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Document # INST-12-769-5 02/21
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ProFreeze™ CDM Medium

Chemically defined, non-animal origin freeze medium (2X)

Instructions for use

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I. Introduction

BioWhittaker® serum-free chemically defined ProFreeze™-CDM™ Medium (2X) 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.

For answers to frequently asked questions and citations regarding these products, please visit our knowledge center: **https://knowledge.lonza.com**

II. Instructions for Use

Preparation of Complete Freeze Medium

Complete 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 freeze medium. The complete 2X freeze medium should be used within 8 hours after addition of the DMSO. Keep the complete 2X freeze medium on ice during use.

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.

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 2-8°C culture medium at 5.0 to 20.0 x 10^6 cells/mL. Keep chilled.
3. Slowly mix equal volumes of chilled complete 2X ProFreeze™ CDM Medium and chilled cell suspension by **adding the complete 2X 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 standard protocol.
5. Place cryovial into a vapor phase liquid nitrogen freezer for long-term storage.

NOTE: Storage at -60°C to -80°C is not recommended for long-term storage.

Cell recovery / thawing

1. Remove cryovial from liquid nitrogen storage and wipe it with ethanol or isopropanol.
2. In a sterile field, briefly twist the cap a quarter turn to relieve pressure and then retighten. Quickly thaw the cryovial in a 37°C water bath, being careful not to submerge the entire vial. Watch your cryovial closely; when the last sliver of ice melts, remove it. Thawing the cells for longer than 1.5 minutes can result in less than optimal results.
3. Wipe the cryovial with ethanol or isopropanol and open it in a sterile field.
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.

NOTE: 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.

III. Storage

ProFreeze™ Medium should be stored at 2 - 8°C.

IV. Ordering information

Cat. no.	Product	Size
BP12-769E	ProFreeze™ CDM Medium; NAO chemically defined freeze medium	500 mL

Product use statement

THESE PRODUCTS ARE FOR FURTHER MANUFACTURING (FFM) OR LABORATORY PURPOSES ONLY. Performance characteristics have not been established.