

## 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 vitro cell culture, in order to preserve the biological activity of cells for a long time, the cells must be cryopreserved, and resuscitated when necessary. At present, the most commonly used method for cell cryopreservation is liquid nitrogen method. In the process, add an appropriate amount of protective agent to slowly cool the cells to the specified temperature range, so as to achieve the purpose of protecting the cells.

The Serum-free Cell Freezing Medium is a special cryopreservation product developed by Pricella for hundreds of types of cells by continuously optimizing experimental conditions for cell cryopreservation and resuscitation in the long-term cell research.

The product adds cell sedimentation stabilizer, which can delay the sedimentation rate of cells in the process of cryopreservation, prevent cells from squeezing each other and affect the effect 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 cell 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 for 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 placed at -80°C, and transferred to liquid nitrogen the next day to complete the entire process, saving a lot of time and energy.

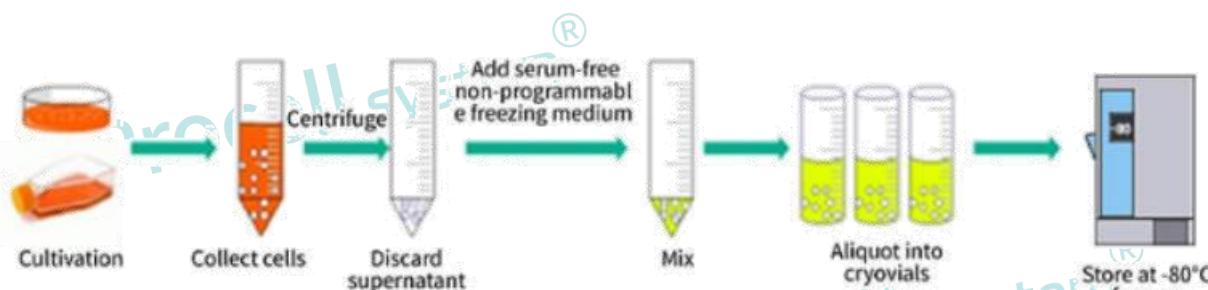
## Special Advantages

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. 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. Prepare the freezing medium and set it aside at room temperature or pre-cool it at 4°C.
2. Centrifuge to collect cells in the logarithmic growth phase (for adherent cells, digest and centrifuge; for suspension cells, centrifuge directly at 1200 rpm or 250 × g for 3 minutes), and then prepare them into a single-cell suspension and count the cells.
3. Centrifuge the cell suspension at 1000 rpm for 5 minutes, and discard the supernatant.
4. Add Freezing Medium to the cell pellet, making the cell density  $5 \times 10^5$ - $1 \times 10^7$  cells/mL, and mix by gentle pipetting.
5. Aliquot the cell suspension into sterile cryovials in amounts of 0.5-1.0 mL, securely tighten the lids, and properly label the vials.
6. Transfer the vials directly into the refrigerator at -80°C overnight (no need for a cell freezing container), and then store them in liquid nitrogen.

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



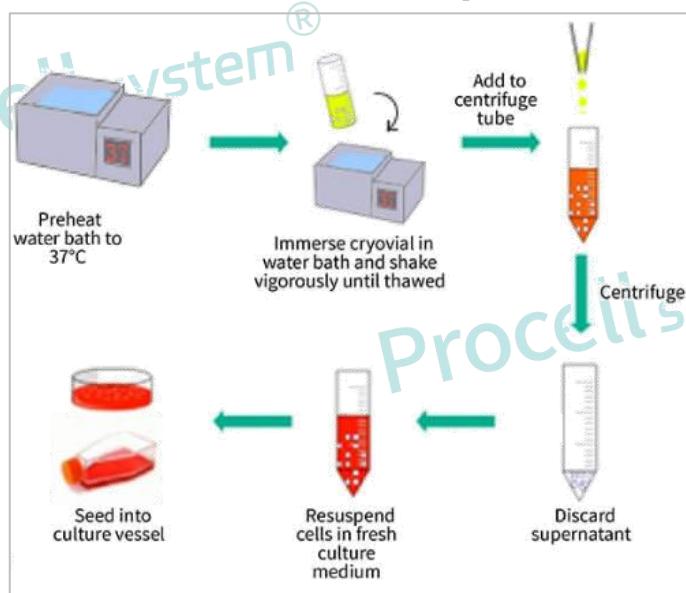
## Cell Resuscitation Section

Cell resuscitation emphasizes "rapid thawing"; the faster the cells thaw, the less damage they will incur. The process of cell resuscitation is not complicated, but each step must be performed meticulously to maximize the survival rate.

1. Preheat the water bath to 37°C.
2. Prepare a 15 mL centrifuge tube and add 8-10 mL of culture medium.
3. Remove the cryovial from the -80°C freezer or liquid nitrogen, place it in a polyethylene (PE) glove, and quickly immerse it in the water bath.

4. Vigorously shake the cryovial to ensure it thaws within 1 minute.
5. Wipe off the water, transfer the cryovial to a biological safety cabinet, and use a pipette to slowly add the cell suspension to the centrifuge tube prepared in step 2.
6. Centrifuge at 1200 rpm for 3 minutes.
7. Discard the supernatant, resuspend the cells in fresh culture medium, and seed them into a new sterile culture vessel.
8. Gently shake the culture vessel to evenly distribute the cells, and then place it in a cell culture incubator at 37°C with saturated humidity to allow them to grow.

Illustration of Cell Resuscitation Operation



## Notes

1. This product is for research use only.
2. This product is sterilized by 0.1 µm filtration.
3. It is necessary to pay attention to the aseptic operation and avoid the contamination.
4. Avoid repeated freezing and thawing. It is recommended to defrost at 2-8°C and aliquot the solution and store at -5~20°C.
5. It is not recommended to store this product at room temperature for a long time.
6. Since this product contains DMSO, please take protective measures to avoid direct contact with skin.