# Cell Freezing Medium II

**Catalog Number:**
B71295

## PRODUCT DESCRIPTION

Cell Freezing Media II is a cost-effective alternative to in-house freezing preparations and are designed for optimum protection and preservation of cells during frozen storage. This ready-to-use freezing media are suitable for the consistent cryopreservation of a broad spectrum of mammalian cells. Each lot of Cell Freezing Medium II is quality tested to assure maximum performance.

Cell Freezing Medium II contains 90% Fetal Bovine Serum and 10% dimethyl sulfoxide. This cryopreservation medium is typically used for sensitive cell lines.

## STORAGE AND HANDLING

The Cell Freezing Medium II is supplied in gamma irradiated, sterile PETG or PETE bottles. We recommend storage at a temperature of -5 °C to -20 °C. Avoid multiple freeze-thaw cycles. This product may be aliquoted into sterile bottles for single-use applications. Cell Freezing Medium II can be stored for short periods at 2-8 °C without affecting performance. We recommend that this product be used within 12 hours after thawing or else refrozen. Always use aseptic techniques when handling.

## PRECAUTION

When handling bio-hazardous materials such as human cells, safe laboratory procedures should be followed, and personal protective equipment should be worn.

## LIMITATIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The safety and efficacy of this product in diagnostic or other clinical uses has not been established.
- Results may vary due to variations among tissue/cells derived from different donors or sources.



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## PRODUCT USE

### CELL PREPARATION

Actively growing, healthy cell cultures should be used in the freezing procedure. This is best accomplished by maintaining cells in their log-phase of growth. For B-cell hybridomas, the preferred cell density is 4-5 x $10^5$ cells/mL with a viability exceeding 70%. Centrifuge the appropriate volume of cells (200 x g for 5-10 minutes), discard the culture supernatant and resuspend the cell pellet in cold (2-8 °C) Cell Freezing Medium II. A final cell density in the freezing medium of 1-10 x $10^6$ cells/mL is recommended. Transfer 1-2 mL of the cell suspension into labeled cryovials. Allow the cells to equilibrate 2-8 °C for 5-10 minutes, including your pipetting time.

### CELL FREEZING

Optimum recovery of viable cells following freezing is best accomplished by freezing the cells at an appropriate cooling rate. This may be accomplished using a low temperature freezer (-70 °C) by placing the vials in a Styrofoam box overnight and transferring the vials into liquid nitrogen storage in the morning. Alternatively, a programmable freezing unit may be used to freeze the cells at 1 °C per minute until the cell suspension reaches a temperature of -30 °C and then at 5-20 °C per minute until the temperature reaches -100 °C. The vials are then transferred to a liquid nitrogen freezer for long term storage.

### CELL PREPARATION

Rapidly thaw each vial of cells in a 37 °C water bath. Transfer the contents to a 10X volume of growth medium and centrifuge the cell suspension at 200 x g for 5-10 minutes. After discarding the supernatant, resuspend the cells in fresh growth medium at a final cell density of 2-4 x $10^6$ viable cells/mL and transfer the suspension into a cell culture flask.

**Note:** *Cells that are sensitive to osmotic shock should be diluted slowly (3-5 minutes) with fresh medium immediately after thawing and before centrifugation.*