cyagen | OriCell

# User Manual

## OriCell™ NCR DMSO-free And Protein-free Cryopreservation Medium For General Use

**Catalog No. NDPF-10001**

*We help you discover life*

## Introduction

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 ™ NCR DMSO-free And Protein-free Cryopreservation Medium For General Use significantly reduces the damage of ice crystals to cells during cryopreservation, effectively improving cell recovery rates and viability. Furthermore, its exogenous protein-free and DMSO-free formulation minimizes the risk of contamination and cytotoxicity. Extensive validation data demonstrate that this product causes minimal cellular damage, ensuring high post-thaw recovery rates and optimal cell viability. Unlike traditional cryopreservation media, this product eliminates the need for time-consuming controlled-rate freezing (programmed cooling). Cells can be directly resuspended and stored at -80 °C overnight before being transferred to liquid nitrogen to complete the preservation process.

OriCell™ NCR DMSO-free And Protein-free Cryopreservation Medium For General Use is suitable for a wide range of cell types, including commonly used cell lines, stem cells, and somatic cells. Post-thaw viability may vary depending on the cell type. It is particularly recommended for cells sensitive to DMSO.

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

- Stable performance and simple operation.
- Chemically defined formulation without exogenous proteins and DMSO.
- Achieves up to 90% post-thaw viability across most mammalian cells.
- Effectively preserves the multipotent differentiation potential of stem cells post-thaw.
- Efficient and time-saving. Cells can be stored directly at -80 °C, eliminating the need for programmed cooling steps or specialized devices.

## QC

- 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

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

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

## Cell Cryopreservation

### Materials Required

- OriCell™ NCR DMSO-free And Protein-free Cryopreservation Medium For General Use (Cat. No.: NDPF-10001)
- Clean, sterile and high-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 × g for 3–5 minutes).
3. Aspirate the supernatant.
4. Add an appropriate volume of cryopreservation medium (Cat. No.: NDPF-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 directly into a -80 °C freezer. Transfer them to liquid nitrogen for long-term storage after 24 hours.

# Cell Recovery

## 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 = $1.118 \times 10^{-5} \times r$**

× $RPM^2$ (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% $CO_2$ and saturated humidity.