

USER MANUAL

Product: AFTERFREEZE

Reference number: 144 (100 mL), 145 (500 mL), 425 (100 mL), 397 (500 mL).

Size: 100 mL bottle, 500 mL bottle.

Intended Use:

For research and manufacturing purposes only.

Overview Product:

AfterFreeze is a complete cell thawing solution that does not require the addition of any other components to the medium before use. It is used for thawing cells that have been cryopreserved at temperatures of -86°C and -196°C in the MSCCryosave OTS environment and the cells can be directly used without washing off the MSCCryosave OTS and AfterFreeze solutions. With our unique technology, AfterFreeze does not contain any salt ions, amino acids, or proteins of animal origin in its composition.

The solution has a low endotoxin level (≤ 0.25 EU/mL), is free from Mycoplasma contamination, meets the sterility criteria (0 CFU).

Note: The viability of the cells depends on the condition of the mesenchymal stem cells before preservation and the proper execution of the freezing and thawing procedures.

Known Applications:

AfterFreeze, in combination with MSCCryosave OTS, has been evaluated for use on adipose-derived mesenchymal stem cells, bone marrow-derived mesenchymal stem cells, and umbilical cord-derived mesenchymal stem cells. On the cell lines that have been studied, the cell survival rate after cryopreservation is over 90% when stored at -86°C or -196°C for up to 6 months. The manufacturer is currently monitoring longer-term storage to assess the viability of cells over extended periods.

Reconstitution, Dilution, and Mixing:

The product is delivered at a 1X concentration, requiring no further dilution or supplementation.

Materials and Reagents Required But Not Provided:

Not applicable.

Handling and Storage:

Transport at room temperature.

Store at room temperature. Do not refrigerate the product.

Recommended shelf life: best before 24 months from manufacture date.

Instructions for Use:

Scenario 1: Cells stored in MSCCryoSave OTS and cryopreserved at -86°C .

1. Take the vials/bags containing the cells out of the -86°C freezer and place them on a clean surface (wood, stone) to allow them to thaw naturally. Do not place the tubes/bags on stainless steel or metal surfaces.
2. Wait for the vials/bags to thaw naturally. To expedite the thawing process, you can gently hold and shake the vials/bags occasionally.

Avoid rapid thawing by placing them in a water bath or incubator. The estimated thawing time for a 1.8 mL cryovial is approximately 17 minutes, for a 5 mL tube is around 10 minutes, and for a 20mL bag is approximately 20 minutes when thaw naturally.

3. Once the thawing process is nearly complete and there are still small ice crystals remaining, proceed to mix the AfterFreeze solution with the MSCCryoSave OTS containing cells solution at a 1:1 ratio (by volume).
4. Thoroughly mix the AfterFreeze and MSCCryoSave OTS solutions. This resulting cell suspension can be directly used without the need for washing. At this

step, you can take a sample to count the cell quantity and viability. The cell suspension can be further diluted with physiological saline solution, Lactate Ringer's solution, Stem Cell Infusion Medium, or other isotonic infusion solutions.

To ensure a high cell viability rate, it is recommended to use the thawed cells within 45 minutes after thawing.

Scenario 2: Cells stored in MSCCryoSave OTS and cryopreserved at -196°C

Since MSCCryoSave OTS does not contain cryoprotective agents, it is necessary to perform a slow thawing process. The cells stored at -196°C should be taken out and placed in a -86°C freezer overnight (> 12 hours) to gradually increase the temperature from -196°C to -86°C. The next day, the thawing process can be carried out following the steps outlined in Scenario 1.

Precautions:

This is a specialized solution used for thawing mesenchymal stem cells preserved in the MSCCryoSave OTS environment (catalog numbers: 182, 185). It should not be used for thawing other cell lines preserved in different environments than MSCCryoSave OTS.









AfterFreeze is a product used for thawing cells that have been cryopreserved using MSCCryoSave OTS at a 1:1 mixing ratio. AfterFreeze does not contain any salt ions, amino acids, or proteins.

First Aid Measures:

Not applicable.

Hazards Identification:

The symbols on produce labels are explained below:

			
Use By:	Batch code	Keep away from light	Catalog number
			
Temperature Limitation	Consult instructions for use	Caution, consult accompanying documents	Sterilized using aseptic processing techniques

Related products:

Product Name	Reference Number
AfterFreeze OTS	
100 mL	261
500 mL	176
MSCCryoSave OTS	
100 mL	182, 398
500 mL	185
Cryosave I	
100 mL	136
Cryosave II	
100 mL	138
MSCCryoSave OTS Neutral	
100 mL	381
MSCCryoSave OTSTH	
100 mL	183

To purchase other products, please visit:

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