

Snap freezing of environmental microbial samples in liquid

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Method Article

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Abstract

This protocol describes the snap freezing process for liquid-phase environmental microbial samples. Compared with standard freezing, it better maintains the integrity of microbial cells.

Introduction

Snap freezing is the technique in which a sample is rapidly frozen using dry ice, a dry ice/ethanol slurry or liquid nitrogen. Samples frozen in this manner include bacteria 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 the degradation of molecules such as RNA or proteins. Freezing tubes in a CoolRack keeps all tubes upright and organized, and provides uniform effortless freezing at ultra-low temperature.

Reagents

Equipment

Materials

- CoolRack thermo-conductive modules CF45 (Corning, Cat# 432051)
- Ice Pan w/Lid, Midi 4L (Corning, Cat# 432110)
- Styrofoam box
- Liquid nitrogen (LN2)
- Crushed dry ice
- Dewar flask
- Cryogenic tubes
- Forceps
- Cryo/Freezer Boxesbox (Fisher, Cat# 03395464)

Personal protection equipment

- Clear full-face shield

- Waterproof insulated gloves (must be waterproof; no good if they absorb the N₂; Any gloves worn should be loose-fitting and very easy to remove)

Procedure

Safety tips

- NEVER seal the containers tightly to avoid pressure buildup.
- Pay close attention to N₂'s warning about *adequate ventilation*. Oxygen deficiency caused by inert gas displacement is totally insidious and strikes with zero warning. It will kill you and you won't have any idea that it is happening. Watch out for oxygen depletion, cold nitrogen gas will fill in low areas.
- Prevent LN₂ splatter from hitting your skin. If you are pouring from a container, as the LN₂ hits the room temperature vessel it will splatter and boil until the vessel is the same temp as the LN₂.

Snap freezing

Label: sample name, date frozen, my initial

1. Aliquot 1 mL samples into 2 mL cryogenic tubes
2. Fill an insulated pan with approximately 2 cm of LN₂ and place a CoolRack in the LN₂. (The liquid nitrogen level should be kept halfway up the side of the CoolRack module (approximately 2 cm) to maintain cryogenic temperature.)
3. The CoolRack will cool to snap freezing temperature of -150 °C in about 15 minutes. (To preserve LN₂ and reduce the time it takes to reach snap freezing temperature, the CoolRack may be pre-chilled in a -80 °C freezer.)
4. Put freezer box on dry ice in Styrofoam box
5. Place the cryogenic tubes in the CoolRack for snap freezing
6. Leave each sample in the CoolRack for 1 min. After the sample snap freeze, transfer the tube into the freezer box using forceps.
7. Put the freezer box into a -80 °C freezer.

Sample Warming

1. Put the tubes at 4 °C to thaw.

Troubleshooting

Time Taken

Anticipated Results

References

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