

### NUTRIFREEZ™

# CAPTURE YOUR CULTURE OF EXCELLENCE

A powerful, fully defined, animal component-free, serum-free freezing solution optimized for the cryopreservation of various cell types.

BI Biological Industries Culture of Excellence



## NUTRIFREEZ™

### POWERFUL CRYOPRESERVATION MEDIA OPTIMIZED FOR ENHANCED PERFORMANCE ACROSS A BROAD RANGE OF CELLS

Biological Industries' NutriFreez<sup>™</sup> brand of cryopreservation media has set the standard for high-quality freezing solutions. NutriFreez D10 Medium is the first fully defined, optimized freezing solution designed and validated for the cryopreservation of various cell types, including hESCs, iPSCs, and hMSCs. NutriFreez D10 Medium maintains animal component-free conditions during cryopreservation, essential to maintaining consistency when culturing cells in a xeno-free system. With proven reliability and consistency, NutriFreez D10 Medium provides a high-performance protective environment for cells during the freezing, storage, and thawing process.



# Optimized for maximum cell recovery and protection

Developed to protect a wide range of human and animal-derived cells, including highly sensitive cell types such as primary cells, lymphocytes, and stem cells (hMSCs and hPSCs).

# Unique, serum-free and fully defined formulation

High-quality, animal component-free, protein-free, serum-free freezing formulation designed for research, cell banking, and therapeutic applications. Contains 10% DMSO.

### Widely referenced, manufactured under cGMP

With over 300 references, NutriFreez is manufactured under cGMP conditions. Customization and scale-up support is available.

NUTRIFREEZ<sup>™</sup> D10 CRYOPRESERVATION MEDIUM | 05-713-1

# NutriFreez<sup>™</sup> D10 Medium: CAPTURE YOUR CULTURE OF **EXCELLENCE**.



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### Attain superior viability and recovery.



Figure 1. hMSCs in NutriFreez D10 Medium exhibit superior viability after the critical recovery period of three days post-thaw with a cell increase of >7-fold (A) and  $\geq$  95% viability directly post-thaw (B) when compared to other commercial serum-free solutions. When compared to homebrew freezing solutions, PBMCs cryopreserved in NutriFreez D10 Medium exhibit significantly higher viability rates direct post-thaw (C).

### Obtain more cells in less time.



**Figure 2**. hMSCs cryopreserved in NutriFreez D10 Medium exhibit normal morphology and exceptional proliferation rates, three days post thaw as they recover from cryopreservation when compared to other commercial serum-free solutions.

# Minimize cellular impact for long-term viability.

**Figure 3.** Viability comparisons of hMSCs derived from bone marrow (A) and adipose tissue (B) after long-term cryopreservation in NutriFreez D10 Medium exemplify the precise and controlled freezing environment the media produces to allow for >90% viability after up to 5 years of storage.





## NutriFreez™ D10 Medium: THE ULTIMATE CRYOPRESERVATION MEDIUM FOR STEEM CELLS.

### Human Mesenchymal Stem Cells

Precise formulation for consistently normal morphologies.



**Figure 4.** Dependably normal morphologies across hMSCs derived from multiple tissue types including adipose tissue (AT), bone marrow (BM), and dental pulp (DP) following cryopreservation in NutriFreez D10 Medium and three days post thaw.







Figure 5. hMSCs derived from adipose tissue (AT), bone marrow (BM), and dental pulp (DP) exhibit  $\ge$  91% viability following cryopreservation in NutriFreez D10 Medium direct post thaw and three days post thaw (A). Not only are these cells highly viable, but they also maintain multipotency marker expression measured via FACS analysis following cryopreservation (B).

### Controlled microenvironments for superior recovery.



**Figure 6.** When compared to homebrew and serum-free competitor freezing solutions, primary hMSCs (from healthy donors) cryopreserved in NutriFreez D10 Medium exhibited the best post-thaw viability and recovery rates in addition to increased cell attachment and growth performance. Images depict comparison of cell recovery of NutriStem D10 Medium, homebrew, and competitor freezing solution at 6 days post thaw.

Data provided by Prof. Shirley H.J. Mei and research team Yuan Tan and Mahmoud Salkhordeh, Regenerative Medicine Program, Ottawa Hospital Research Institute. Reference: Salkhordeh, et. al. May 2018. Evaluation of different cryopreservation agents for mesenchymal stem cell as final study product. Cytotherapy.

### **Human Pluripotent Stem Cells**

### Preserve cellular function in flexible formats.

Figure 8. Colonies of hESCs were cryopreserved in NutriFreez D10 Medium and were thawed into NutriStem® hPSC XF Medium on Matrigel, exemplifying the cells' ability to maintain excellent attachment, normal morphology, and exceptional proliferation marker expression.



H1 hESC Phase

BGO1V/hOG Phase

BGO1V/hOG Oct4-GFP reporter

### Capture cell potential downstream.

Figure 9. H1 hESC, identified by spontaneously formed (after 18 days) embryoid bodies sectioned and stained with H&E, maintain trilineage differentiation potential post cryopreservation in NutriFreez D10 Medium, enhancing the possibilities of downstream processing.



EC=neural rosettes, ME=primitive vessels, END=primitive parenchyma (100X)



### Maintain cellular capabilities.

Figure 10. ACS-1019 cells demonstrate excellent single cell recovery, attachment, and morphology following cryopreservation in NutriFreez D10 Medium.

### **Primary Cells & Various Cell Lines**



Optimized for superior viability across cell types.

**Figure 11.** HUVECs show  $\ge$  94% viability and high cell yield direct post thaw and normal morphology 4 days post thaw; cells cultured in EndoGo<sup>TM</sup> XF Medium (top). HDMECs show  $\ge$  96% viability and high cell yields direct post thaw and normal morphology 4 days post thaw; cells cultured in EndoGo<sup>TM</sup> XF Medium (bottom).



### Consistent production over long-term storage.

**Figure 12**. Various cell lines exhibit excellent attachment ability (left) as well as enhanced viability (right) following cryopreservation in NutriFreez D10 Medium when compared to homebrew freezing solutions (A). Various cell lines stored long-term exhibit high viability after 4-years of cryopreservation (B).





## NutriFreez™ D10 Medium: UNIVERSAL FORMULATION. RELIABLE RESULTS.



#### **ORDERING INFORMATION**

PRODUCT	CAT. #	SIZE
NutriFreez™ D10 Cryopreservation Medium	05-713-1A	500 mL
	05-713-1B	100 mL
	05-713-1E	50 mL

Bulk orders, custom packaging (bioprocessing bags), and scale-up support is available upon request.

To place an order in the US, contact Biological Industries USA at: tel: 860-316-2702; fax: 860-269-0596; email: orders@bioindusa.com.

Outside the US, contact your local distributor.

### **Frequently Asked Questions**

#### Q: Does NutriFreez D10 Medium contain Phenol Red?

A: Yes. Phenol red is used for pH monitoring during freeze and thaw cycles. If cells are thawed and the solution exhibits yellow/purple coloration, it may indicate that the freezing process was inadequate, and cell viability should be checked.

#### Q: Can I get NutriFreez D10 Medium without Phenol Red?

**A:** Yes, we can provide a custom version for those applications requiring a phenol red-free formulation as well as different packaging sizes.

#### Q: Why do I need an animal component-free freezing solution?

A: Serum is a "black box" that contains numerous components that are not necessary for cryopreservation and may even harm the cells during freezing and thawing. Animal component-free solutions ensure enhanced viability and recovery, in addition to reproducibility and consistency.

#### Q: Should I keep cells in -20°C / -80°C for long periods of time?

A: As with any freezing solution, long-term cryopreservation in NutriFreez D10 Medium is most efficient at -196°C or in liquid nitrogen. Storage in -20°C / -80°C for long periods may affect cell viability due to prolonged exposure to DMSO, an effect that is ameliorated by preserving cells in liquid nitrogen (-196°C).

#### Q: Do I need to add anything to NutriFreez D10 Medium?

**A:** No, it is a ready-to-use formulation; simply pellet the cells and resuspend in the solution.

#### Q: What type of cells can I cryopreserve in NutriFreez D10 Medium?

A: NutriFreez<sup>™</sup> D10 Medium has been used successfully with many cell types, including, but not limited to: Human Mesenchymal Stem Cells (hMSC) derived from Bone Marrow (BM-MSC), Adipose Tissue (AT-MSC), Umbilical Cord Tissue (UC-MSC), and Dental Pulp Tissue (DP-hMSC), Human Embryonic Stem Cells (hESC), Induced Pluripotent Stem Cells (iPSC), Human Peripheral Blood Mononuclear Cells (PBMC), Human Endothelial Cells (EC), T cells, including Chimeric Antigen Receptors (CAR-T) cells and Tumor-Infiltrating Lymphocytes (TIL), Neurons, Astrocytes, Hybridomas, CHO cells, Vero cells, multiple mammalian cell lines, including: MRC-5, HEK-293, HepG2, HeLa, BSC-1, BGM, 3T3, MA-10, BHK-21, as well as other extremely sensitive cell types.

### **Q:** Can I thaw my cells frozen in NutriFreez D10 Medium and inject them, as part of a clinical application?

A: No. NutriFreez D10 Medium is considered an ancillary material, meaning it may come into contact with the cell or tissue product during manufacturing, but is not intended to be part of the final product formulation.

#### Q: Can you freeze tissues using NutriFreez D10 Medium?

**A:** No, NutriFreez is used for freezing cells and clusters of cells (e.g. hPSC colonies). It is not intended for tissue samples.

### Q: What do you recommend: Liquid Nitrogen (LN2) in a liquid phase or vapor phase?

**A:** It is best to preserve cells in the vapor phase of the LN2, mainly due to the fact that mycoplasma can survive in extreme temperatures, and thus have the potential to be found in liquid phase.

#### Q: How do you validate NutriFreez D10 Medium?

A: Each lot is tested for: pH, sterility, appearance, and performance.

#### Q: Is NutriFreez D10 Medium manufactured under GMP conditions?

A: Yes, NutriFreez is manufactured under cGMP and under US FDA IVD part 864.9225 Cell-freezing apparatus and reagents for in vitro diagnostic use. It is listed in Europe under CE IVD class I, thus complying with European In-Vitro Diagnostic Devices Directive (98/79/EC) requirements, manufactured under ISO 13485 QMS and in compliance with applicable cGMP guidelines. It is manufactured under controlled environments and processes in accordance with: - ISO 13408 – Aseptic Processing of Health Care Products; - ISO 14644 – Airborne Particulate Cleanliness Classes in Clean Rooms and Clean Zones.



Biological Industries USA 100 Sebethe Drive Cromwell, CT, 06416 Tel: 860-316-2702 Email: orders@bioindusa.com Biological Industries Kibbutz Beit-Haemek 25115, Israel Tel: 972-(0)4-9960595 Email: info@bioind.com Biological Industries Shanghai 3F, Building 1, No.80, Lane 1505 Zuchongzhi Rd, Pudong District Shanghai, China, 201203 Tel: (021) 58785545

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