

## Freezing Medium (Serum-free & animal origin-free)

Cat. No.: PB180438

Size: 10mL×5 / 50mL / 100mL

### General Information

Product Form	Liquid
Concentration	Ready-to-use
pH	7.4-7.6
Bacterial detection	Negative
Fungal detection	Negative
Mycoplasma detection	Negative
Endotoxin level	< 3 EU/mL
Shipping Conditions	Ice bag
Storage	2-8°C
Expiry date	12 months

### Introduction

In order to preserve the biological activity of cells in vitro, cells must be cryopreserved for a long time and then revived and cultured when needed. Currently, the most commonly used technique for cell freezing is liquid nitrogen cryopreservation, which primarily involves adding an appropriate amount of protectant to slowly cool the cells to a specified temperature range, thereby protecting the cells.

The Serum-free Cell Freezing Medium is a specialized cryopreservation product developed by Procell for use with hundreds of cell types, continuously optimizing experimental conditions for cell cryopreservation and resuscitation in long-term cell research.

The product contains a cell sedimentation stabilizer, which can delay the sedimentation rate of cells during the cryopreservation process, preventing cells from squeezing each other and affecting the effectiveness of cell cryopreservation. In addition, a variety of cryoprotectants, such as cell membrane protectors, permeable intracellular membrane protectors, and non-permeable cell protectors, are added. These components combine with water molecules in the solution to hydrate and weaken the crystallization process of water, increasing the viscosity of the solution and reducing the formation of ice crystals, which can greatly reduce the damage of ice crystals to cells during cryopreservation, and effectively improve the survival rate of cells after resuscitation. The product formula is clear, does not contain serum or animal-derived protein, which can reduce contamination risk from bacteria, viruses, and mycoplasma, and ensure the safety of frozen cells. It is not only suitable for conventional cell lines, primary cells, but also serum-free culture cells and protein-expressing cells.

Compared with traditional cryopreservation media, there is no need for tedious and time-consuming gradient freezing steps, and no need for expensive program cooling equipment. Cells can be directly resuspended and stored long-term in a -80°C ultra-low temperature freezer, but it is essential to ensure the stability of the freezer's temperature. For more prolonged and stable cell storage, frozen cells can be placed in a -80°C ultra-low temperature freezer overnight (>16 hours) before transferring them to a liquid nitrogen tank for long-term storage.

### Product Features

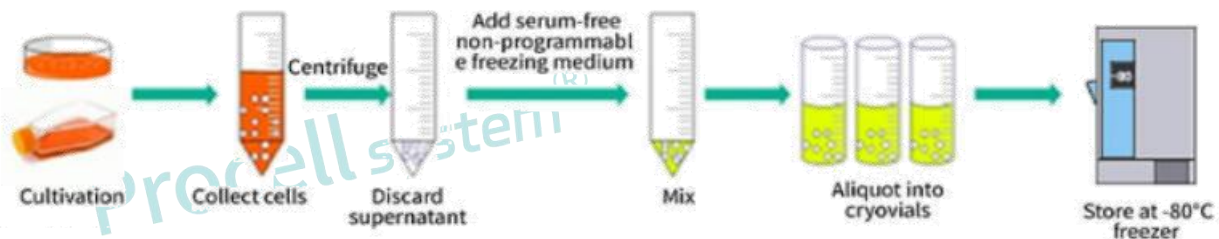
1. Ready-to-use cell cryopreservation medium, which can be accessed at any time and remains stable for over a year at 2-8°C.
2. Eliminates the need for cumbersome cryopreservation procedures or expensive programmed freezers, allowing direct placement in a -80°C freezer, thereby saving considerable time and effort.
3. The recovery rate of cells can exceed 90%, making it ideal for cryopreservation of most mammalian cells.
4. Contains no serum, ensuring minimal variability between batches.
5. Capable of effectively maintaining the multilineage differentiation potential of stem cells.
6. Features clearly defined chemical components and is devoid of any exogenous proteins, reducing the risk of cellular contamination and minimizing the impact of exogenous proteins on normal cell growth and differentiation.

## Instructions for use

### 1. Cell cryopreservation

- 1) Select cells in the logarithmic growth phase (approximately 90% confluence) and ensure that the medium is changed once within 24 hours before cryopreservation. Collect the cells and prepare them as a single-cell suspension (trypsin digestion may be required for adherent cells). Count the cells and ensure viability is greater than 90%.
- 2) Centrifuge the cell suspension at 1000 rpm for 5 minutes, and discard the supernatant.
- 3) Add Freezing Medium to the cell pellet, making the cell density  $2-5 \times 10^6$  cells/mL, and mix by gentle pipetting.
- 4) Aliquot the cell suspension into sterile cryovials in amounts of 0.5-1.0 mL, securely tighten the lids, and properly label the vials.
- 5) Immediately place the aliquoted cell cryovials into a -80°C ultra-low temperature freezer and ensure the stability of the ultra-low temperature freezer's temperature.

#### Illustration of Operating with Serum-Free Non-Programmable Freezing



### 2. Cell Resuscitation Section

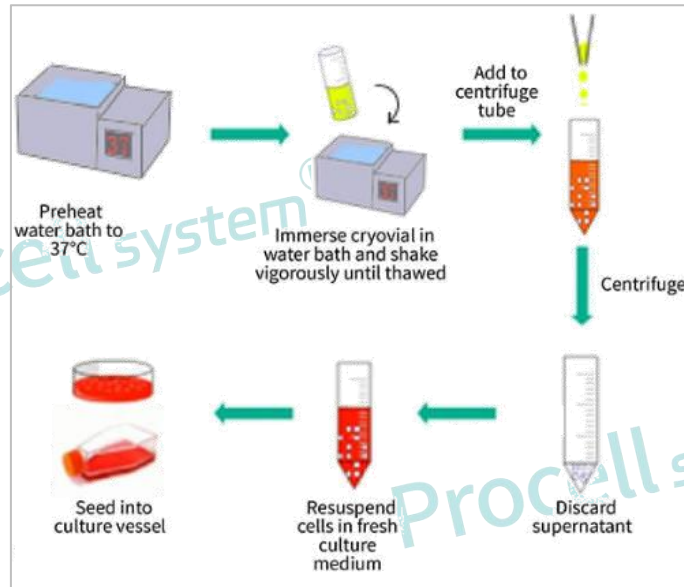
- 1) Preheat the water bath to 37°C, prepare clean disposable PE gloves, and add 9 mL of pre-warmed sterile culture medium to a sterile centrifuge tube.
- 2) Remove cells from the liquid nitrogen tank and place them into a PE glove, quickly immerse them in a 37°C water bath, and shake the cryovial to accelerate thawing, aiming for complete dissolution within one minute.

**Note:** If the distance between the refrigerator or liquid nitrogen tank and the water bath is far (more than 1 minute by walking), the cells should first be placed on dry ice before being transported to the water bath. If cell cryovials are placed directly on the hand or in a pocket, it may lead to a gradual temperature increase, causing ice crystal damage to the cells. Placing the cell cryovials inside PE gloves is to prevent contamination. This step should be done as quickly as possible, ideally with a timer set aside. If it does not melt within one minute, it may be due to too much cryopreservation solution or insufficient shaking.

- 3) In the laminar flow hood, add the revived cell suspension to the centrifuge tube containing fresh

- medium, centrifuge at 1200 rpm for 3 minutes, and discard the supernatant after centrifugation.
- 4) Resuspend cells with an appropriate amount of complete medium corresponding to the cells, transfer them into a sterile container (culture flask or dish), add sufficient medium, and incubate in a cell culture incubator.

Illustration of Cell Resuscitation Operation



## Product stability and storage conditions

1. This product should be stored in the dark at 2-8°C, with a shelf life of one year; to ensure product quality, please avoid freezing and thawing this product.
2. This product should be used within its shelf life. If it exceeds the shelf life, discontinue use to avoid affecting cell cryopreservation survival rate.

## Notes

1. This product is only used for scientific research or further research, not for diagnosis and treatment.
2. This product has been sterilized through three filtrations of 0.1 micrometers. When using this product, sterile procedures should be observed to avoid contamination.
3. For your safety and health, please wear a lab coat and disposable gloves when operating.
4. After aliquoting cryopreserved cells into cryovials, minimize the time they are stored at room temperature or 4°C, and quickly transfer them to an ultra-low temperature freezer set at -80°C, ensuring the stability of the freezer's temperature.
5. Divide the aforementioned cell suspension into sterile cryopreservation tubes at a volume of 0.5 mL or 1.0 mL, tighten the tube caps, and make proper markings.
6. To preserve cells for a longer and more stable period, freeze the cryopreserved cells in a -80°C ultra-low temperature refrigerator overnight (>16 hours) before transferring them to a liquid nitrogen tank for long-term storage.
7. When cryopreserving cells, please tighten the cap of the cryovial to prevent liquid nitrogen from seeping in, which could lead to the risk of the cryovial cracking during cell revival.
8. For sensitive cell lines, precious cell lines, primary cell samples, etc., it is recommended that you conduct at least one week of cryopreservation testing on the cells before use to confirm there are no issues before proceeding with formal cryopreservation.