

# User Manual

## OriCell™ Cryopreservation Medium

### For General Use (9:1)

Catalog No. GCRN-10001



## Introduction

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Cell cryopreservation refers to placing cells in a low-temperature environment for long-term storage. Through years of extensive research, the OriCell™ R&D team has continuously optimized cell cryopreservation and recovery conditions to develop a range of cryopreservation products suitable for a wide variety of cell types.

OriCell™ Cryopreservation Medium For General Use (9:1) is formulated with a serum-to-DMSO ratio of 9:1. This formulation significantly reduces ice crystal damage to cells during the freezing process, effectively improving the post-thaw recovery rate and cell viability. Extensive cryopreservation data have demonstrated that this product minimizes cell damage during freezing, ensures high post-thaw viability, and helps maintain optimal cell functionality. OriCell™ Cryopreservation Medium For General Use (9:1) is suitable for the vast majority of cell types, as well as for the cryopreservation of rare and precious cell samples.

**Note:** This product is intended for research use only and is not for diagnostic, therapeutic, clinical, household, or any other applications.

When citing our products in academic publications, please use the following format: “OriCell™ [Product Name] + [Catalog Number], from Cyagen Biosciences.”

## Product Advantages

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- Stable performance and simple operation.
- Achieves up to 90% post-thaw viability across most mammalian cells.
- Effectively preserves the multipotent differentiation potential of stem cells post-thaw.

## QC

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- Pass the detection of bacteria, fungi, mycoplasma, and endotoxins.
- Pass the detection of osmotic pressure and pH.
- Pass the detection of product quality.

Please refer to "COA" for details.

## General Handling Principles

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1. Maintain strict aseptic technique. Ensure the entire lab and operating areas are kept clean.
2. Follow standardized protocols. Adhere strictly to the instructions provided in the manual.
3. Ensure proper storage and use. Store the product according to specified conditions and use it promptly to ensure optimal performance.

## Product Stability and Storage Conditions

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- Protect from light. The product has a shelf life of 1 year and should be stored at 4 °C.
- Use the product within the shelf life. Do not use it after the expiration date.

## Cell Cryopreservation

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### Materials Required

- OriCell™ Cryopreservation Medium For General Use (9:1) (Cat. No.: GCRN-10001)
- Clean, sterile, and stable quality disposable consumables (pipettes, pipette tips, centrifuge tubes, etc.)
- Clean sealing film

### Steps

1. Harvest cells in the logarithmic (log) growth phase using standard protocols and collect them into a centrifuge tube. Calculate the total number of cells required based on the target seeding density and the size of the cryovials (Recommended density:  $5 \times 10^5$  to  $5 \times 10^6$  cells/mL).
2. Transfer the required volume of cell suspension into a centrifuge tube. Pellet the cells by centrifugation (Recommended conditions:  $250 \times g$  for 3 – 5 minutes).
3. Aspirate the supernatant.
4. Add an appropriate volume of cryopreservation medium (Cat. No.: GCRN-10001) into a centrifuge tube. Resuspend gently but thoroughly to ensure a uniform cell suspension.
5. Aliquot the cell suspension into pre-labeled cryovials.
6. Place the cryovials into a controlled-rate freezing container, then transfer them to a  $-80^\circ\text{C}$  freezer.

After 24 hours, transfer the vials to liquid nitrogen for long-term storage.

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## Cell Recovery

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### Materials Required

- The appropriate complete medium for the specific cell line

### Steps

1. Pre-warm the water bath to 37 °C.
2. Warm the complete medium to 37 °C.
3. Transfer the cells from liquid nitrogen to -80 °C and hold them there for 10 minutes. This will allow any residual liquid nitrogen to evaporate and prevent vial explosion.
4. Add at least 8 mL of pre-warmed complete medium to a 15 mL centrifuge tube for subsequent use.
5. Remove the cryovial containing cells from the -80 °C freezer, immerse it in the 37 °C water bath, and gently and quickly swirl to thaw the cryopreservation medium.

#### Note:

**(1) Gently swirl the cryovial during thawing to ensure rapid and uniform thawing.**

**(2) Avoid submerging the cap in water to prevent contamination.**

**(3) Stop thawing in the water bath when only a single ice crystal (approximately 2 mm in diameter) remains, then continue gently swirling the vial until it is completely thawed.**

6. Once the cell suspension is completely thawed, wipe the outer surface of the cryovial with 75% ethanol.
7. In a biosafety cabinet, open the cryovial and transfer the cell suspension to the prepared centrifuge tube using a Pasteur pipette.
8. Rinse the cryovial once with 1 mL of complete medium to collect residual cells and minimize loss.
9. Centrifuge the cell suspension at 250 × g for 4 minutes.

**Note:** Please calculate the corresponding rotational speed using the formula:  $RCF = 1.118 \times 10^{-5} \times r \times RPM^2$  (where RCF is the relative centrifugal force, r is the rotor radius in cm, and RPM is the rotational speed).

10. Carefully remove the supernatant after centrifugation. Add 2 mL of complete medium, gently resuspend the cell pellet by pipetting up and down to mix thoroughly.
11. Gently rock the culture vessel to ensure a uniform cell distribution.
12. Examine the cells under a microscope, then incubate at 37 °C with 5% CO<sub>2</sub> and saturated humidity.

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