

### ★ Storage

Store at -20 °C.

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- Cell Freezing Media II

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### ★ Introduction

Cell Freezing Medium II contains RPMI1640, 20% FBS Gold and 10% DMSO (dimethyl sulfoxide). This cryopreservation medium is supplied as a ready-to-use solution. It is commonly used for hardy cell lines that are less susceptible to freezing.

### ★ Procedure

1. Thaw Cell Culture Freezing Medium II, mix well and keep at 2°C to 8°C until use.
2. For suspension cells proceed to step 3. For adherent cells, gently detach cells from the substrate on which they are growing using a suitable dissociation reagent. Resuspend cells in complete medium required for that cell type.
3. Transfer cell suspension to a sterile 15-mL centrifuge tube.
4. Determine the viable cell density and percent viability using a Cell Counter (automated or manual methods may be used) and calculate the required volume of Cell Culture Freezing Medium II to give a final cell density of  $1 \times 10^6$  to  $1 \times 10^7$
5. Centrifuge cell suspension at 100-200 x g for 5-10 minutes. Aseptically decant supernatant without disturbing the cell pellet.

**Note:** Centrifugation speed and duration may vary depending on cell type.

6. Resuspend the cell pellet in (2°C to 8°C) chilled Culture Freezing Medium II at recommended viable cell density for specific cell type (typically  $1 \times 10^6$  cells/mL or greater).
7. Dispense aliquots of cell suspension (mix frequently to maintain a homogenous cell suspension) into cryovials according to the manufacturer's specifications (i.e., 1.5 mL in a 2-mL cryovial).
8. Achieve cryopreservation in an automated or manual controlled rate freezing apparatus following standard (approximately 1°C decrease per minute).
9. Transfer frozen cells to liquid nitrogen, (vapor phase) storage at -200°C to -125°C is recommended.

### ★ Related Products

Product Name	Cat No
Cellmaxin, 10mg/ml	C3314
Cellmaxin Plus, 10.5mg/ml	C3319
Fungizols PS (100X)	F2100