General Freezing Medium

Cat. No: PB180436

Size: 10mL×5/10mL×10

General Information

Product Form	Liquid
Concentration	Ready-to-use
pH	7.8-8.0
Bacterial detection	Negative
Fungal detection	Negative
Mycoplasma detection	Negative
Endotoxin level	< 3 EU/mL
Shipping Conditions	Ice bag
Storage Conditions	2-8℃ or -5~-20℃, shading light
Expiry date	2-8℃ for 3 months or -5~-20℃ for 24 months with shading light.

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 great cytotoxic side effects at room temperature. And when the concentration is higher, the cytotoxicity is greater, especially for sensitive cells, such as hybridoma cells. In general, DMSO has a good effect when the final concentration is between 5% and 15%.

The Cell Freezing Medium produced by Pricella is composed of DMSO, FBS 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.

Instructions for use

1. Prepare the freezing medium and set it aside at room temperature or pre-cool it at 4℃.
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 General Freezing Medium to the cell pellet, making the cell density 2-5×10^6 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. Freeze the cells according to the programmed cooling steps for cell cryopreservation (2-8℃ for 40 minutes → -20℃ for 30-60 minutes → -80℃ overnight → store in liquid nitrogen) or alternatively use a programmed freezing container for cooling, and then store them in liquid nitrogen.

Illustration of Manual Gradient Cooling Operation

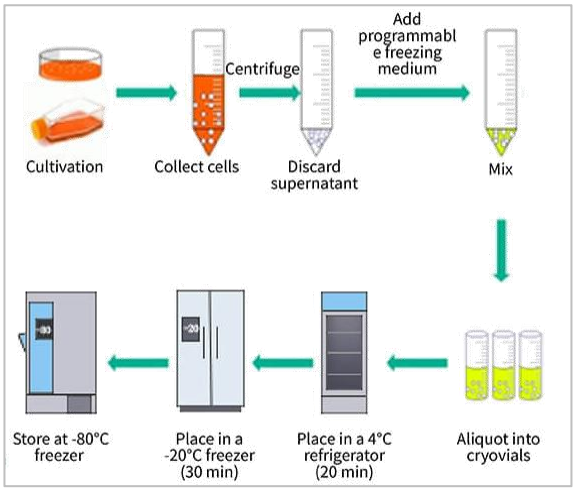
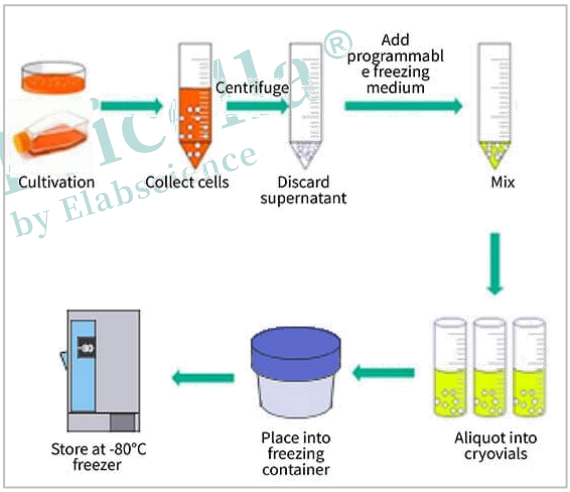


Illustration of Operating with a programmed Freezing Container

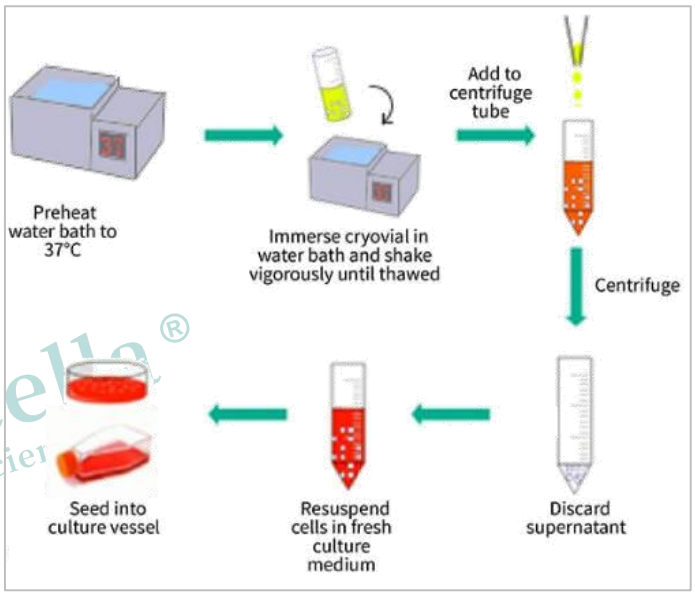


Cell Resuscitation Section

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℃.
2. Prepare a 15 mL centrifuge tube and add an appropriate amount (recommend 7-9 mL) of culture medium.
3. Remove the cryovial from the -80℃ 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 (250×g) for 3 minutes.
7. Discard the supernatant, resuspend the cells in fresh culture medium, and seed them into a new sterile

Illustration of Cell Resuscitation Operation



Notes

1. This product is for research use only.
2. This product is sterilized by 0.1μm filtration.
3. It is necessary to pay attention to the aseptic operation and avoid the contamination during the culture.
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 suitable for long time storage at room temperature.
6. If there is a small amount of protein precipitation during use, it can be used directly without affecting the effect of freezing storage, or it can be used after centrifugation to remove precipitation.