# Serum/Protein-Free Cell Freezing Medium

## P778221

**Storage：** Store at 2-8℃ for 12 months.

### Introduction:

Serum/Protein-Free Cell Freezing Medium adopts a combined formula of permeating protectants, non-permeating protectants, cell sedimentation stabilizers, and cell membrane protectants to provide comprehensive low-temperature protection for cells. It can lower the freezing point of the solution during cryopreservation, improve the permeability of cell membranes to water, allowing intracellular water to permeate out of cells before freezing, thereby preventing or reducing ice crystal damage to cells. Meanwhile, it reduces the concentration of electrolytes around cells during freezing to avoid electrolyte-induced damage, and can also delay the mutual extrusion of cells during cryopreservation and protect cell membranes, thus effectively improving the cell recovery rate and viability after resuscitation.

Traditional cell cryopreservation medium is prepared by mixing culture medium, serum, and DMSO in a certain proportion. Due to disadvantages such as complex serum components and large batch-to-batch variations, its application is limited. Moreover, it requires a programmed cooling cryopreservation method, which is time-consuming and labor-intensive.

Serum/Protein-Free Cell Freezing Medium is a Serum-Free, Animal Origin-Free, Protein-Free, and Chemically Defined cryopreservation medium that does not require programmed cooling. It has obvious advantages compared with traditional cell cryopreservation medium, and the survival rate of cryopreserved cells after resuscitation is also significantly improved.

Serum/Protein-Free Cell Freezing Medium (Stem Cell Special) is recommended for the cryopreservation of various conventional mammalian cells, and is particularly suitable for the cryopreservation of serum-free cultured stem cells and protein-expressing cells.

### Product Features：

**Safety** – Serum-free, animal-origin-free, protein-free, with a chemically defined composition.
**High Efficiency** – Effectively improves cell recovery survival rate; the relative recovery rate of immune cells can reach over 90%.
**Stability** – It does not affect the phenotype and growth status of stem cells, and can maintain the multi-differentiation potential of stem cells.
**Convenience** – Ready-to-use product that requires no programmed cooling. It can be directly stored at -80℃ overnight before being transferred to liquid nitrogen for long-term preservation.
**High Quality** – Strict quality control ensures product performance comparable to imported

alternatives; raw materials are either self-produced or high-purity, pharmaceutical-grade materials with low endotoxin, subjected to rigorous testing and purification; prepared with water for injection, featuring stable processes and minimal batch-to-batch variation.

**Reliability** – Already applied in the cryopreservation of various immune cells such as T cells, NK cells, and CIK cells.

## Usage method:

**Cell Cryopreservation:**

Before cryopreservation, ensure that the cells are in the logarithmic growth phase.

1. Preparation of cell suspension from adherent cells: (Suspension cells can skip this step)

(1) Aspirate and remove the old medium, and wash twice with PBS.

(2) Add a small amount of trypsin to the culture flask, just enough to cover the bottom of the flask. Place the culture flask flat in the incubator for digestion for approximately 1–2 minutes. During this period, observe under a microscope. Once cell gaps expand, cells become rounded, and appear loose, immediately terminate digestion (For individual hard-to-digest cells, the digestion time may need to be extended, but over-digestion should be avoided).

(3) Add an appropriate amount of pre-warmed complete medium to terminate digestion, and gently pipette to resuspend the cells evenly.

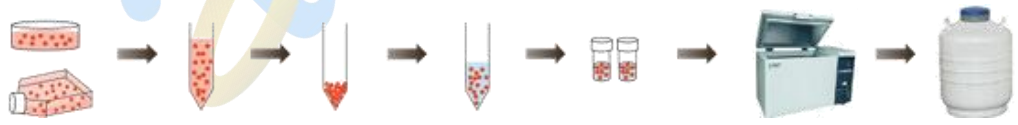
2. Cryopreservation of adherent cells and suspension cells:

(1) Take a small amount of cell suspension for cell counting, and calculate the total number of cells and the required volume of cell cryopreservation solution. The optimal cryopreservation density of cells is $1\times10^6$~$2\times10^7$ cells/ml.

(2) Transfer the required volume of cell suspension to an appropriate centrifuge tube, centrifuge at 200~500 × g for 3 – 5 minutes, discard the supernatant, and collect the cells. (Centrifugation speed and time depend on cell type)

(3) Add an appropriate amount of Serum/Protein-Free Cell Freezing Medium to the cell pellet, gently pipette to resuspend the cells, aliquot into sterile cell cryopreservation tubes, seal tightly, and mark the cell name, passage number, and date.

(4) Store directly in a -80℃ refrigerator, where cells can be preserved for at least one year; or after overnight storage at -80℃, transfer the cells to liquid nitrogen for long-term storage the next day.

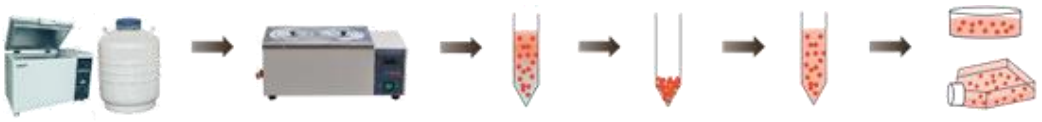


**Cell Revival:**

1. Take out the cryopreservation tube from the liquid nitrogen tank or -80℃ refrigerator, check whether the lid is tightly screwed, and immediately put it into a 37℃ water bath for rapid thawing (to avoid cell death caused by recrystallization of ice crystals). Gently shake

the freezing tube to completely melt it within 1-2 minutes. Wipe the outside of the cryopreservation tube with 75% alcohol and move it into a sterile operating platform.

2. Transfer the thawed cell suspension to a 15ml sterile centrifuge tube, then add 5-10ml of pre-warmed complete medium, gently mix well, centrifuge at 200-500×g for 3-5 minutes, discard the supernatant and collect the cells. (Centrifugation speed and time depend on cell type)
3. Add pre-warmed complete medium, mix well, transfer to a cell culture dish or flask, and place in a cell incubator for cultivation.



## Precautions:

1. During the cryopreservation process, after adding the cryopreservation solution to the cells, please operate as close to an ice pack as possible, because low temperature can prevent the protective agent from causing damage to the cells.
2. Please ensure that the cell cryopreservation tube is completely sealed to avoid explosion of the cryopreservation tube during the revival process.
3. Theoretically, this product is suitable for the cryopreservation of various mammalian immune cells (primary or immortal cell lines). However, due to the wide variety of cell types, it is impossible to verify them one by one. Therefore, we recommend that before using this product for the first time, you should conduct an experimental cell cryopreservation culture for the cells to be cryopreserved for at least one week. Proceed with formal cryopreservation only after confirming its performance.
4. This product is supplied in sterile packaging and does not require filtration. Please note to take it aseptically.
5. For your safety and health, please wear a lab coat and disposable gloves during operation.