Instructions for Use

NutriFreez® D5 Salt-Based Cryopreservation Solution

An Animal Component-Free (ACF), salt-based, chemically defined, protein-free, cryopreservation solution with 5% DMSO

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Animal Component-Free (ACF), salt-based, chemically defined, protein-free, cryopreservation solution with 5% DMSO

REF	05-715-1
	2 - 8°C

Contents

1 Product Description

NutriFreez® D5 Salt-Based Cryopreservation Solution is an optimal All-in-one solution, chemically defined and specially designed for the freezing and thawing of cells which are 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 Mesenchymal Stromal Cells (MSCs) as part of the broad QC tests, it has been widely tested on a variety of additional cells, such as PBMCs, NK, CHO, hPSCs and Vero cells as well.

NutriFreez® 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.

2 Cell Type Applications

NutriFreez® D5 Salt-Based Cryopreservation Solution was tested and found suitable for the cryopreservation of:

- Human Mesenchymal Stromal Cells (hMSCs), from various sources:
  - Bone Marrow (BM-MSCs)
  - Adipose Tissue (AT-MSCs)
  - Umbilical Cord tissue (UC-MSCs)
  - Dental Pulp tissue (DP-MSCs)
- Human Pluripotent Stem Cells (hPSCs)
- Immune Cells (Patient CAR-Ts, healthy PBMCs, and NK cells)
- Mouse Hepatic Organoids
- Colorectal Cancer (CRC) Organoids
- Cord Blood Total Nucleated Cells (TNC)
- CHO cells
- Vero Cells

3 Precaution and Disclaimer

- Do **not** use if a visible precipitate is observed in the freezing solution
- Do **not** use beyond the expiration date indicated on the product label
- Store at 2 – 8°C
- Avoid exposure to light
- Please refer to the Material Safety Data Sheet (MSDS) for hazard information
- Maintain aseptic work conditions
- 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.

4 Storage and Stability

NutriFreez® D5 Cryopreservation Solution should be stored at 2 – 8°C.

5 Features

- A Salt-Based, Protein-Free, Serum-Free, Animal Component-Free, 5% DMSO solution
- Manufactured for the freezing and thawing of cells which are intended for clinical use
- An all-in-one bottle, ready-to-use solution
- High post-thaw viability and recovery of cells
- A Chemically defined solution. Does not contain any antibiotics, antimycotics, hormones or growth factors
- Maintains stem cell multipotency and expansion capabilities

6 Instructions for use

Instructions for Use for the Cryopreservation of Human Mesenchymal Stromal Cells (hMSC)

NOTE

- It is recommended to keep NutriFreez® D5 Cryopreservation Solution on ice at all times during use.

Freezing procedure of hMSC

1. Aseptically remove the hMSC culture medium from the culture vessel or well(s) to be harvested for cryopreservation.
2. Rinse wells with Dulbecco's PBS w/o Ca & Mg (Cat. No. 02-023-1), using approximately 2 mL of DPBS per 10 cm^2 culture surface area, then aspirate the DPBS.
3. Detach adherent hMSC using a sufficient volume of Recombinant Trypsin Solution (Cat. No. 03-078-1) to cover the entire cell culture surface and incubate the cells at room temperature or 37°C for 3 to 5 minutes.

   NOTE Recombinant Trypsin-EDTA Solution (Cat. No. 03-079-1) can be used if the cells are over-confluent or are difficult to detach.

4. Observe the cells under a microscope. If less than 90% of the cells are detached from the culture surface, continue incubating and observe again at 1-minute intervals to check for complete detachment.

   NOTE Incubation times may vary between cells and confluency levels. Begin checking the cultures after 3 minutes. Do not over-incubate the culture, as MSC can be sensitive to enzymatic stress. Tap the vessel periodically to expedite cell detachment and monitor the progress of the enzyme solution.

5. Once the cells are detached from the surface, neutralize the action of the trypsin enzyme by adding a volume of pre-warmed complete medium that is 2 – 4 times the volume of the trypsin solution used.

   NOTE Alternatively, 1X Soybean Trypsin Inhibitor (SBTI) solution (Cat. No. 03-048-1) diluted in DPBS can be used to neutralize the trypsin.

6. Collect the cell suspension and transfer to a centrifuge tube. If needed, rinse the culture vessel with additional media to collect any remaining cells, and transfer to the same tube. Perform a viable cells count if neccessary.
7. Centrifuge at 300 x g for 5 minutes at room temperature, and then aseptically remove supernatant without affecting the cell pellet.
8. Determine the required volume of NutriFreez® D5 Cryopreservation Solution needed according to the desired concentration.
9. Remove the supernatant from the centrifuge tube and quickly but gently resuspend the pellet in cold NutriFreez® D5 Cryopreservation Solution according to the freezing volume determined in the previous step.
10. Dispense aliquots of this suspension into cryovials (e.g., 1.0 mL of suspension in a 1.5 mL cryovial).

    NOTE If freezing multiple cryovials, keep the cells on ice at all times. Gently mix the resuspended cell solution frequently to ensure even distribution throughout the vials. Immediately transfer filled cryovials to ice before aliquotting the remaining cell solution.

11. Freeze the cells gradually (1 – 2°C per minute) by using a controlled rate freezing system and store the vials in liquid nitrogen (vapor phase). Alternatively, place the vials in appropriate freezing container (e.g. Mr. Frosty) and transfer to -80°C for at least 2 hours and up to 24 hours.
12. After 2 – 24 hours, transfer the cryovials into liquid nitrogen (vapor phase).

    NOTE Long-term storage at -80°C is not recommended.

Thawing of Cryopreserved hMSCs

1. Briefly warm 5 – 10 mL of complete MSC NutriStem® XF Medium (Cat. No. 05-200-1 and 05-201-1) or other growth culture medium, in a 50 mL centrifuge tube.
2. Rapidly thaw a cryovial of hMSC in a 37 °C water bath, by gently shaking the vial and remove the vial when only a small frozen cell pellet remains. Do **not** vortex cells.
3. Disinfect the vial by wiping it down with a cloth moistened with 70% Ethanol or Isopropanol.
4. In a sterile biological safety cabinet, transfer the contents of the cryovial drop by drop into culture medium in the previously prepared centrifuge tube. Gently rock to continually mix the cells as the new cell droplets are added to the tube.
5. Centrifuge cells at 300x g for 4 – 5 minutes at room temperature.

   NOTE It is possible to skip the centrifugation step after thawing by simply transferring the thawed cells directly onto a culture vessel with medium at a ratio of at least 1:10 (for the dilution of the DMSO). Replace the culture medium when the cells are attached and spread (usually after 2 hours post seeding).

6. Remove supernatant and resuspend cell pellet in 0.5 – 1 mL of complete MSC NutriStem® XF Medium or other culture medium.
7. Perform a viable cell count (e.g., using Trypan Blue Exclusion Assay).
8. Add the desired volume of complete MSC NutriStem® XF Medium or other culture medium.
9. Culture cells as desired and incubate in a humidified CO_2 incubator (37°C).
10. Refresh culture medium 48 hours after plating.

7 Quality Control

Each lot is tested for pH, sterility, recovery and morphology of human Mesenchymal Stromal Cells (hMSCs). For full specifications please check the lot specific Certificate of Analysis (CoA).

Each lot is tested for pH, sterility and performance using hMSC-BM.

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

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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.
CE	Indicates that the product meets the requirements of the applicable EC directives.
IVD	Indicates a product that is intended to be used as an in vitro diagnostic medical device.
	Indicates the need for the user to consult the instructions for use.

9 Related Products

Product	Cat. No.
Dulbecco's PBS (w/o Ca & Mg)	02-023-1
Soybean Trypsin Inhibitor (SBTI)	03-048-1
Cell dissociation solution – non enzyamtic	03-071-1
MSC NutriStem® XF Basal Medium	05-200-1
MSC NutriStem® Supplement Mix	05-201-1
MSC Attachment Solution	05-752-1
Recombinant Trypsin Solution	03-078-1
Recombinant Trypsin-EDTA Solution	03-079-1
NutriFreez® D10 Cryopreservation Medium	05-713-1

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The information and figures contained in these instructions correspond to the version date specified below.
Sartorius reserves the right to make changes to the technology, features, specifications and design of the equipment without notice.
Masculine or feminine forms are used to facilitate legibility in these instructions and always simultaneously denote all genders.

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