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ProFreeze™-CDM™ NAO
Chemically Defined Freeze Medium (2X)

Instruction for Use

Introduction

BioWhittaker® serum-free chemically defined ProFreeze™-CDM™ Medium (2X) (12-769E) is suitable for cryopreserving all cell types in the absence of FBS (fetal bovine serum). However, it may be used to greatest advantage with cells that were cultured in a serum-free and animal component-free environment. This protein-free freezing medium contains no animal derived components, insulin, or hydrolysate, and maintains high cell viability upon recovery from frozen storage. ProFreeze™-CDM™ Medium requires addition of 15% reagent or spectrophotometric grade dimethylsulfoxide (DMSO) at time of use. This 100 ml bottle will make 117.6 ml of complete 2X concentrated freezing medium after the addition of 17.6 ml DMSO. Store ProFreeze™-CDM™ Medium at 2-8°C and, for best results, keep on ice during use.

Instructions for Use

Preparation of Complete Freezing Medium

Complete 2X freeze medium is prepared by supplementing ProFreeze™-CDM™ Medium with 15% DMSO. For example, 1.5 ml DMSO + 8.5 ml ProFreeze™-CDM™ Medium will make 10 ml of 2X complete freezing medium. Prepare only enough for a single day's use. Keep the 2X freeze medium on ice before use.

Cryopreservation

1. Harvest log phase cells (viability ≥ 90%) by centrifugation at 100 to 200 x g for 10 minutes.
2. Resuspend the cell pellet in 4℃ culture medium at 5.0 to 20.0 x 10^6 cells/ml. Keep chilled.
3. Slowly mix equal volumes of chilled complete ProFreeze™-CDM™ Medium and chilled cell suspensions by **adding the ProFreeze™-CDM™ Medium to the cell suspension.** The resulting final DMSO concentration is 7.5% and the final cell concentration is 2.5 to 10.0 x 10^6 cells/ml.
4. Dispense into 1 ml cryovials and freeze according to your customary protocol.
5. Move frozen cells into a vapor phase liquid nitrogen freezer for long-term storage. The warmer storage temperatures of -60℃ to -80℃ are inadequate.

Cell Recovery / Thawing

1. Remove cryovial from liquid nitrogen storage and quickly transport it to a 37℃ water bath. Keep the neck of the cryovial dry.
2. Allow it to thaw undisturbed (1-2 min).
3. Disinfect the cryovial with 70% alcohol and open it aseptically in a biosafety cabinet.
4. Gently mix the 1 ml cell suspension and then add it to 9 ml of growth medium.
5. Perform a cell count and viability test before dispensing the 10 ml cell suspension into one or more culture vessels.
6. Centrifugation to remove DMSO is generally not recommended since most cells easily tolerate this dilute DMSO concentration. In addition, centrifugation adversely affects the integrity of the cell membrane, which is fragile after cryopreservation.

CAUTION: DMSO is a combustible liquid and is readily absorbed through the skin. Keep away from open flames and avoid contact with the skin. Wear suitable protective clothing.

Ordering Information

Cat. No.	Description	Size
12-769E	ProFreeze™-CDM™ Medium; NAO chemically defined freeze medium (2X)	100 ml

Product Use Statement

THIS PRODUCT IS FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use *in vitro* diagnostic or clinical procedures.