

NutriStor[®] Cold Storage Solution

A serum-free, animal component-free (ACF) and protein-free solution, for short-term, non-cryogenic (2–8 °C) storage of cells



NutriStor[®] Cold Storage Solution is designed for the storage of sensitive cells without the need of repeated freeze-thaw cycles and multiple centrifugation steps. It has been tested on a variety of cells (including peripheral blood mononuclear cells (PBMCs), chimeric antigen receptor T-cells (CAR-Ts) and mesenchymal stromal cells (MSCs) from various sources and optimized to ensure maximum safety and high viability after cold storage.

NutriStor[®] Cold Storage Solution diminishes temperature induced cell stress responses that take place during hypothermic storage and shipping of cells, and provides maximum stability at 2–8 °C. It has been proven very effective in reducing post-storage necrosis and apoptosis that often occurs in cells during these procedures.

With components that provide pH buffering, osmotic support, energy substrates, and ionic concentrations, cells stored in this ACF solution show high recovery rates, normal cell characterization, and excellent performance.

- Ready-to-use
- Chemically defined, Animal Component-Free, Serum-Free, Protein-Free formulation
- Provides maximum safety during cold storage (2–8 °C)
- Ensures high viability and recovery rates after cold storage
- Ensures cell safety during shipment
- Reduces post storage necrosis and apoptosis
- Optimal for a wide range of applications
- DMF will be available
- Outperforms predominant market competitor

Relevant Applications

- Short-term cell storage (avoids the need for cryopreservation and freeze-thaw cycles)
- Shipment of cells in cold storage rather than in LN2
- Clinical applications (e.g., Immunotherapy, CAR-T, Tissue regeneration, etc.)
- All applications involving PBMCs, hMSCs and T-cells

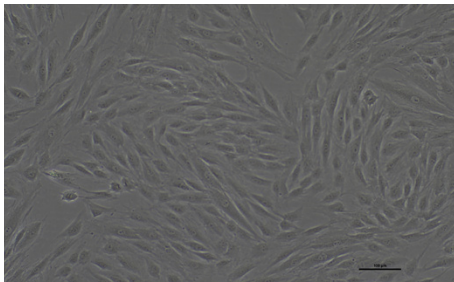
Relevant Process Steps

- Cell suspension
- Cell storage
- Cell shipment
- Cell recovery in culture media (dependent on cell type)

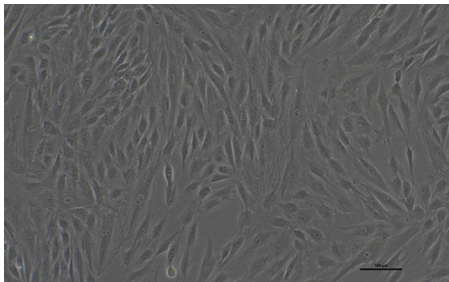
Technical Specifications

Volume	10 mL	100 mL	250 mL	500 mL
Bottle Bag	bottle	bottle	bag	Bag bottle

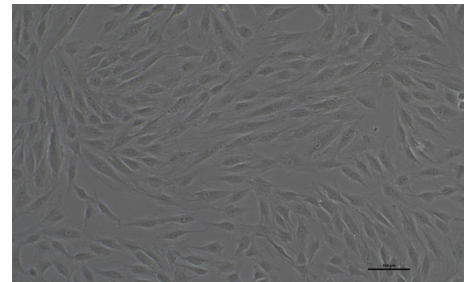
Figure 1: A comparison between cells stored in NutriStor® Cold Storage solution and NutriFreez® D10 cryopreservation media.



Sample 1
Cells after storage in NutriStor®.
Initial seeding: 44.0×10^3 cells/cm²



Sample 2
Cells after storage in NutriStor®.
Initial seeding: 44.8×10^3 cells/cm²



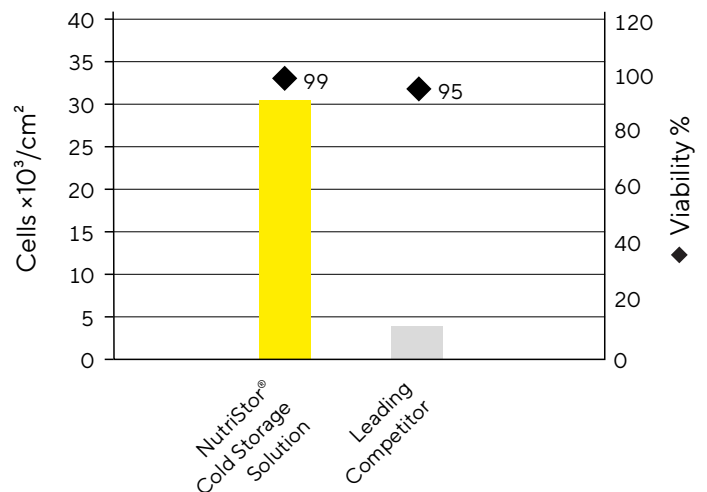
Positive control
Cells after cryopreservation in NutriFreez® D10.
Initial seeding: 41.8×10^3 cells/cm²

Note: Representative images (×100) of human BM-MSCs after a 3-day storage in NutriStor® Cold Storage solution. After storage, the cells were seeded for recovery with MSC NutriStem® XF Medium. Cells were counted and images were taken 3 days post-seeding. The cells stored in NutriStor® Cold Storage Solution showed impressive results, with high recovery and proliferation rates, and normal morphology.

Figure 2: NutriStor® Cold Storage Solution – Viability and Recovery.

Note: An evaluation of viability and recovery rates of human BM-MSCs stored in NutriStor® Cold Storage Solution compared to cells stored in the leading market competitor. Cells were stored for 4 days in storage solution, at 4 °C (Stored cell concentration was ~330k cells/vial [1 mL/vial]). After storage live cells were counted (viability) and seeded in 12 well plates, at a concentration of 5×10^3 cells/cm² (2×10^4 cells/well) in 1 mL/well MSC NutriStem® XF Medium. Plates were pre-coated with MSC Attachment Solution. Although both groups showed excellent viability rates, cells stored in NutriStor® Cold Storage Solution showed much higher recovery rates, compared to cells stored in the leading competitor.

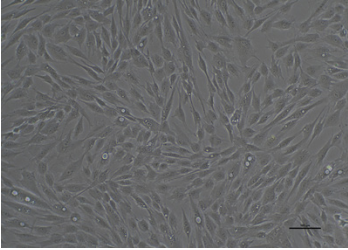
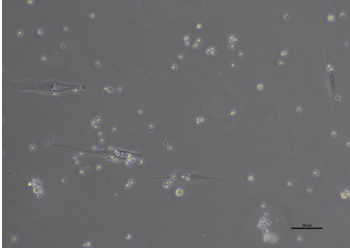
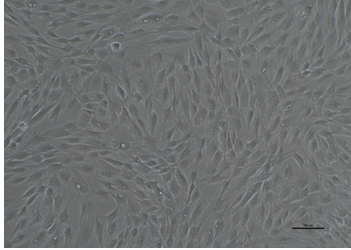
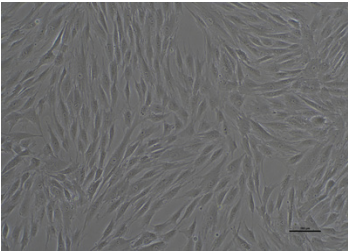
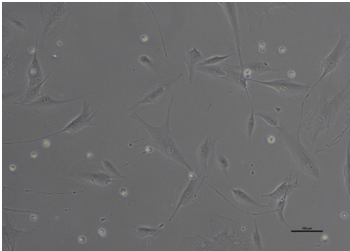
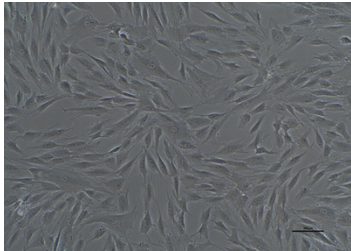
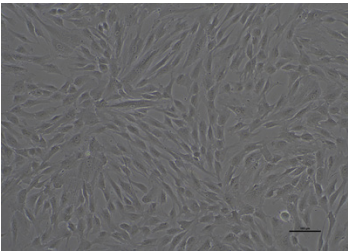
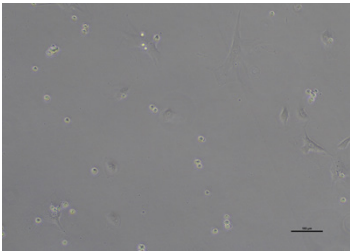
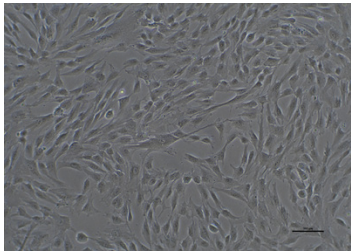
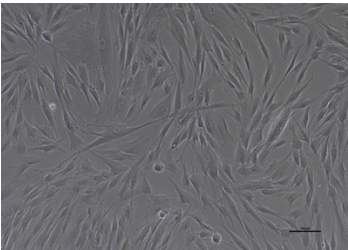
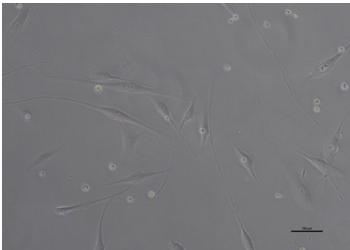
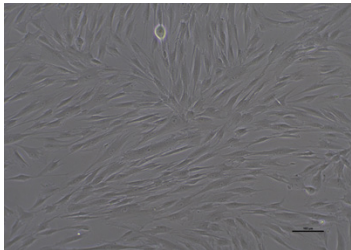
BM-MSC viability and recovery after cold storage



The following assays characterize various cell functions and characteristics after storage in different storage solutions. All assays (figures 3–6) are based on human MSCs (Bone Marrow (BM), Adipose Tissue (AT), Cord Tissue (CT) or Dental Pulp (DP) derived) stored for 4 days in NutriStor® Cold Storage Solution or in the current market leading competitor solution (2–8 °C).

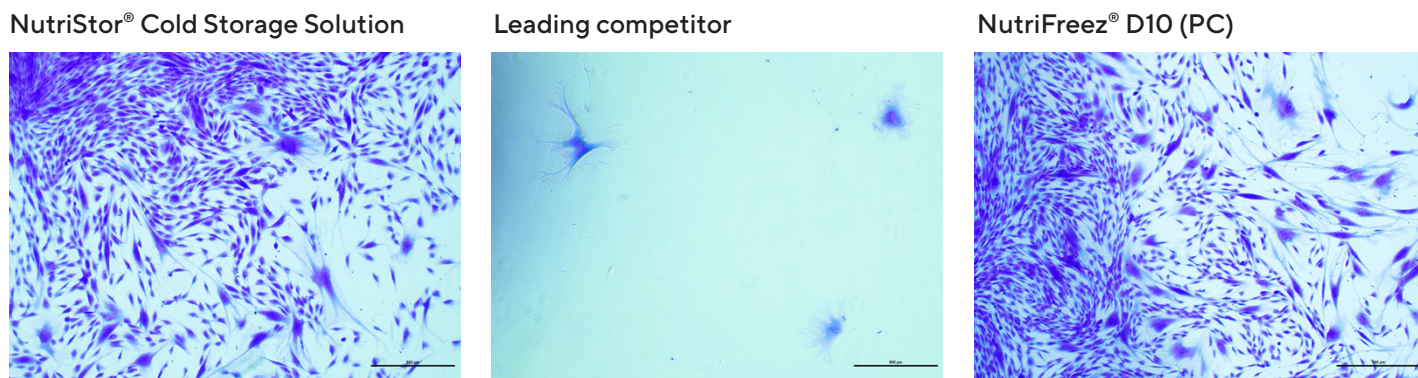
As a positive control (PC) cells from the same source were stored in NutriFreez® D10 (–150 °C). Figure 7 represents the same comparison based on Peripheral Blood Mononuclear Cells (PBMCs) with NutriFreez® D5 cryopreservation solution as a positive control.

Figure 3: Recovery of hMSCs from different sources.

NutriStor® Cold Storage Solution	Leading competitor	NutriFreez® D10 (PC)
AT-MSCs		
		
52×10 ³ cells/cm ²	1×10 ³ cells/cm ²	59×10 ³ cells/cm ²
BM-MSCs		
		
36×10 ³ cells/cm ²	5×10 ³ cells/cm ²	43×10 ³ cells/cm ²
CT-MSCs		
		
49×10 ³ cells/cm ²	3×10 ³ cells/cm ²	57×10 ³ cells/cm ²
DP-MSCs		
		
34×10 ³ cells/cm ²	5×10 ³ cells/cm ²	40×10 ³ cells/cm ²

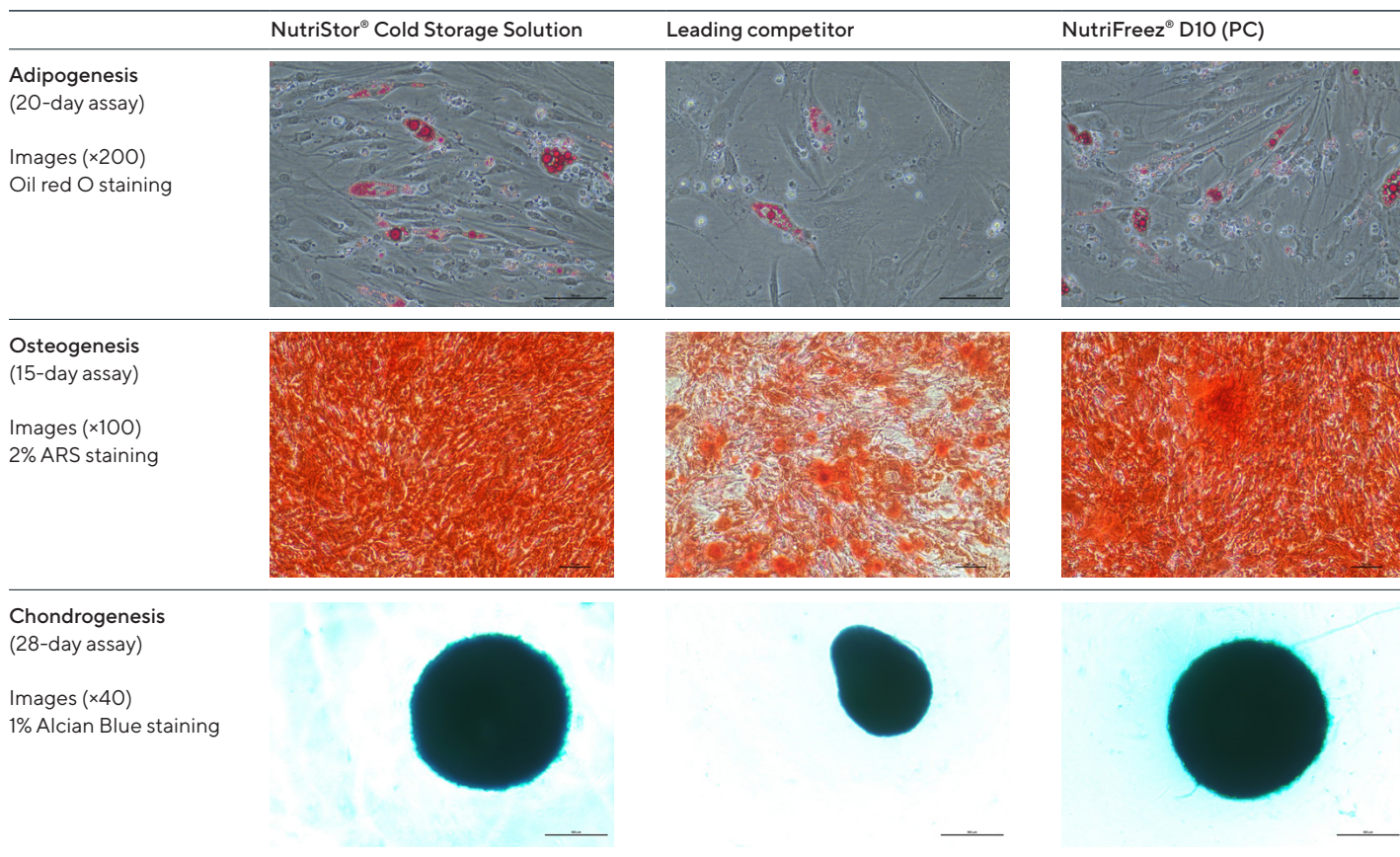
Note: Representative images of AT/BM/CT and DP MSCs, after storage in either NutriStor® Cold Storage Solution or leading Competitor (2–8 °C). Images and cell counts (presented on each image) indicate that Cells stored in NutriStor® Cold Storage Solution showed significantly better recovery rates than leading competitor.

Figure 4: CFU-F assay. Self-renewal potential of hMSCs after cold storage.



Note: Representative images (×40) of mature BM-MSC colony forming units, stained with 0.5% Crystal violet. Images were taken post 14 days of CFU-F assay. After storage in cold storage solution, cells were seeded at a concentration of 100 cells/well, to assess colony-forming potential. Cells stored in NutriStor® Cold Storage Solution showed much higher colony-forming potential than Leading competitor. Morphology was similar in NutriStor® and PC wells. Similar results were obtained while testing AT/CT and DP-derived MSCs.

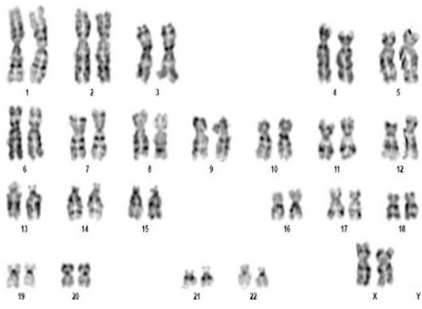
Figure 5: Differentiation potential of MSCs after cold storage.



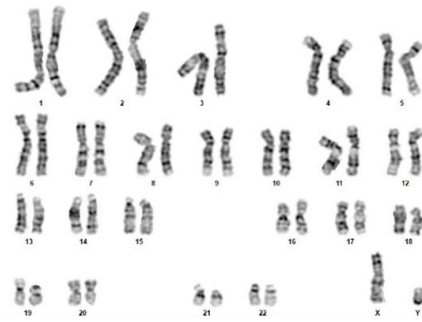
Note: Representative images of BM-MSCs, after various differentiation assays (Adipogenesis, Osteogenesis and Chondrogenesis). Mature differentiated cells were observed in all storage and frozen samples. Cell morphology and confluency were similar in NutriStor® Cold Storage Solution and PC samples. Very few mature adipocytes were observed in the leading competitor samples, with a different cell morphology (elongated) and many dead cells present. Osteogenesis results of the leading competitor showed a very low differentiation capacity. In the chondrogenesis assay the NutriStor® and NutriFreez® chondrocyte colony shape and size indicated that the differentiation was significantly more efficient. Similar results were observed in the AT/CT and DP-derived MSC samples.

Figure 6: Genomic stability of various hMSCs.

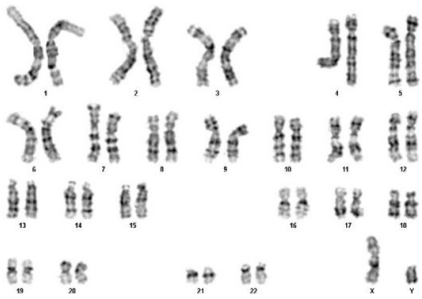
AT-MSCs



BM-MSCs



CT-MSCs



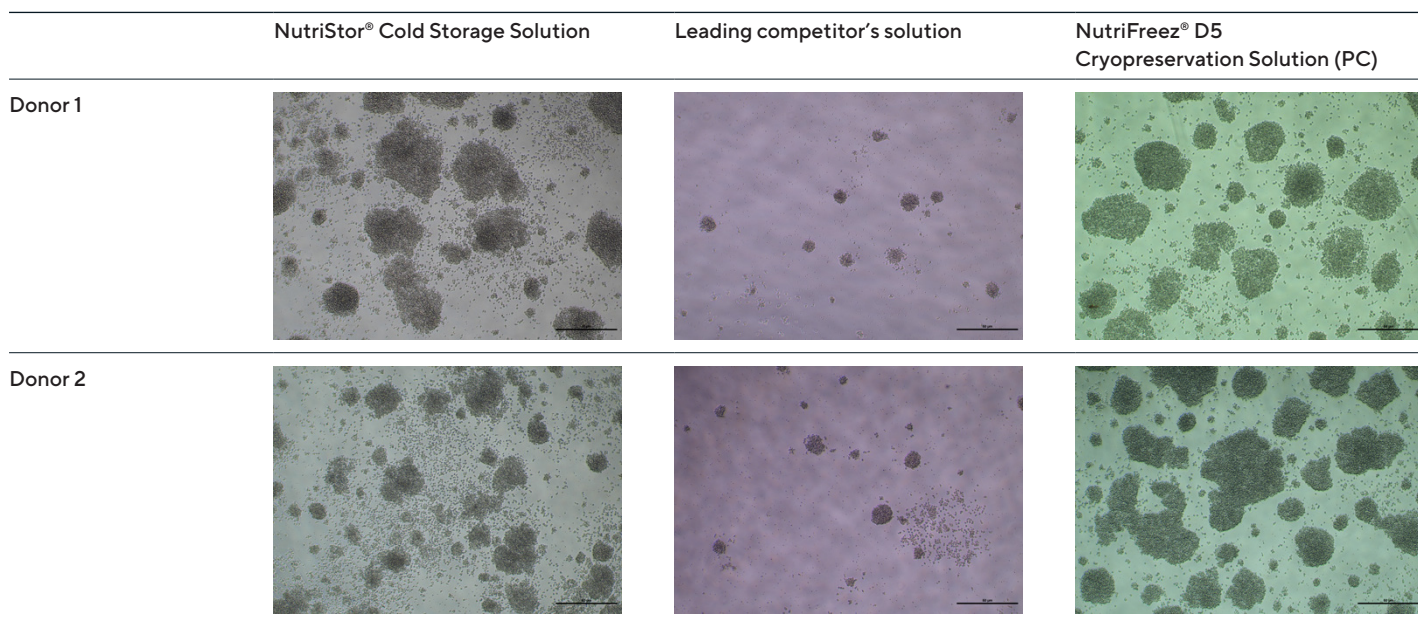
DP-MSCs



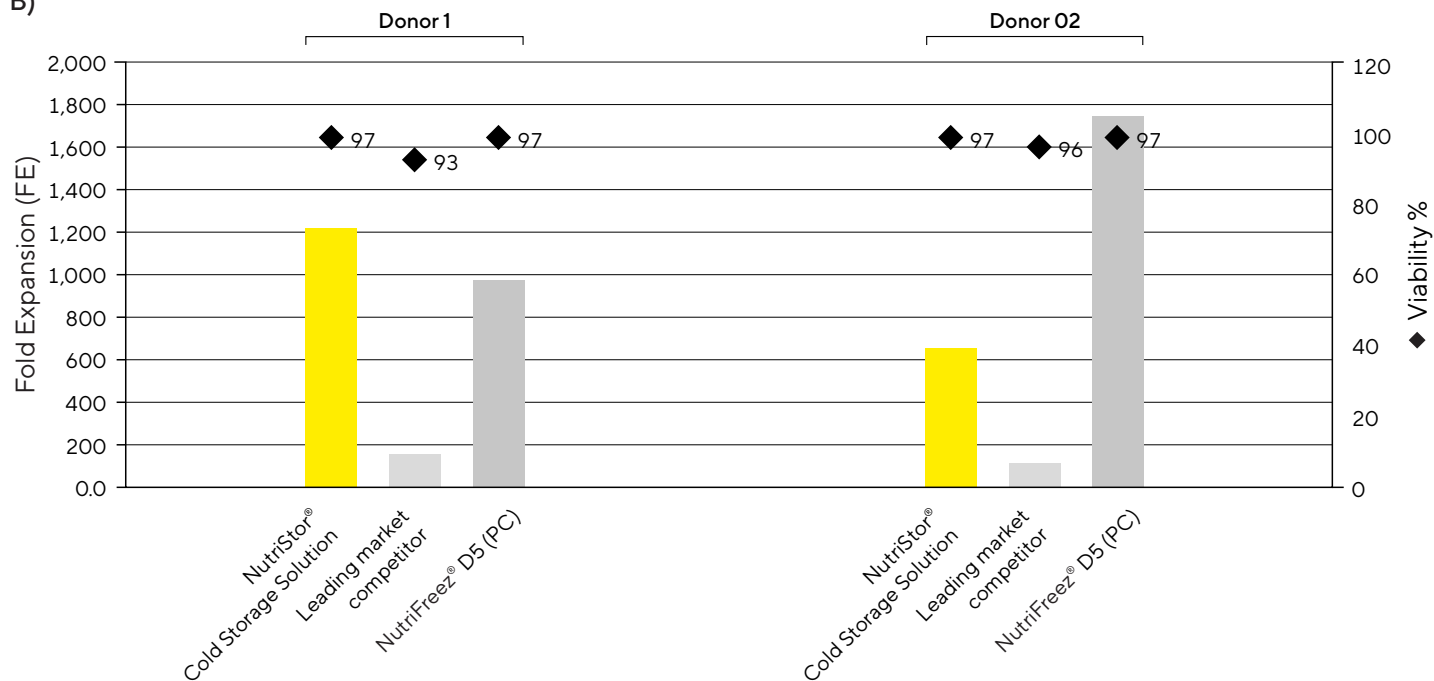
Note: G-banding Karyotype analysis of AT, BM, CT, and DP-derived MSCs after 4 days of storage in NutriStor® Cold Storage Solution (2–8 °C). Images show MSCs maintain genomic stability after being stored in NutriStor® Cold Storage Solution. Normal 46XY or 46XX karyotype was observed in all samples.

Figure 7: PBMC culture viability and recovery, NutriStor® Cold Storage Solution vs. Leading competitor.

A)



B)



Note: Fresh cells from two healthy donors were stored in NutriStor® Cold Storage Solution or in the leading competitor's solution, for 4 days, at 4° C, $5-6 \times 10^6$ cells/mL per vial [1 mL/vial]). NutriFreez® D5 cryopreservation solution served as a positive control. The cells were seeded post-storage in Nutri-T XF medium in 24 well plates, at a concentration of 50×10^3 cells/well, and cultured for 8 days with a passage on day 5. (A) Light microscopy images taken on day 5 post-seeding. PBMCs stored in NutriStor® Cold Storage Solution or PC showed typical culture morphology and higher proliferation rates than the competitor's solution. (B) Cells were counted, and fold expansion (FE) was evaluated as a recovery indicator on day 8. Though viability results were good in both solutions, PBMCs stored in NutriStor® showed higher viability and significantly higher FE results than the cells stored in the competitor's solution.



Ordering Information

Item	Description	Volume	Order Number
NutriStor® Cold Storage Solution	10 mL bottle	10 mL	05-F3F3001-1D
NutriStor® Cold Storage Solution	100 mL bottle	100 mL	05-F3F3001-1B
NutriStor® Cold Storage Solution	500 mL bottle	500 mL	05-F3F3001-1A
NutriStor® Cold Storage Solution	250 mL FlexSafe® bag	250 mL	05-F3F3001-1-0.25L
NutriStor® Cold Storage Solution	500 mL FlexSafe® bag	500 mL	05-F3F3001-1-0.5L



Peripherals and Accessories

Product Name	Order Number
MSC NutriStem® XF Media	05-200-1 + 05-201-1
4Cell® Nutri-T GMP	05-F3F2111-1K

Instructions for Use

NutriStor[®] Cold Storage Solution

An animal component-free (ACF), chemically defined, protein-free, cold storage solution for cells



2976141-000-01



SARTORIUS

Animal component-free (ACF), chemically defined, protein-free,
cold storage solution for cells

REF

05-F3F3001-1



2-8 °C

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1 Product Description

NutriStor® Cold Storage Solution is an optimized serum-free, protein-free, animal component-free (ACF), salt-based, chemically defined formulation.

It is designed for the short-term storage of cells (up to 7 days, recommended 3–4 days), in cold temperatures (2–8 °C) by providing a stable, defined and protective environment. NutriStor® Cold Storage Solution enables the storage and maintenance of cells without the need of repeated freeze-thaw cycles and multiple centrifugation steps. It has been tested on a variety of cells (including peripheral blood mononuclear cells (PBMCs), chimeric antigen receptor T-cells (CAR-Ts) and mesenchymal stem cells (MSCs)) from various sources and ensures high viability, recovery rates and performance after cold storage.

NutriStor® Cold Storage Solution is optimally formulated and does not contain any antibiotics, hormones, growth factors, serum or proteins.

2 Cell Type Applications

- Human Mesenchymal Stem Cells (hMSCs), from various sources:
 - Bone Marrow (BM-MSCs)
 - Adipose Tissue (AT-MSCs)
 - Umbilical Cord Tissue (UC-MSCs)
 - Dental Pulp Tissue (DP-MSCs)
- Human Peripheral Blood Mononuclear Cells (PBMCs)
- T Cells (including CAR-Ts)

3 Intended Use and Safety

- For research or further manufacturing use only. **Not** approved for human or veterinary use. **Not** approved for application in humans or animals, or for use in in-vitro diagnostical or clinical procedures.
- Do **not** use if a visible precipitate is observed in the storage solution.
- Do **not** use if there is any package leak or any exposure to environment conditions. The sterility of the product might be comprised.
- Refer to the Material Safety Data Sheet (MSDS) for hazard information.
- Maintain aseptic work conditions.

4 Storage and Stability

- Store at 2–8 °C.
- Avoid exposure to light.
- Refer to the manufacturing date indicated on the product label and CoA (Certificate of Analysis).

5 Features

- Ready-to-use
- Chemically defined, serum-free, protein-free, animal component-free
- Maintains high viability and recovery of cells.
- Maintains the unique features of cells (e. g. multipotency of hMSCs).
- Diminishes temperature-induced cell stress responses that take place during cold storage of cells.

6 Instructions for Use

NOTE Keep NutriStor® Cold Storage Solution at 2–8 °C until use.

Cold Storage Procedure

1. To maintain aseptic work conditions, wipe the outer packaging with a cloth moistened in 70 % Ethanol | 70 % Isopropanol before opening the vial.
2. Centrifuge the cells to obtain a cell pellet, at 300–400 xg for 4–5 minutes, then aseptically decant supernatant without affecting the cell pellet. The centrifugation conditions are according to the cell type used (e. g. hMSCs at 300–400 xg for 4–5 minutes, PBMCs at 150 xg for 8 minutes).
3. Suspend the pellet in cold (2–8 °C) NutriStor® Cold Storage Solution and mix thoroughly.
4. Transfer the cells to the desired storage vials (e. g. cryovials, 1–2 mL/vial).

NOTE The maximal cell concentrations that were tested in the storage vials:

- hMSCs: $\sim 5 \times 10^6$ cells/mL (2 mL/vial)
 - Healthy PBMCs: $\sim 5 \times 10^6$ cells/mL (2 mL/vial)
 - Patient's CAR-Ts: $\sim 25 \times 10^6$ cells/mL (1 mL/vial)
5. Store the vials at 2–8 °C until required. Vials can be stored up to 7 days, 3–4 days are recommended.

NOTE NutriStor® Cold Storage Solution can be used for storage of an adherent culture. In this case the culture medium should be replaced with NutriStor® Cold Storage Solution before storing at 2–8 °C. After completion of the storage at 2–8 °C, remove the NutriStor® Cold Storage Solution and replace it with culture medium (no wash step is required). Incubate cells at 37 °C in a CO₂ incubator for 24 hours prior to additional in-vitro tests.

Post Storage Procedure

The cells in the vials are ready to use and further procedures are application dependent.

- We recommend performing viable cells count to evaluate the cell number, viability, and yield.
- Using PBMCs, 24 hours recovery in culture medium (e. g. 4Cell Nutri-T Medium) is recommended prior to additional in-vitro tests.
- Optional: Centrifuge the cells prior to further cell seeding.
 - The centrifugation conditions are according to the cell type used (e. g. hMSCs at 300–400 xg for 4–5 minutes, PBMCs at 150 xg for 8 minutes).

Culture the cells according to the recommended seeding density and the common practice.

7 Quality Control

Each lot is visually examined and tested for pH, osmolality, sterility, endotoxins and mycoplasma as well as performance (using BM-MSCs). For full specifications see the lot specific Certificate of Analysis (CoA).

8 Quality Assurance

- Manufactured under ISO 13485 QMS and in compliance with applicable cGMP guidelines.
- Manufactured under controlled environments and processes in accordance with:
 - ISO 13408 – Aseptic Processing of Health Care Products
 - ISO 14644 – Cleanrooms and associated controlled environments



Manufacturer

Biological Industries Israel Beit Haemek Ltd.
Kibbutz Beit Haemek 2511500, Israel

8.1 Product Label Symbols

REF

Indicates the manufacturer's catalogue number so that the product can be identified.

LOT

Indicates the manufacturer's batch code so that the batch or lot can be identified.

NOTE Synonyms for batch code are lot number and batch number.



Indicates the date after which the product is **not** to be used.



Indicates the temperature limits to which the product can be safely exposed.

STERILE A

Indicates a product that has been manufactured using accepted aseptic techniques.



Indicates the need for the user to consult the instructions for use.

9 Related Products

Product	Cat. No.
MSC NutriStem® XF Medium	05-200-1
MSC NutriStem® XF Supplement Mix	05-201-1
4Cell® Nutri-T Medium	05-11F2001-1
4 Cell® Nutri-T GMP Medium	05-F3F2111-1

