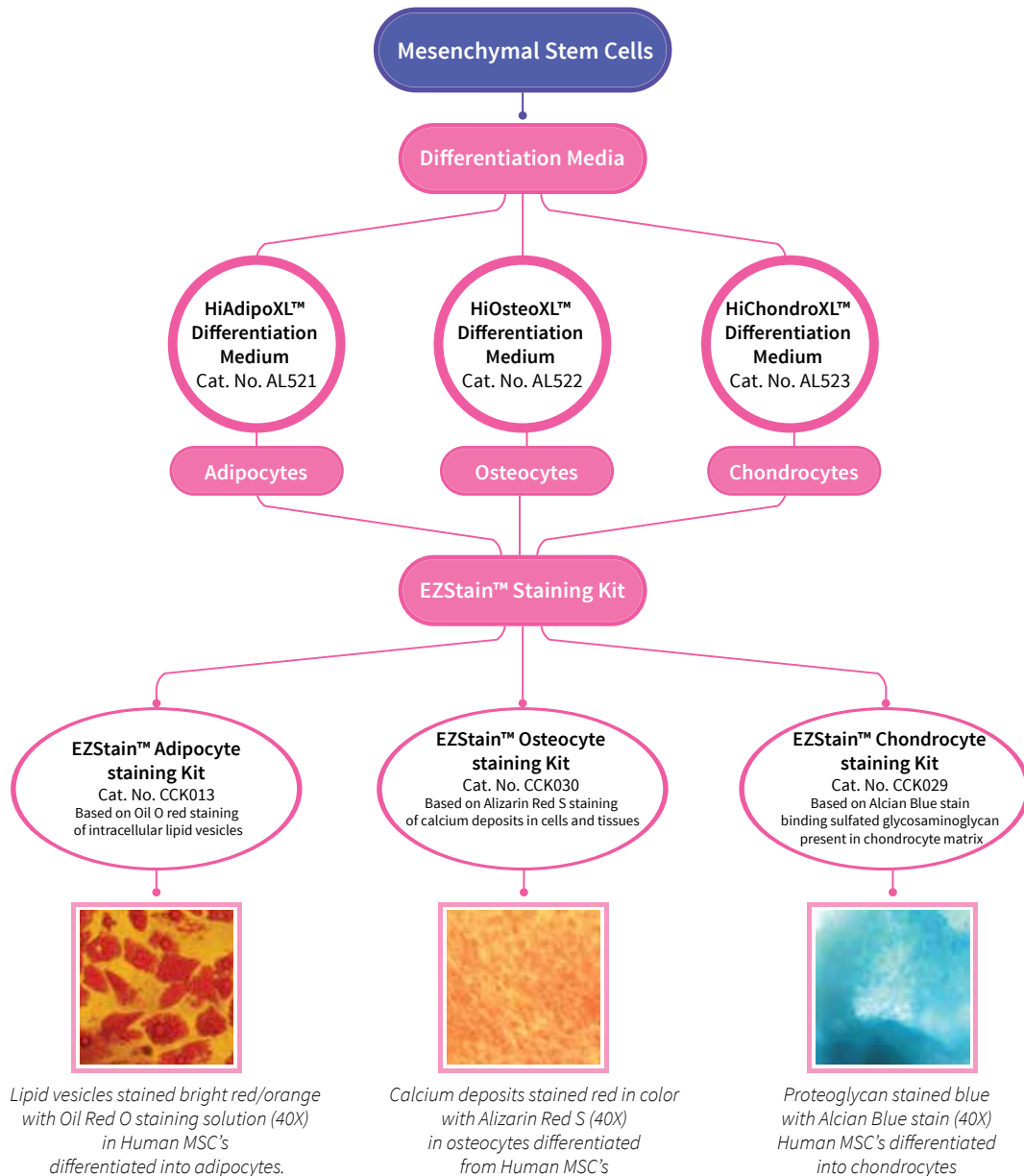
Ability to form colonies in culture



Comparative performance of MSC tested FBS on clonal efficiency of mesenchymal stem cells

Differentiation & Staining

Differentiation potential of clonally expanded mesenchymal stem cells into adipocytes, osteocytes and chondrocytes is considered as a reliable functional criterion to identify MSCs. HiMedia offers Differentiation Media that help MSCs differentiate into one of these specific lineages which can be validated using EZStain Staining Kits. Besides, HiDiff™ Assay kits enable users to differentiate MSCs and detect Adipogenesis or Osteogenesis in one go.



All-in-one: EZXpand™ Mesenchymal Stem Cells Culture Kits

EZXpand™ culture kits are designed for hassle-free culture of Adipose derived- and Wharton's Jelly-derived mesenchymal stem cells using single kit.

Kit components

- Mesenchymal stem cells - 0.5 million cryopreserved cells OR proliferating cells in T25cm² flasks (depending upon the choice of user)
- Expansion medium
- Antibiotic-antimycotic solution

Product Category	Product Name	Product Code
Cells	HiFi™ Human Wharton's Jelly Mesenchymal Stem Cells (HWJ-MSC)	CL001
	HiFi™ Human Adipose Derived Mesenchymal Stem Cells (HAD-MSC)	CL007
	HiFi™ Human Dental Pulp Stem Cells (HDP-SC)	CL008
Expansion Media	HiMesoXL™ Mesenchymal Stem Cell Expansion Medium	AL512
	HiMesoXL™ Mesenchymal Stem Cell Expansion Medium, Reduced Serum	AL519
MSC Qualified Sera	Fetal Bovine Serum, EU Approved	RM10435
	Fetal Bovine Serum, EU Approved	RM10832
	Fetal Bovine Serum, USDA Approved	RM10845
	Fetal Bovine Serum, Australia origin	RM10846
	Fetal Bovine Serum, US origin	RM10938
Cell Culture Kits	EZXpand™ Human Adipose-derived Mesenchymal Stem Cell Culture Kit	CCK024
	EZXpand™ Human Wharton's Jelly derived Mesenchymal Stem Cell Culture Kit	CCK025
Differentiation Media	HiAdipoXL™ Adipocyte Differentiation Medium	AL521
	HiOsteoXL™ Osteocyte Differentiation Medium	AL522
	HiChondroXL™ Chondrocyte Differentiation Medium	AL523
Differentiation Supplements	HiAdipoXL™ Adipocyte Differentiation Supplement	TCL167
	HiOsteoXL™ Osteocyte Differentiation Supplement	TCL168
	HiChondroXL™ Chondrocyte Differentiation Supplement	TCL169
Differentiation Media for preadipocytes	HiAdipoXL™ Adipogenic Differentiation Medium for 3T3-L1 cells	AL537
Differentiation Kits	HiDiff™ Adipogenesis Assay Kit	CCK011
	HiDiff™ Osteogenesis Assay Kit	CCK049
Staining Kits	EZstain™ Adipocyte Staining Kit	CCK013
	EZstain™ Chondrocyte Staining Kit	CCK029
	EZstain™ Osteocyte Staining Kit	CCK030
Freezing Medium	CryoXL™ Stem Cell Freezing Medium	TCL107





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- Australia

- North America
- South America
- Europe



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