

ExCell Bio

OptiVitro[®]

Serum-free Cryopreservation Medium UC04

For Research and Manufacturing Use

Not Intended for Diagnostic and Therapeutic Use

User Manual

Catalog Number UC000-N056

UC000-N056S



| Product Description

OptiViro[®] Serum-free Cryopreservation Medium UC04 is a ready-to-use cell cryopreservation medium. Designed to prepare and preserve cells in ultra low temperature environments (–80 to –196 °C), UC04 medium provide a safe, protective environment for cells and tissues during the freezing, storage, and thawing process. Through modulating the cellular (including peripheral blood mononuclear cells (PBMC), T cells, NK cells, mesenchymal stem cells (MSC), etc.) biochemical response to the cryopreservation process, UC04 medium provide enhanced cell viability and functionality. UC04 medium is serum-free, protein-free, and animal components free with simple and definite ingredients. Additionally, the viability of various cells cryopreserved in UC04 could be up to 90% or more.

| Contents and Storage

Catalog No.	Amount	Storage	Shelf Life
UC000-N056	100 mL	2~8°C; Protect from Light	18 months

| Principle

OptiViro[®] Serum-Free Cryopreservation Medium UC04 protects the cells through the cryoprotectant. The cryoprotectant combines with water molecules, contributing to hydration, weakening the crystallization process of water, ultimately, increasing the viscosity of the solution, and reducing the formation of ice crystals. Additionally, the cryoprotectant can maintain certain molarity inside and outside the cell, reduce the concentration of electrolyte in the unfrozen solution inside and outside the cell, protect the cell from the damage of the solute, and maintain the survival of the cell at low temperature.

| Benefits

- **Security:** OptiViro[®] Serum-Free Cryopreservation Medium UC04 contains 7.5% (v/v) dimethyl sulfoxide (DMSO) and is formulated without serum, protein, or heterologous components.
 - **Simple:** The ingredients of OptiViro[®] Serum-Free Cryopreservation Medium UC04 are simple and definite, which is more conducive to the evaluation and application in clinical research.
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- **Efficient:** OptiVibro® Serum-Free Cryopreservation Medium UC04 is intended for freezing and storing a variety of immune cells including T cells, NK cells, etc.. UC04 is also applicable to other cells lines, including hMSC, HEK293, Vero, CHO, etc.. Please verify before using more cell lines.
 - **Convenience:** Ready-to-use, no additional preparation required.

| Instructions for Use

Cryopreservation

The following operations take the cryopreservation of T cells as an example to briefly describe the guidelines for OptiVibro® Serum-Free Cryopreservation Medium UC04.

1. Observe the cell status and record cell viability before freezing, collect T cells with good growth status, centrifuge at 300g for 5 minutes, discard the supernatant, and keep the cell pellet.

Note: The status of cells before cryopreservation directly affects the viability and growth of cells after thawing. Keeping the viability of T cells above 95% before cryopreservation and being in the logarithmic phase of expansion is more conducive to obtaining a better cryopreservation effect.

2. Add an appropriate volume of PBS solution to resuspend the cells, centrifuge at 300g for 5 minutes to collect the cells, and discard the supernatant.

Note: Washing is an optional step, which can be operated as needed, and Cryopreservation Medium UC04 can also be used for resuspension and washing. It is recommended to use cryogenic centrifugation when washing cells with the cryopreservation solution.

3. According to the cryopreservation density, add an appropriate amount of cryopreservation medium UC04, and repeatedly pipette to disperse the cells evenly.

Note: The cryopreservation density can be adjusted as required. The recommended cryopreservation density for T cells is $1 \times 10^6/\text{mL} \sim 1 \times 10^8/\text{mL}$. For higher-density cells, please use it after verification.

4. Collect the cell suspension to the cryovials, tighten the cap of the tube, and mark it.
5. Place the cryovials in the programmed cooling box (ExCell, CS041-0001) at -80°C overnight (Or store for more than 6h).

6. Transfer cryovials from -80°C to liquid nitrogen (-196°C) for long-term storage.

Note: For long-term storage of cells, it is recommended to resuscitate and identify the cell state every 5-10 years.

Thawing

1. Preparation: Turn on the water bath and adjust the temperature so that the temperature is stable at 37°C, pre-warm the cell culture medium at 37°C, and confirm the location where the cells are stored.
2. After taking out the cells and confirming the label, quickly transfer the cells to 37°C water, shake the cryovials constantly, and observe the thawing of the ice cubes (it takes about 2~3min).
3. When the ice in the cryovial is about to melt completely, take it out of the water bath, clean the outer surface with 75% alcohol, and then place it into a Bio-safety Cabinet.

Note: When shaking, avoid immersing the cap of the cryovial in the water bath, shorten the thawing time as much as possible, and avoid heating the cryopreservation solution after it dissolves.

4. Use 75% alcohol to clean the surface of the cryovial again.
5. Open the cryovial, mix gently with a pipette, and add the cell suspension to the pre-warmed complete culture medium, in the end, gently pipette the suspension to mix the cells evenly.

Note: Add drop by drop and operate gently. It is recommended to add 10mL of complete culture medium to each milliliter of cryopreservation solution.

6. Centrifuge at 300g for 5min, collect the cells and discard the supernatant,300g.
7. Add an appropriate amount of medium to resuspend the cells again, count the cells, and calculate the cell density.
8. According to the cell type or research needs, seed the cells at an appropriate density into an appropriate culture vessel, shake the well, and then place in the incubator for culture.