

### ★ Storage

Store at -20 ~ -80°C.  
Prease prevent repeated freeze-thaw cycles.

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- Product Manual
- Cell Freezing Media III

ALL PRODUCTS SOLD BY GenDEPOT ARE INTENDED FOR RESEARCH USE ONLY UNLESS OTHERWISE INDICATED. THIS PRODUCT IS NOT INTENDED FOR DIAGNOSTIC OR DRUG PURPOSE

### ★ Shipping Condition

Frozen on dry ice.

### ★ Introduction

Cell Freezing Media III contains 90% Fetal Bovine Serum Opti-Gold and 10% dimethyl sulfoxide. This cryopreservation medium is typically used for sensitive cell lines since it offers more cryoprotection than Cell Freezing Media I.

### ★ 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  $4-5 \times 10^5$  cells/ml with a viability exceeding 70%. Centrifuge the appropriate volume of cells (200xg 5-10 minutes), discard the culture supernatant and resuspend the cell pellet in cold (4-8 °C) Cell Freezing Medium. A final cell density in the freezing medium of  $1-10 \times 10^5$  cells/ml is recommended. Transfer 1-2 ml of the cell suspension into labeled cryovials. Allow the cells to equilibrate in the freezing medium at 4-8 °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 over -night 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 pre 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 Thawing

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 200xg for 5-10 minutes. After discarding the supernatant, resuspend the cells in fresh growth medium at a final cell density of  $24 \times 10^6$  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.

### ★ Related Products

Product Name	Cat No
Albumin	A0100
Ceracryo Cell Freezing Media, Xeno Free	C0667
Ceraplex, Premium Cell Culture Supplement (10X)	C3318
Cellmaxin Plus, 10.5mg/ml	C3319
Scraptase, Non-animal Original Dissociation Reagent (1X)	CA110
Ceracol Collagen Type I Solution, 1mg/ml	CA081
Fibronectin, Bovine, Stabilized Solution, 1mg/ml	CA082
Fibronectin, Human, Stabilized Solution, 1mg/ml	CA083
Insulin-Transferrin-Selenium Solution I (1,00X), Animal Free	CA201
DMEM, No glucose, with L-Glutamine and Phenol red	CM001
DMEM, High Glucose, with L-glutamine and Sodium Pyruvate	CM002
DMEM/Ham's F-12 (1:1 Mixture), with L-Glutamine and HEPES	CM017
DMEM/Ham's F-12 (1:1 Mixture); No Phenol red	CM017
MCDB 131 Medium	CM034
Glutaplex, 200mM (100X)	G7000
Fetal Bovine Serum, Premium, US Origin	F0600
FBS, Mouse Embryonic Stem cell and iPSC Optimized	F0650
FBS OptiGold	F0900
FBS OptiGold, Premium, Heat Inactivated	F0910
Fungizol PS (100X)	F2100
Glutaplex, 200mM (100X)	G7000
LIF, Mouse Recombinant, 10 MIU/ml	L4501
WATER, 0.1um Final Filtered, cell culture tested	W0900