Bacteria Cryopreservation Using a CoolRack® Module

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 a much faster rate (-10 to 1000°C/minute, as compared to -1°C/minute. Snap freezing with a thermo-conductive CoolRack® tube module will provide sample vessel stability, organized and consistent freezing parameters, rapid hands-free sample processing while avoiding lost or contaminated samples. Snap freezing is performed on a pre-cooled CoolRack module, 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.

Materials

- Bacterium preparation
- Cryoprotective agent
- CoolBox™ CFT30 ice-free cooling system
- Green CoolBox 30 System freezing cartridge
- CoolRack® CFT30 module
- Cryolabels and/or cryomarkers
- ThermalTray™ LP platform (optional)
- 50 mL reagent reservoirs
- CoolSink® LX55 reagent reservoir holder (optional)
- TruCool® cryogenic vials
- TruCool® hinged cryostorage boxes
- 37°C waterbath
- -80°C freezer

Bacteria Preparation

Follow the laboratory protocol for viral growth and/or purification. Refer to Centers for Disease Control and Prevention (CDC) guidelines for utilization of pathogens in specific Biosafety Level (BSL). *Pathogens are infectious agents and should always be manipulated under a biosafety cabinet with laminar flow.*

Bacteria Freezing

1. As a general rule, maintain the bacterium preparation at 4°C by placing it in a reagent reservoir and place the reservoir on a thermo-conductive CoolSink LX55 module for uniform and stable cooling. Rest the CoolSink LX55 on ice directly, or on a ThermalTray LP platform for greater stability to minimize ice contact with the reservoir and it's contents.

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) into a pre-labeled TruCool cryogenic vial. To avoid titer reduction maintain the vials at 4°C in a CoolRack CFT30 in the CoolBox. The CoolRack CFT30 module will ensure uniform temperature to all vials and minimizes contamination and spill accidents by allowing one-handed opening/closing of the cryogenic vials.

3. While bacteria samples are kept cold at 4°C in the CoolRack CFT30, equilibrate a second CoolRack CFT30 on dry-ice for 10 minutes. **Note: with this protocol, there is no need to make a dry-ice/ETOH slurry.** Place the vials directly on the pre-equilibrated CoolRack CFT30 module on dry ice and freeze the samples for 3-5 min.

4. Transfer the frozen samples to a TruCool hinged cryostorage box and place it in the -80°C freezer for long-term storage.

Bacteria Thawing

1. Place the cryogenic vials from the -80°C freezer in a CoolBox CFT30 with a pre-frozen green cartridge inside it 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 CoolRack CFT30 module equilibrated to <4°C on ice. Samples are ready for experimental procedures or titer assessment.

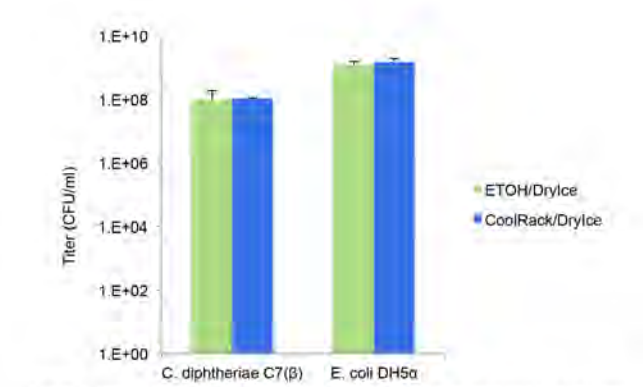

Figure 1: Graph showing the Titer (CFU/ml) of 2 different bacterial strains C.diptheriae C7 and E. coli DH5 using the 2 freezing methodologies.