



CryoStem™ MSC Freezing Medium

A chemically defined, animal- component free and protein-free freezing solution, designed for the cryopreservation of hMSCs

Cat. No.: 05-712-1E 50 ml
05-712-1D 10 ml

Store at: 2-8°C

Instructions for Use

Product Description

There are many problems associated with the use of animal sera e.g. the fear of contamination with viral agents such as BSE, Hepatitis, HIV, BVD, or other potential adventitious agents, especially regarding human mesenchymal stem cells (hMSCs) which are mainly intended for verity of clinical application. The culture of hMSCs in serum-free medium (e.g MSC NutriStem® XF medium, Cat. No. 05-200-1 and 05-201-1) eliminates those risks. Furthermore, it allows hMSCs to be grown under a defined set of conditions. When cultured cells under serum free conditions, it is important to cryopreserve the cells also in a solution free of serum.

The novel CryoStem™ MSC Freezing Medium contains no serum, but rather methylcellulose and DMSO. After freezing and thawing, a very high percentage of viable hMSCs are obtained with excellent attachment ability as well as growth performance. In fact, comparative studies have shown that in most cases higher viabilities and adhesion percentages are obtained in comparison to serum-containing freezing medium. Therefore, the use of serum-free CryoStem™ MSC Freezing Medium is also recommended for hMSC culture employing serum-supplemented growth media.

Features

- A complete ready-to-use solution.
- Without any animal or human derived components.
- Suitable for various sources of hMSCs (such as bone marrow, adipose tissue, umbilical cord tissue).
- Suitable for cells cultured in serum-free and serum-containing media.
- High cell viability after thawing.
- Optimized for hMSCs culterd in MSC NutriStem® XF Medium.

Precautions and Disclaimer

1. For in vitro diagnostic use.
2. Do not use if a visible precipitate is observed in the CryoStem™ MSC Freezing Medium.
3. Do not use beyond the expiration date indicated on the product label.

Instruction for use

Cryopreservation of hMSC Cultures

1. Detach adherent hMSC using MSC Dissociation Solution (Cat. No. 03-075-1) or Non-Enzymatic MSC Dissociation Solution (03-077-1) according to manual instruction.
2. Pellet hMSC by centrifugation at 300-400g for 4-5 minutes. Carefully discard supernatant.
3. Re-suspend the pellet in cold MSC Freezing Solution at the desired concentration (recommended: $0.5-1.0 \times 10^6$ cells/ml; 1ml/vial).
4. Immediately place the cryovials in appropriate freezing container (e.g., "Mr. Frosty") and store at -80°C for overnight.
5. Transfer the cryovials into liquid nitrogen.
6. Viability and recovery of cryopreserved hMSCs should be checked 24 hours after storage of vials in liquid nitrogen by following the thawing procedure outlined below.

Thawing of Cryopreserved hMSC

1. Pre warm 5-10 ml of complete MSC NutriStem® XF Medium (05-200-1 and 05-201-1) in a 50ml conical tube.
2. Rapidly thaw frozen vial of hMSCs in a 37°C water bath, with agitation until a small amount of ice remains.
3. Slowly add (drop by drop while gently swirling) the cells into the pre-warmed complete MSC NutriStem® XF Medium. Resuspend the cells by carefully pipetting up and down.
4. Centrifuge cells at 300-400xg for 4-5 minutes at room temperature.
5. Remove supernatant and re-suspend cell pellet in 0.5-1 ml of complete MSC NutriStem® XF Medium.
6. Perform a viable cell count (e.g., using Trypan Blue Exclusion Assay).
7. Add the desired volume of complete MSC NutriStem® XF Medium.
8. Culture cells as desired and incubate in a humidified CO_2 incubator (37°C).

Note:

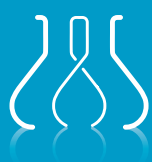
It is possible to avoid the centrifugation step after thawing. In this case skip steps 8.14-8.1.5 and transfer the thawed cells (from Step 8.1.3) directly into the pre-coated culture flask (using MSC Attachment Solution, Cat. No. 05-752-1) with the required volume of the complete MSC NutriStem® XF Medium, at a ratio of at least 1:10 (for the dilution of the DMSO).

Quality Control

This product is tested for performance using MSC cells. Each lot is tested for cell viability, cell attachment and proliferation after cryopreservation and thawing.

Auxiliary products

Product name	Cat. No.
MSC NutriStem® XF Basal Medium and Supplement Mix	05-200-1 and 05-201-1
MSC Attachment Solution	05-752-1
Recombinant Trypsin Solution	03-078-1
Recombinant Trypsin-EDTA Solution	03-079-1
Dulbecco's PBS (w/o Ca & Mg)	02-023-1



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