Consumables & Instruments

APPLICATION NOTE

Snap Freezing Using Dry Ice or Liquid Nitrogen

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Introduction

Snap freezing is the technique in which a sample is rapidly frozen using dry ice, a dry ice/alcohol slurry or liquid nitrogen. Samples frozen in this manner include bacterial and viral stocks, cell lysates, proteins, and tissues. Snap freezing reduces the chance of water present in the sample forming ice crystals during the freezing process, and better maintains the integrity of the sample. In the case of tissue or lysates, snap freezing slows the actions of proteases and nucleases to inhibit degradation of molecules such as RNA or proteins.

Typically, snap freezing is performed either directly in dry ice or in a slurry containing dry ice and ethanol or isopropanol. Liquid nitrogen is commonly used for snap freezing tissue pieces. Azenta Life Sciences' Thermoconductive Tube Rack modules, Thermoconductive Tray platforms and ice bucket portfolio are easily adapted to all snap freezing techniques. Alcohol is completely eliminated from the process, providing easier handling, sample organization and better reproducibility.

Current method: Tubes directly in dry ice or slurry

Non-uniform dry ice contact may result in different freezing rates leading to poor reproducibility. Samples are placed randomly which could result in misidentification.

Thermoconductive Tube Rack method: Dry ice

Thermoconductive Tube Rack modules rapidly adapt to the dry ice temperature allowing you to snap freeze your samples without direct contact with the dry ice. Samples stay organized and freeze upright in a very reproducible manner. (Fig. 1)

Thermoconductive Tube Rack method: Liquid nitrogen (LN2)

After approximately 15 minutes, a room temperature rack module resting on a Thermoconductive Tray SLP or LP will equilibrate to approximately -140°C when placed in LN2. Samples stay organized and freeze upright in a very reproducible manner plus the risk of contamination is reduced. (Fig. 2)

Thermoconductive Tube Rack Module in Dry Ice

Performance Test:

Tests were performed with a 0.5 mL Sarstedt tube (#72.785) containing 0.25 mL water. The interior water temperature was measured using a thermocouple probe inserted through a hole introduced into the cap and held in an axial orientation by a custom cap adaptor. Vials were placed in a dry well in the Thermoconductive Tube Rack module (green) or directly into dry ice (red) and the temperature recorded. Data shown are from three different vials.

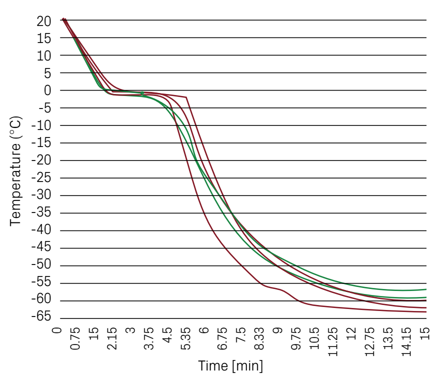

Figure 1

Quick Protocal - Dry Ice

Using a Thermoconductive Tube Rack module with dry ice in an ice pan or Cooling Workstation base.

1. Place pelleted or crushed dry ice in your ice pan or Cooling Workstation base.
2. Place rack module on top of the dry ice allowing it to equilibrate to the temperature of dry ice; approximately 5-7 min.
3. Place your samples in rack module and wait approximately 3-4 minutes until frozen.

Performance Test:

A Thermoconductive Tube Rack for 45 Cryo Tubes on a Low-profile Tray was placed in a pan containing 5cm of LN2. When the LN2 evaporated to the depth of 0.5cm (52 minutes) it was re-filled to 5cm. The Tube Rack temperature remained between -139.0°C and -140.2°C during the subsequent 115 minute interval for the LN2 to again reach a level of 0.5 cm.

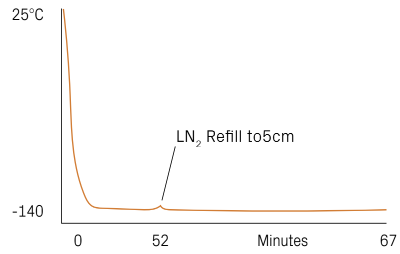

Figure 2

Note: It is important to adhere to laboratory safety protocols when handling dry ice or liquid nitrogen. Thermoconductive Tube Rack and Thermoconductive Tray modules may cause skin burns when cooled to ultra-low temperatures. Use extreme caution and appropriate protective clothing and equipment.

Quick Protocol - LN2

Using a Thermoconductive Tube Rack module and Thermoconductive Tray platform with LN2.

1. Place the tray platform in the 9L ice pan. Rest the rack module on top.
2. Pour LN2 around the tray until the fins are covered. Replenish as necessary. Wait approximately 12-15 min. for module to equilibrate to -140̊C.
3. Place your samples in rack module and wait approximately 3-4 minutes until frozen.

Bacteria Freezing in a Thermoconductive Tube Rack Module on Dry Ice

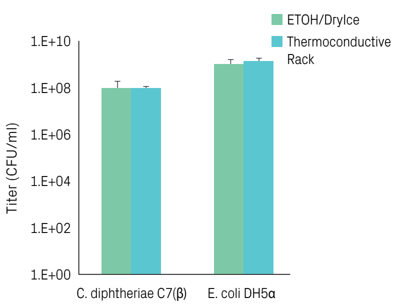

Figure 1. Graph showing the Titer (CFU/mL) of 2 different bacterial strains (C.diptheriae), C7 and E. coli DH5 using the 2 freezing methodologies

Performance Test:

Corynebacterium diphtheriae strain C7 (Beta) and Escherichia coli strain DH5 alpha were grown in heart infusion broth (HI) in Luria broth (LB) respectively, at 37°C overnight. 80% glycerol was added to obtain a final concentration of 15%. Aliquots of each strain were frozen on dry ice and ethanol (ETOH) slurry and in a Thermoconductive Tube Rack equilibrated to dry ice temperature (10 min). The frozen vials were stored in a -80°C freezer and were thawed on ice at the sixth day. 10-fold dilutions were made in HI or LB from 10-1 to 10-8 and 100 uL of each dilution was plated on HI or LB agar. Colonies were counted after 24 h incubation at 37°C. Results represent average of three samples.

Conclusion:

Bacteria titers recovered from freezing with Azenta's Thermoconductive Tube Rack module on dry ice are equivalent to conventional freezing in ETOH/dry ice, as shown in Figure 1. The rack method provides reproducible, ethanol-free freezing of bacterial samples while minimizing risk of contamination and keeping cryogenic vials organized and dry. Samples can also be safely transferred to storage areas while still seated in rack module.

Virus Freezing in a Thermoconductive Tube Rack Module on Dry Ice

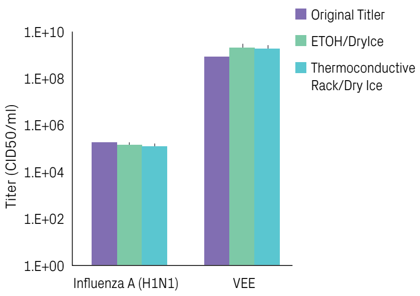

Figure 2. Graph showing the Titer (TCID50/ mL) of 2 different virus (influenza A and VEE) using the 2 freezing methodologies compared to the original titer value.

Performance Test:

2.5 x 105 TCID50 of influenza virus A/PuertoRico/8/34 (H1N1) and 1.3x109 TCID50 of VEE (Venezuelan equine encephalitis) virus frozen using the classical method of ETOH and dry ice slurry or Azenta's Thermoconductive Tube Rack equilibrated on dry ice (10 min). The frozen samples were stored overnight (influenza virus) or three days (VEE) in a -80°C freezer. Samples were thawed and the titers assayed by TCID50. Results represent an average of three samples.

Conclusion:

Virus titers recovered from freezing with Azenta's Thermoconductive Tube Rack module on dry ice are equivalent to conventional freezing in ETOH/dry ice, as shown in Figure 2. The rack method provides reproducible, ethanol-free freezing of viral samples while minimizing risk of contamination and keeping cryogenic vials organized and dry. Samples can also be safely transferred to storage areas while still seated in rack module.

Product Selection Guide

Thermoconductive Tube Racks for Microcentrifuge Tubes

BCS-137	**Thermoconductive Tube Rack, holds 30 x 500ul microcentrifuge tubes,** conical wells**, gray**
BCS-127	**Thermoconductive Tube Rack, holds 15 x 1.5 conical tubes,** conical wells**, gray**
BCS-128	**Thermoconductive Tube Rack, holds 30 x 1.5 conical tubes,** conical wells**, gray**
BCS-163	**Thermoconductive Tube Rack, holds 6 x 1.5 or 2ml microcentrifuge tubes,** cylindrical wells**, gray**
BCS-125	**Thermoconductive Tube Rack, holds 15 x 1.5 or 2ml microcentrifuge tubes,** cylindrical wells**, gray**
BCS-108	**Thermoconductive Tube Rack, holds 30 x 1.5 or 2ml microcentrifuge tubes,** cylindrical wells**, gray**
BCS-102	**Thermoconductive Tube Rack, holds 90 x 1.5 or 2ml microcentrifuge tubes,** cylindrical wells**, gray**
BCS-116	**Thermoconductive Tube Rack, holds 96 x 1.5 or 2ml microcentrifuge tubes,** cylindrical wells**, SBS compatible, row and column indexing, gray****

*** Thermoconductive Tube Rack for 96 Microcentrifuge Tubes has A-H and 1-12 row and column indexing*

Product Selection Guide *cont.*

Thermoconductive Tube Racks for Cryo or FACS Tubes

BCS-126	**Thermoconductive Tube Rack, holds 15 cryo tubes or FACS tube modules,** cylindrical wells**, gray**
BCS-138	**Thermoconductive Tube Rack, holds 30 cryo tubes or FACS tube modules,** cylindrical "gripping" wells for one-hand opening/closing vials, **gray**†
BCS-105	**Thermoconductive Tube Rack, holds 45 cryo tubes or FACS tube modules,** cylindrical wells**, gray**

†"gripping" wells for one-hand vial opening/closing

Thermoconductive Trays

BCS-252	**Thermoconductive Tray, slim with lowprofile,** for use in 9L ice pan with LN2**, gray**

Ice Pans

BCS-113x	**Ice Pan without Lid, Rectangle 4L**
BCS-111x	**Ice Pan without Lid, Rectangle 9L**

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