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Culture of Excellence

NutriFreez™ D10 Cryopreservation Medium

Animal Component-Free (ACF), chemically defined, protein-free, cryopreservation medium with 10% DMSO

REF 05-713-1

2-8°C

Instructions for Use

Product Description

NutriFreez™ D10 Cryopreservation Medium is a unique animal component-free, chemically defined, serum free, protein-free, cryopreservation formulation containing Methylcellulose and 10% Dimethyl Sulfoxide (DMSO). NutriFreez™D10 Cryopreservation Medium is designed to maintain a multitude of cells types, even extremely sensitive cells, in ultra-low-temperatures (-196°C) by providing a defined, protective environment. NutriFreez™ D10 Cryopreservation Medium ensures high viability, recovery rates and performance with a variety of cells, including primary human cells, and mesenchymal stem cells. NutriFreez™ D10 Cryopreservation Medium is optimally formulated and does not contain any antibiotics, antimycotics, hormones, growth factors, serum, or protein.

Cell Type Applications

- Human Mesenchymal Stem Cells (hMSC), freshly isolated - expanded and commercial from various sources:
  - Bone Marrow (BM-MSC)
  - Adipose Tissue (AT-MSC)
  - Umbilical Cord Tissue (UC-MSC)
  - 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: MRC-5, HEK-293, HepG2, HeLa, BSC-1, BGM, 3T3, MA-10, BHK-21 as well as other extremely sensitive cell types

Features

- Ready-to-use
- Animal component-free,Chemically defined, serum-free, protein-free
- Manufactured in complince with applicable cGMP guidlines
- Sterility, endotoxin, and cell-based quality control testing
- High post-thaw viability and recovery of cells
- Maintains stem cell pluripotency and expansion capabilities

Precaution and Disclaimer

- Do not use if a visible precipitate is observed in the freezing medium
- Do not use beyond the expiration date indicated on the product label
- Store at 2°C to 8°C
- Avoid exposure to light
- Please refer to the Material Safety Data Sheet (MSDS) for hazard information
- Maintain aseptic work conditions
- Caution: not for direct administration into humans or animals.

Quality Control

- Certificate of Analyses is available for each lot.
- Each lot is tested for: pH, sterility, appearance, performance: freezing of animal cells (Vero cells) and human stem cells (hPCS (H1 and hMSC).

Quality Assurance

- For in vitro diagnostic use, research use or for use as ancillary material in manufacturing Cell-Gene or Tissue-based products.
- Notified under US FDA IVD part 864.9225 Cell-freezing apparatus and reagents for in vitro diagnostic use.
- Listed in Europe under CE IVD class I, thus comply with European In-Vitro Diagnostic Devices Directive (98/79/EC) requirements.
- 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 – Airborne Particulate Cleanliness Classes in Clean Rooms and Clean Zones;
- DMF (Drug Master File) has been acknowledged by the FDA.

EC REP Authorized Representative in the European Community:
MedNet GmbH Borkstrass 48163 Muenster
Germany

Manufacturer:
Biological Industries Israel Beit Haemek Ltd.
Kibbutz Beit Haemek 2511500, Israel
T.+972.4.9960595 F.+972.4.9968896
Email: info@bioind.com

Product Label Symbols

IVD	Indicates a medical device that is intended to be used as an in vitro diagnostic medical device.
CE	The product meets the requirements of the applicable EC directives
REF	Indicates the manufacturer's catalogue number so that the medical device can be identified.
STERILE A	Indicates a medical device that has been manufactured using accepted aseptic techniques.

I. General Instructions for Use for the Cryopreservation of Mammalian Cells

Notes:

- **For freezing human Induced Pluripotent and Embryonic Stem Cells (hPSC) see section II.**
- **For freezing human Mesenchymal Stem Cells (hMSC) see section III.**
- Keep NutriFreez™ D10 Cryopreservation Medium on ice at all times during use.
- For freezing adherent cells, detach cells using a dissociation solution according to the manual instructions.

Freezing procedure

1. To maintain aseptic work conditions, wipe the outer packaging with a cloth moistened in 70% Ethanol/70% Isopropanol before opening the NutriFreez™ D10 Cryopreservation Medium.
2. Centrifuge cells to obtain a cell pellet, 300-400xg for 4-5 minutes, then aseptically decant supernatant without affecting the cell pellet.
3. Suspend the pellet in cold (2 - 8°C) NutriFreez™ D10 Cryopreservation Medium, mix thoroughly, and transfer the suspension to a cryovial (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.

4. 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 overnight. The following day transfer cryovials into liquid nitrogen (vapor phase recommended).

Note: long-term storage at -80°C is not recommended.

5. It is recommended to determine the efficiency of cryopreservation by thawing one vial after 24 hours of storage in liquid nitrogen and following the thawing procedure outlined below.

Thawing of cryopreserved cells:

1. Briefly warm culture medium of choice in a 37°C water bath.
2. Rapidly thaw the cryovial of cells 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. Suspend the cells in warmed growth culture medium at a ratio of at least 1:10 (cell suspension to culture medium).
5. Centrifuge cells to obtain a cell pellet, 300-400 x g for 4-5 minutes, then aseptically decant supernatant without affecting the cell pellet and resuspend in growth medium as desired.
6. Culture the cells according to the recommended seeding density.

II. Instructions for Use for the Cryopreservation of Human Induced Pluripotent and Embryonic Stem Cells (hPSC)

Notes:
- hPSC may be frozen as clumps or single cells with high viability and minimal differentiation post thaw.
- The single cells can be thawed onto recombinant Laminin coated culture ware without the addition of ROCK inhibitors. In case of using other matrices (e.g. Matrigel™), Rock inhibitor is required.
- Keep NutriFreez™ D10 Cryopreservation Medium on ice at all times during use.

The procedure describes the cryopreservation of cells cultured in a 6-well plate.

1. Using a vacuum aspirator and a sterile aspirator pipette, remove the hPSC 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# 02-023-1), using approximately 2mL of DPBS per 10cm^2 culture surface area, then aspirate out the DPBS.
3. Determine the desired viable cell density and calculate the required volume of NutriFreez™ D10 Cryopreservation Medium needed for a concentration of approximately 1×10^6 viable cells/mL.
4. Add dissociation solution as desired. Cells may be detached using the enzyme and method that the culture has been routinely passaged with.

   In case of using Collagenase, Dispase or EDTA, incubate at 37°C or at room temperature until the edges of the colonies begin to loosen from the plate.

Note: Incubation times may vary between cell lines, colony size and the detachment solution used. Begin checking the culture after 3 minutes.

5. Cells cultured on Laminin may be detached using Recombinant Trypsin-EDTA Solution (Cat# 03-079-1) to yield a single cell suspension.

Note: Once the cells are detached from the surface, neutralize the enzyme by adding 2-4 volumes of pre-warmed complete medium to the volume of the trypsin solution used. Alternatively, 1X Soybean Trypsin Inhibitor (SBTI) solution (Cat. No. 03-048-1) diluted in DPBS can be used to neutralize the trypsin.

6. Transfer the clumps or cell suspension to a centrifuge tube.
7. Centrifuge at 200 x g for 5 minutes at room temperature then aseptically decant supernatant without affecting the cell pellet.
8. Resuspend the cell pellet in the pre-determined volume of cold NutriFreez™ D10 Cryopreservation Medium on ice (1mL for every 1×10^6 viable cells). In case of aggregates; do not break up cell masses any more than necessary, two or three gentle pipetting motions are usually sufficient.
9. Dispense aliquots of this suspension into cryo vials (e.g., 1.0mL of suspension in a 1.5mL 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.

10. 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 an appropriate freezing container (e.g. Mr. Frosty) and transfer to -80°C overnight.
11. The following day, transfer vials to liquid nitrogen storage (vapor phase).

Note: Long term storage at -80°C is not recommended

Thawing of Cryopreserved hPSC

1. Briefly warm NutriStem® hPSC XF Medium, or other growth culture media of choice, in a 37°C water bath.
2. Add 9ml of warmed NutriStem® hPSC XF Medium, or other growth culture media, into a centrifuge tube.
3. Rapidly thaw the cryovial of cells 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.
4. Disinfect the vial by wiping it down with a cloth moistened with 70% Ethanol or Isopropanol.
5. In a sterile biological safety cabinet, transfer the contents of the cryovial drop by drop into the 9mL of 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.
6. Centrifuge the cells at 200 x g for 5 minutes. Remove and discard supernatant.
7. Gently resuspend the cell pellet in NutriStem® hPSC XF Medium (Cat# 05-100-1) or other growth culture media, and plate as desired.
8. Refresh culture medium 48 hrs after plating.

III. Instructions for Use for Cryopreservation of Human Mesenchymal Stem Cells (hMSC)

Note:
- Keep NutriFreez™ D10 Cryopreservation Medium on ice at all times during use

Freezing procedure of hMSC

1. Using a vacuum aspirator and a sterile aspirator pipette, 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# 02-023-1), using approximately 2mL of DPBS per 10 cm culture surface area, then aspirate out 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 after a short incubation with Recombinant Trypsin Solution.

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 will 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 to 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.
7. Centrifuge at 300x g for 5 minutes at room temperature, and then aseptically decant supernatant without affecting the cell pellet.
8. Resuspend the pellet in the amount of complete media (approximately 3-5mL) required to perform a cell count to determine the total viable cell number (e.g, using Trypan Blue Exclusion Assay).
9. Centrifuge the cell suspension at 300x g for 5 minutes at room temperature.
10. Determine the required volume of NutriFreez™ D10 Cryopreservation Medium needed for a concentration of approximately 1×10^6 viable cells/mL.
11. Remove the supernatant from the centrifuge tube and quickly but gently resuspend the pellet in cold NutriFreez™ D10 Cryopreservation Medium according to the freezing volume determined in the previous step.
12. Dispense aliquots of this suspension into cryovials (e.g., 1.0mL of suspension in a 1.5mL 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.

13. 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 overnight.
14. The following day, transfer the cryovials into liquid nitrogen (vapor phase).

Note: long-term storage at -80°C is not recommended.

It is recommended to determine the efficiency of cryopreservation by thawing one vial after 24 hours of storage in liquid nitrogen and following the thawing procedure outlined below.

Thawing of Cryopreserved hMSCs

1. Briefly warm 5-10mL of complete MSC NutriStem® XF Medium (Cat. No. 05-200-1 and 05-201-1) or other growth culture medium, in a 50mL 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, then resuspend the cells by carefully pipetting up and down.
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).

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 hrs. after plating.

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
NutriStem® hPSC XF	05-100-1
LaminStem™ 521	05-753-1
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
NutriStem® V9 XF Medium	05-105-1