Bacteria Cryopreservation Protocol

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Abstract

Snap freezing, or flash freezing, is the process by which samples are lowered to temperatures below -70°C very rapidly using dry ice or liquid nitrogen. Snap freezing achieves the same endpoint as slow rate-controlled freezing, but at approximate rate of -10-1000°C/min, compared to -1°C/min. Snap freezing with a CoolRack® module will provide sample vessel stability, organization and consistent freezing parameters, rapid hands-free sample processing while avoiding lost or contaminated samples. Snap freezing is performed on a pre-cooled CoolRack, which ensures fast heat transfer. This method can provide excellent specimen integrity and a wide array of options for analysis, including extraction of proteins, DNA and RNA for use in research and diagnostics. The following protocol describes a general procedure for cryopreserving bacteria for long-term storage. Verify with your laboratory SOP for specific needs for each strain.

Introduction

Snap freezing, or flash freezing, is the process by which samples are lowered to temperatures below -70°C very rapidly using dry ice or liquid nitrogen. Snap freezing achieves the same endpoint as slow rate-controlled freezing, but at approximate rate of -10-1000°C/min, compared to -1°C/min. Snap freezing with a CoolRack® module will provide sample vessel stability, organization and consistent freezing parameters, rapid hands-free sample processing while avoiding lost or contaminated samples. Snap freezing is performed on a pre-cooled CoolRack, which ensures fast heat transfer. This method can provide excellent specimen integrity and a wide array of options for analysis, including extraction of proteins, DNA and RNA for use in research and diagnostics.

Reagents

Bacterium preparation Cryoprotective agent TrueCoolTM cryovial

Equipment

CoolBoxTM CFT30 ice-free cooling station CoolRack® CFT30 CoolRack® CF Cryolabels and/or cryomarkers ThermalTrayHP platform \(optional) CoolSinkTM BX50 \(optional) 37°C waterbath -80°C Freezer

Procedure

Bacteria Preparation Follow the laboratory protocol for bacterial growth and preparation. Refer to CDC \(Centers for Disease Control and Prevention) guidelines for utilization of pathogens in specific BSL \(Biosafety Level). Pathogens are infectious agents and should be manipulated in a controlled and safe environment. Bacteria Freezing 1. As a general rule, maintain the bacterial preparation at 4°C by placing it in a boat-reservoir in CoolSinkBX50 and/or ThermalTray on ice. 2. Prepare a bacteria glycerol stock by

diluting the bacterial preparation with a sterile glycerol solution for a final 15-50% v/v glycerol concentration. Dispense 1 ml of stock \(or desired amount) in a pre-labeled TrueCool cryovial. To avoid titer reduction maintain the vials on a CoolRackCFT30 in the CoolBox at 4°C. The CoolRackCFT30 reduces contamination and spill accidents by allowing one-handed opening/closing of the cryovials. 3. While bacteria samples are kept cold at 4°C in the CoolRackCFT30, equilibrate a CoolRackCF on dry-ice for 10 minutes. Note: with this protocol, there is no need to make a dry-ice/ethanol slurry. Place the cryotubes directly on the pre-equilibrated CoolRackCF and snap-freeze the samples for 3-5 minutes. 4. Transfer the frozen samples to a storage box and place it in the -80°C freezer for long-term storage. Bacteria Thawing 1. Place the cryovials from the -80°C freezer in a CoolBoxCFT30 with a -12°C frozen cartridge inside to maintain the vials at the correct temperature and allow transport of the vials in a safe manner. 2. Place the vials directly in a 37°C water bath, and manually slowly agitate the vials to enable the thawing process. Just before the whole liquid is completely thawed, remove the vial from the 37°C water bath and place it on a CoolRackCFT30 previously equilibrated on ice. Samples are ready for experimental procedures or titer assessment.

Troubleshooting

For more information please contact us at info@biocision.com

Anticipated Results

See Figure 1 for results of experiment to compare freezing methods for bacteria using BioCision's CoolRack on dry ice and the classic method of dry ice/ethanol slurry Full experiment can be found "here":http://www.biocision.com/uploads/docs/Bacteria_Freezing_on_Dry_Ice.pdf.

Figures

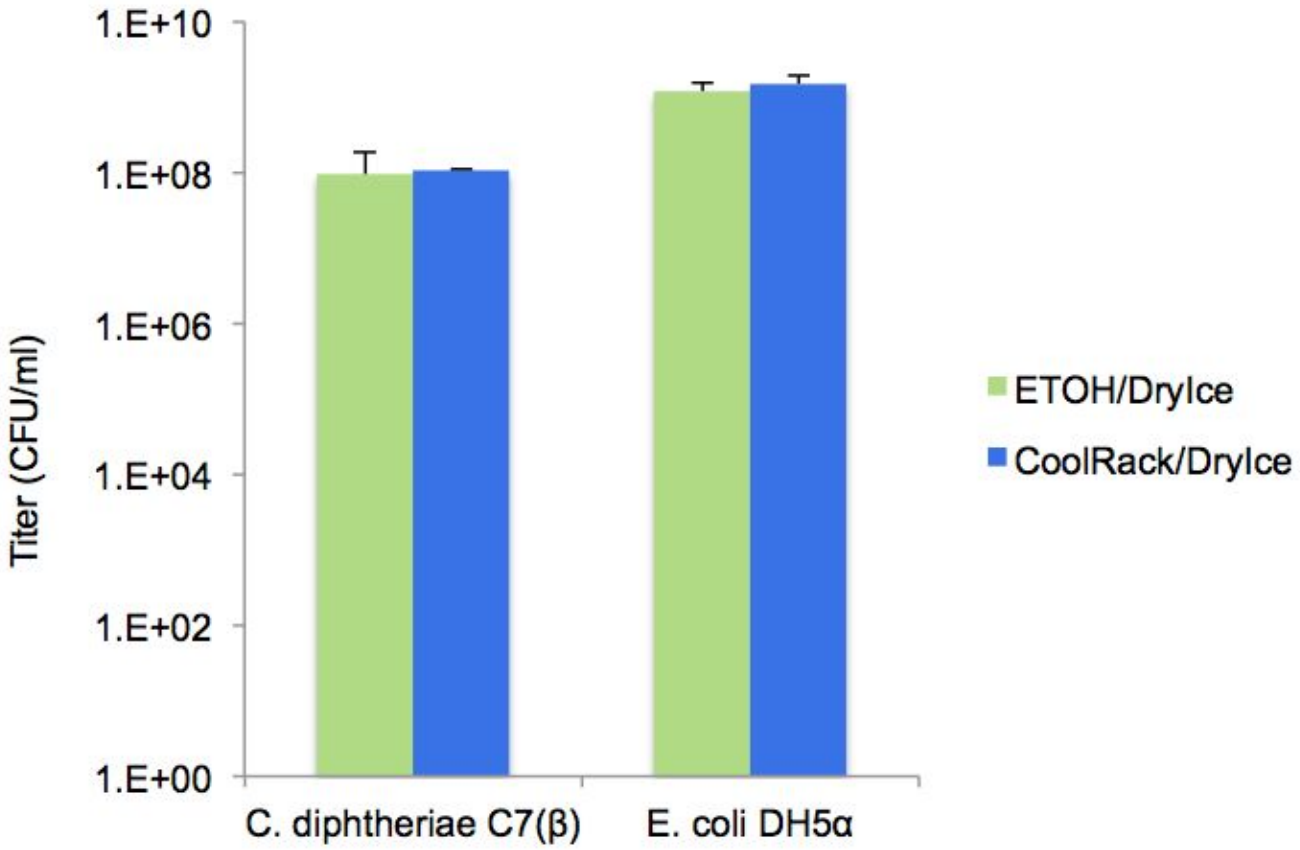

Figure 1: Graph showing the Titer (CFU/ml) of 2 different bacterial strains C.diptheriae C7 and E. coli DH5 using the 2 freezing methodologies.

Figure 1

Figure 1: Experiment to Compare Freezing Methods for Bacteria Using BioCision's CoolRack on Dry Ice and the Classic Method of Dry Ice/Ethanol Slurry