General Freezing Medium

Cat. No. : PB180436

Size : 10mL×5 / 10mL×10

Introduction

Cryopreservation is the main method of cell preservation, which is of great significance for cell preservation, introduction, culture and experimental research. In order to protect cells from freezing damage, cryoprotectants are often added. Currently, the most widely used osmotic protectant is DMSO (dimethyl sulfoxide). Its protective mechanism is to penetrate into cells before the cell freezing solution is completely solidified, create a certain molar concentration inside and outside the cells, and reduce the concentration of electrolytes in the unfrozen solution inside and outside the cells. In this way, the cells are protected from the damage of high electrolyte concentration, and at the same time, the water in the cells will not leak excessively, to avoid excessive dehydration and shrinkage of cells. However, DMSO has a greater cytotoxic effect at room temperature, and at higher concentrations it has a greater cytotoxic effect, especially on sensitive cells such as hybridoma cells. In general, DMSO has a good effect when the final concentration is between 5% and 15%.
The General Freezing Medium produced by Procell is composed of DMSO, Nutrients and DMEM/F12 basal medium. The product has been verified by nearly one thousand kinds of cells. It is suitable for the cryopreservation of various mammalian primary cells, passaged cell lines and hybridoma cells. The performance is stable and it's easy to use. More importantly, the cell survival rate is high.

General Information

Product Form	Liquid
Size	10mL×5 / 10mL×10
Concentration	Ready-to-use
DMSO	5%
Nutrients	40%
DMEM/F12 basal medium	55%
Cell viability	≥ 90%
Bacterial detection	Negative
Fungal detection	Negative
Mycoplasma detection	Negative
Shipping Conditions	Ice bag
Storage Conditions	2-8°C or -5~-20°C, shading light
Expiry date	2-8°C for 3 months or -5~-20°C for 24 months with shading light

Instructions for use

1. Cell cryopreservation

1) Prepare the freezing medium and set it aside at room temperature or pre-cool it at 4°C.
2) Select cells in the logarithmic growth phase (approximately 90% confluency). Refresh the culture

medium within 24 hours prior to harvesting. Harvest cells and prepare a single-cell suspension (use trypsin digestion for adherent cells if necessary). Count cells and ensure viability > 90%.

3) Centrifuge the cell suspension at 1000 rpm for 5 minutes, and discard the supernatant.
4) Add General Freezing Medium to the cell pellet and resuspend gently by pipetting. Adjust the cell density to 2-5×10^6 cells/mL.
5) Aliquot the cell suspension into sterile cryovials (0.5 mL or 1.0 mL per vial). Tighten caps securely and label each vial clearly.
6) Freeze the cells according to the programmed cooling steps for cell cryopreservation :2-8°C for 40 minutes→-20°C for 30-60 minutes→-80°C overnight (>16 h)→store in liquid nitrogen. Alternatively, use a programmed freezing container for cooling, and then store them in liquid nitrogen.

2. Cell Resuscitation Section

1) Preheat the water bath to 37°C. Prepare clean disposable PE gloves. Add 9 mL of pre-warmed sterile culture medium into a 15 mL sterile centrifuge tube.
2) Remove the cryovial from the -80°C freezer or liquid nitrogen, place it inside a polyethylene (PE) glove, and quickly immerse it into the 37°C water bath. Gently shake the vial to accelerate thawing, ensuring complete dissolution within 1 minute.

   Tips: If the distance between the liquid nitrogen tank/refrigerator and the water bath exceeds 1 minute on foot, transport the cells on dry ice first to avoid temperature fluctuations. Do not carry cryovials in hands or pockets, as gradual temperature rise may form ice crystals that damage cells. Use a PE glove to hold the vial to prevent contamination. Work rapidly during thawing, use a timer. If thawing exceeds 1 minute, check if the cryoprotectant volume is excessive or shaking is insufficient.
3) Transfer the thawed cell suspension into the prepared centrifuge tube with fresh medium in a clean bench. Centrifuge at 1200 rpm for 3 minutes. Discard the supernatant after centrifugation.
4) Resuspend the cell pellet in an appropriate volume of complete medium (specific to the cell type). Transfer the suspension to a sterile culture vessel (flask or dish) and add medium to the optimal volume. Incubate in a CO_2 incubator under standard culture conditions.

Notes

1. This product is only used for scientific research or further research, not for diagnosis and treatment.
2. This product has undergone triple 0.1 μm filtration sterilization. Maintain aseptic techniques during use to avoid contamination.
3. Thaw the product at 2-8°C, mix thoroughly before use, and avoid repeated freeze-thaw cycles. For small-volume usage, aliquot before freezing.
4. Avoid prolonged storage at room temperature. After initial thawing, the product can be stored at 2-8°C in the dark for up to 3 months.
5. If slight protein precipitation occurs, the product can be used directly without affecting cryopreservation efficacy. Alternatively, remove precipitates by centrifugation before use.
6. Wear a lab coat and disposable gloves during operation to ensure personal safety.
7. After aliquoting cells into cryovials, minimize their exposure to room temperature or 4°C. Transfer them promptly to a -80°C ultra-low temperature freezer, and ensure the freezer maintains stable temperature conditions.

8. For long-term storage, you should store the cells overnight (>16 hours) in the -80°C ultra-low temperature freezer after perform programmed cooling of cryopreserved cells. Subsequently, transfer the cryovials to a liquid nitrogen tank for long-term preservation.
9. Ensure the cap of the cryovial is tightly sealed before freezing to prevent liquid nitrogen infiltration. Leakage of liquid nitrogen may cause cryovial explosion during cell thawing.
10. For special cells (e.g., sensitive cell lines, rare/valuable cell lines, primary cells), conduct a preliminary cryopreservation test for at least 1 week prior to formal freezing. Proceed to full-scale cryopreservation only after confirming successful cell viability and functionality.