Snap Freezing Using Dry Ice or Liquid Nitrogen

Application Note
October 2013

DRY ICE
(or Liquid Nitrogen)
Snap-Freezing

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. BioCision CoolRack® modules, ThermalTray™ 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.

CoolRack method: Dry ice
CoolRack 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)

CoolRack method: Liquid nitrogen (LN2)
After approximately 15 minutes, a room temperature CoolRack module resting on a ThermalTray 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)

CoolRack Module in Dry Ice

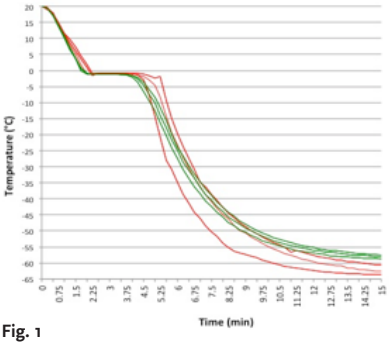

Fig. 1

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 CoolRack module (green) or directly into dry ice (red) and the temperature recorded. Data shown are from three different vials.

QUICK PROTOCOL - DRY ICE

Using a CoolRack module with dry ice in an ice pan or CoolBox™ base.

1. Place pelleted or crushed dry ice in your ice pan or CoolBox base.
2. Place CoolRack 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 CoolRack module and wait approximately 3-4 minutes until frozen.

CoolRack Module in Liquid Nitrogen

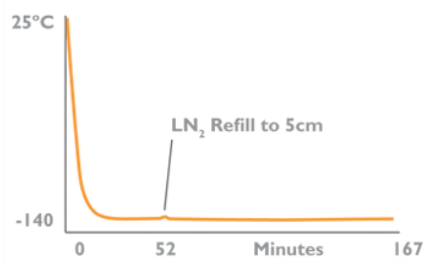

Fig. 2

Performance Test:
A CoolRack CF45 on a ThermalTray LP 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 CoolRack CF45 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.

QUICK PROTOCOL - LN2

Using a CoolRack module and ThermalTray platform with LN2.

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

⚠ *It is important to adhere to laboratory safety protocols when handling dry ice or liquid nitrogen. CoolRack and ThermalTray modules may cause skin burns when cooled to ultra-low temperatures. Use extreme caution and appropriate protective clothing and equipment.*

Bacteria Freezing in a CoolRack Module on Dry Ice

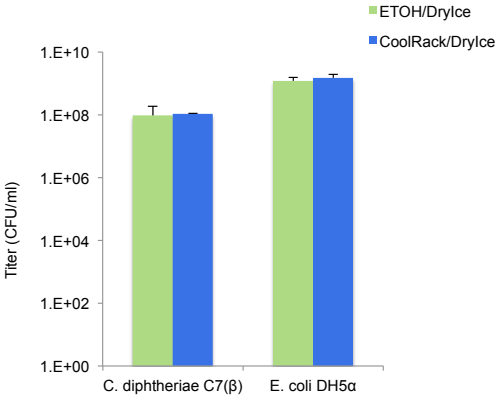

Fig. 1 Graph showing the Titer (CFU/mL) of 2 different bacterial strains (C. diptheriae), C7 and E. coli DH5 using the 2 freezing methodologies.

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 CoolRack 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 BioCision's CoolRack module on dry ice are equivalent to conventional freezing in ETOH/dry ice, as shown in Figure 1. The CoolRack 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 CoolRack module.

Virus Freezing in a CoolRack Module on Dry Ice

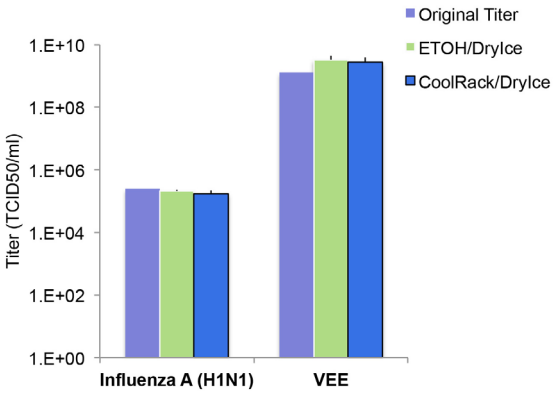

Fig. 2 Graph showing the Titer (TCID50/mL) of 2 different virus (influenza A and VEE) using the 2 freezing methodologies compared to the original titer value.

Performance Test:
2.5 x 10^5 $TCID_{50}$ 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 BioCision's CoolRack 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 $TCID_{50}$. Results represent an average of three samples.

Conclusion:
Virus titers recovered from freezing with BioCision's CoolRack module on dry ice are equivalent to conventional freezing in ETOH/dry ice, as shown in Figure 2. The CoolRack 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 CoolRack module.

Product Selection Guide

Item No.	Description	For Use With	Dimensions
CoolRack M-PF thermo-conductive tube modules for conical microcentifuge tubes			
BCS-137	CoolRack M30-PF (500 uL)	30 wells, 0.5 mL conical microfuge tubes	L 12.0 x W 10.2 x H 3.8 cm.
BCS-127	CoolRack M15-PF	15 wells, 1.5 mL conical microfuge tubes	L 10.2 x W 6.4 x H 3.8 cm.
BCS-128	CoolRack M30-PF	30 wells, 1.5 mL conical microfuge tubes	L 12.0 x W 10.2 x H 3.8 cm.
CoolRack M thermo-conductive microfuge tube modules for 1.5 mL and 2.0 mL tubes			
BCS-163	CoolRack M6	6 wells, 1.5 mL or 2.0 mL microfuge tubes	L 6.0 x W 4.3 x H 3.8 cm.
BCS-125	CoolRack M15	15 wells, 1.5 mL or 2.0 mL microfuge tubes	L 10.2 x W 6.4 x H 3.8 cm.
BCS-108	CoolRack M30	30 wells, 1.5 mL or 2.0 mL microfuge tubes	L 12.0 x W 10.2 x H 3.8 cm.
BCS-102	CoolRack M90	90 wells, 1.5 mL or 2.0 mL microfuge tubes	L 26.8 x W 11.2 x H 3.8 cm.
BCS-116	CoolRack M96ID	96 wells, 1.5 mL or 2.0 mL microfuge tubes	L 25.4 x W 15.2 x H 3.8 cm.
CoolRack CF thermo-conductive tube modules for cryogenic vials			
BCS-126	CoolRack CF15	15 wells, cryogenic vials	L 10.2 x W 6.4 x H 3.8 cm.
BCS-138	CoolRack CFT30	30 wells, cryogenic vials with "grip" well design for one-hand tube open/close	L 12.0 x W 10.2 x H 3.8 cm.
BCS-105	CoolRack CF45	45 wells, cryogenic vials	L 17.3 x W 9.7 x H 3.8 cm.
ThermalTray platform			
BCS-252	ThermalTray SLP	thermo-conductive platform to support CoolRack modules in LN2	L 28.0 x W 14.0 x H 3.2 cm.
Ice pans			
BCS-113x	Midi 4L Rectangular Pan	Compatible with ice, dry ice and liquid nitrogen	L 31.1 x W 22.2 x H 11.4 cm.
BCS-111x	Maxi 9L Rectangular Pan	Specify color (x): "PL" purple, "GR" green, "OR" orange, "PK" pink	L 40.6 x W 31.8 x H 11.4 cm.

⚠ *It is important to adhere to laboratory safety protocols when handling dry ice or liquid nitrogen. CoolRack and ThermalTray modules may cause skin burns when cooled to ultra-low temperatures. Use extreme caution and appropriate protective clothing and equipment.*

BioCision, LLC 12 E. Sir Francis Drake Boulevard, Unit B, Larkspur, CA 94939 USA sales@biocision.com www.biocision.com