

# NutriFreez<sup>®</sup> D5 Salt Based Cryopreservation Solution



## Executive Summary

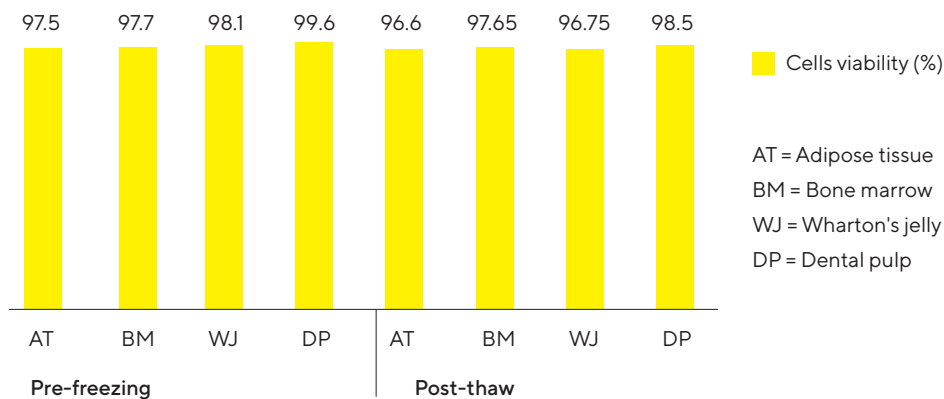
NutriFreez<sup>®</sup> D5 Salt Based Cryopreservation Solution is an optimal all-in-one solution, chemically defined and specially designed for freezing and thawing of cells intended for cell therapies and clinical applications. It provides a defined, protective environment in ultra-low temperatures (-196 °C), thus ensuring high viability, recovery rates, and performance even for extremely sensitive cells. While every lot is tested on MSCs as part of the broad QC tests, NutriFreez<sup>®</sup> D5 has been widely tested on various other cells, such as hPSCs, PBMCs, NK, and Vero cells. NutriFreez<sup>®</sup> D5 is manufactured under cGMP conditions and offers industry-leading performance. The product is optimally formulated, contains 5% DMSO and does not contain any antibiotics, antimycotics, hormones, growth factors, serum, or protein.

## Unique Selling Points

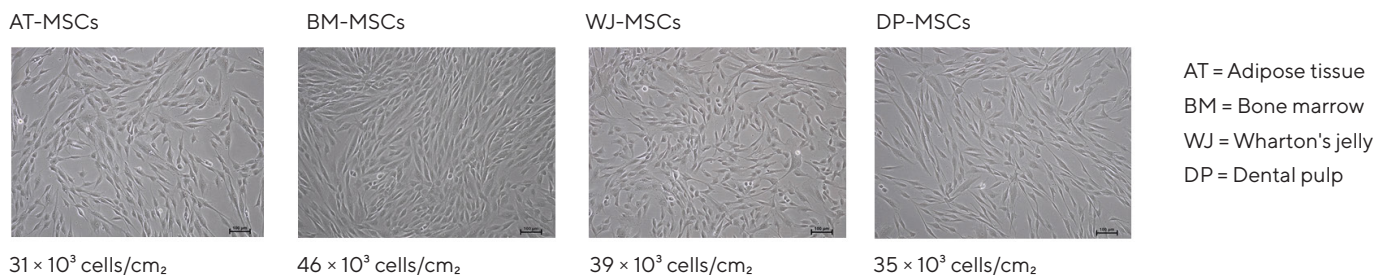
- Ready-to-use, simple protocol
- Fully defined medium
- Ideal for serum-free applications
- Animal component-free
- Serum-free and protein-free
- cGMP manufactured
- Sterility, endotoxin, and cell-based quality control testing
- Lot-to-lot consistency
- Drug Master File coming soon

# Suitability of NutriFreez® D5 Salt Based Cryopreservation Solution for hMSCs from Various Sources

## A. NutriFreez® D5 Salt Based Cryopreservation Solution



## B.



**Figure 1:** Cell viability and morphology after freezing in NutriFreez® D5 Salt Based Cryopreservation Solution hMSCs derived from a variety of sources were frozen in NutriFreez® D5 Solution and Thawed (after being frozen for one week). The cells were seeded in a XF culture system.

A. A comparison of cell viability (%) before and after freezing.

B. Representative images (X100) taken 3 days post seeding (cell recovery). The numbers represent the proliferation results (viable cells count). High proliferation and normal morphology are observed in all the cells.

**NutriFreez® D5 Salt Based Cryopreservation solution promotes high viability and recovery of hMSCs from various sources.**

## Relevant Applications

### Freezing, thawing, and recovering of:

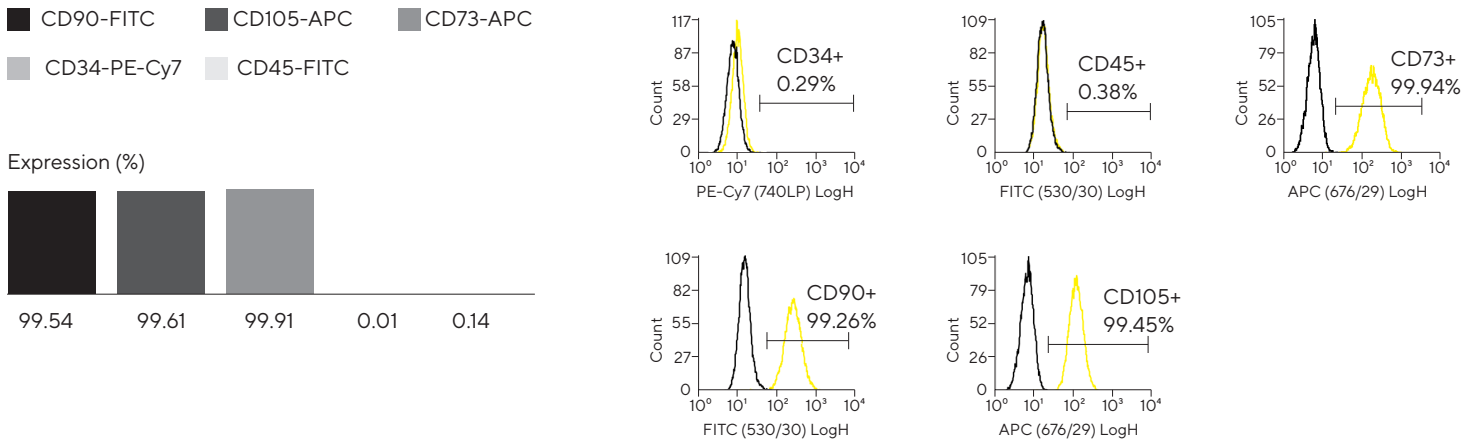
- hMSCs from various sources (e.g. AT, BM, CT, DP)
- hPSCs
- PBMCs
- NK cells
- Vero cells
- Long term Cryopreservation of cells intended for therapeutic applications
- Mouse hepatic organoids
- Colorectal cancer (CRC) organoids
- Cord Blood Total Nucleated Cells (TNC)
- CHO cells

## Relevant Process Steps

- Freezing cells
- Thawing frozen cells and recovery

# Immunophenotyping of hMSCs After Cryopreservation in NutriFreez® D5 Salt Based Cryopreservation Solution

Flow cytometry analysis of hMSCs after being frozen in NutriFreez® D5 Salt Based Cryopreservation Solution

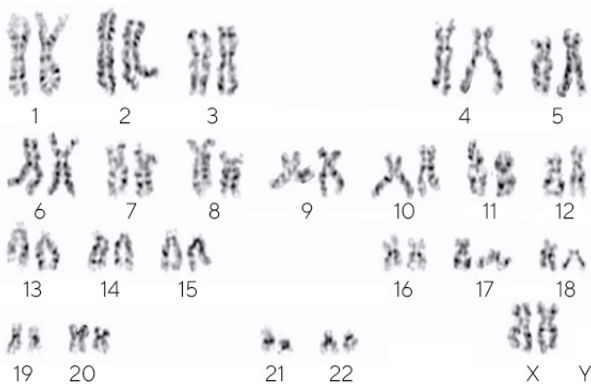


**Figure 2:** Immunophenotyping results of AT and BM MSCs using flow cytometry analysis – Immunophenotyping results (flow cytometry data and summary of marker expression) of Bone marrow and adipose tissue derived MSCs after being frozen in NutriFreez® D5 cryopreservation solution.

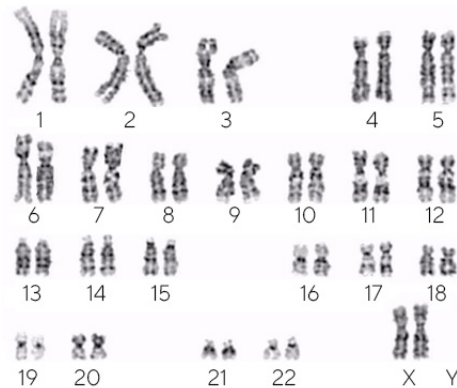
**hMSCs maintain classical profile of MSC markers with low percentage of hematopoietic contamination after being frozen in NutriFreez® D5 Salt Based Cryopreservation Solution.**

## Karyotyping

MSCs from two donors were frozen in NutriFreez® D5 Salt Based Cryopreservation Solution and NutriFreez® D10 Cryopreservation Medium, thawed, expanded to passage P6 and prepared for karyotyping (performed at Rambam healthcare campus). The analysis results demonstrated the karyotype of all tested samples to be normal.

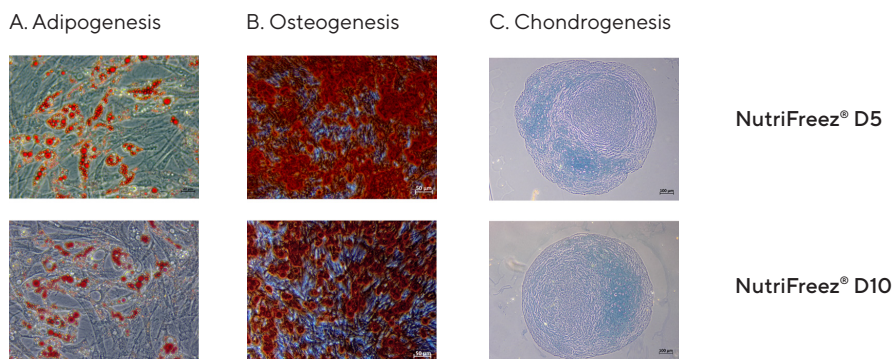


**Figure 3:** NutriFreez® D10 Cryopreservation Medium



**Figure 4:** NutriFreez® D5 Salt Based Cryopreservation Solution

# MSCs After Cryopreservation in NutriFreez® D5 and Differentiation into Adipocytes, Osteocytes and Chondrocytes



**Figure 5:** Differentiation potential of cryopreserved AT-MSCs into the adipogenic, chondrogenic and osteogenic lineages. Representative images of the differentiated AT-MSCs originating from a donor's MSCs, cryopreserved in NutriFreez® D5 Salt Based Solution. The cells were thawed at P2 and for passage P3/P4 seeded and grown in adipogenic, chondrogenic and osteogenic differentiation medium (B), in order to evaluate the cryopreserved AT-MSCs stemness (Bourin et al., 2013). The cells were stained as follows: Adipocytes were stained on day 10 of adipogenic differentiation with Oil Red O Stock to visualize lipids and fat deposits (A); In osteocytes the calcium deposits in the cells were stained with Alizarin Red S on day 18-21 of osteogenic differentiation (B); in chondrocytes the sulfated proteoglycan present in cartilage tissue is stained on day 21 of chondrogenic differentiation with Alcian Blue (C).

## Technical Specifications

Attribute			
Volume (ml)	10 ml	100 ml	500 ml
% DMSO	5	5	5
QC Cells	hMSC	hMSC	hMSC

## Ordering Information

Item	Description	Package   Volume   Quantity   Size	Order Number
NutriFreez® D5	A Salt Based Protein Free, 5% DMSO Cryopreservation Solution	10 ml	05-715-1D
NutriFreez® D5	A Salt Based Protein Free, 5% DMSO Cryopreservation Solution	100 ml	05-715-1B
NutriFreez® D5	A Salt Based Protein Free, 5% DMSO Cryopreservation Solution	500 ml	05-715-1A

# Peripherals and Accessories

Product Name		Description	Order Number
Dulbecco's PBS (w/o Ca & Mg)		A buffer solution	02-023-1
Soybean Trypsin Inhibitor (SBTI)		A trypsin inhibitor solution	03-048-1
Cell dissociation solution - non enzymatic		A non-enzymatic cell dissociation solution	03-071-1
MSC NutriStem® XF Basal Medium		A media for the expansion of MSCs	05-200-1
MSC NutriStem® Supplement Mix		Supplementation for NutriStem Basal Medium	05-201-1
MSC Attachment Solution		A solution for the attachment of hMSCs on culture plates	05-752-1
Recombinant Trypsin Solution		A solution for the dissociation of adherent cells	03-078-1
Recombinant Trypsin-EDTA Solution		A solution for accelerated dissociation of adherent cells	03-079-1
NutriFreez® D10 Cryopreservation Medium		A solution for cryopreservation of cells (10% DMSO)	05-713-1
NutriStem® hPSC XF Medium		A media for the expansion of hPSCs	05-100-1

## Contact Details


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