Corning® CoolRack® and Corning ThermalTray™

Snap-freezing of Tissue Samples Protocol

CORNING

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. Snap-freezing with a Corning CoolRack module will provide sample vessel stability, organization, consistent freezing parameters, and rapid hands-free sample processing while avoiding lost or contaminated samples. Snap-freezing is performed on a pre-cooled CoolRack module, which ensures fast temperature transfer. This method can provide excellent specimen integrity and a wide array of options for tissue analysis including extraction of proteins, DNA, and RNA. This procedure is intended to ensure that tissue samples collected will be frozen in a safe and efficient manner while eliminating the risks of contamination and variation in molecular integrity. The following protocol describes a general procedure for snap-freezing. For non-standard sample types, always refer to tissue-specific protocols.

Materials

- Fresh tissue (harvest as fast as possible)
- Corning cryogenic vials
- Corning CoolRack – See Compatibility Chart (Table 1)
- Corning CoolBox™ or Ice Tray
- Cryolabels or cryomarker
- Corning ThermalTray LP platform
- 37°C water bath
- Crushed ice
- Liquid nitrogen or dry ice

Methods

Tissue Collection

NOTE: Ensure the resected tissue never desiccates or is contaminated by surrounding tissue or other samples. Use clean scalpels and forceps between samples to avoid cross-contamination.

1. Prepare the CoolRack using one of the procedures shown below (dry ice or liquid nitrogen).
2. Place crushed ice in an ice pan and insert the ThermalTray into ice. Allow to cool until water vapor begins to collect on the surface.
3. Place a thermally conductive dissecting mat on the ThermalTray to protect the surface and the edges of the cutting tools.

Table 1. Corning CoolRack CF Cryogenic Vials Modules

Cat. No.	Description	Capacity
432049	CoolRack CF15	15 cryogenic vials or FACS tubes
432050	CoolRack XT CFT24	24 cryogenic vials or FACS tubes, with “gripping” wells for one-hand vial opening/closing, SBS-compatible
432052	CoolRack CFT30	30 cryogenic vials or FACS tubes, with “gripping” wells for one-hand vial opening/closing
432051	CoolRack CF45	45 cryogenic vials or FACS tubes

4. Cut tissue from each anatomic site to the appropriate size (usually <5 mm thick), and place specimens onto the dissecting mat for detailed dissection.

NOTE: The low temperature will pre-chill the tissue and significantly reduce the rate of dehydration.

5. Place isolated segments of tissue into separate sterile labeled cryogenic vials for snap-freezing.
6. Close the container and place the specimen container into a Corning® CoolRack® well.

NOTE: Unless intended for another method of preservation, fresh tissue should be frozen as soon as possible (within 30 minutes from resection is optimal).

Snap-freezing on Dry Ice

NOTE: Using a Corning CoolBox™ in this process allows protected handling of the CoolRack and minimal requirements for dry ice. Depending on environmental conditions, a CoolBox with 200 cc of crushed dry ice will maintain the CoolRack and sample cryogenic vials at -78°C for up to 6 hours with the lid open and up to 10 hours with the lid closed. To extend the cooling duration, simply replenish the dry ice. Dry ice requirements and cooling duration will vary with other insulated containers. Always use dry ice to transfer the cryogenic vials to permanent storage to avoid temperature rise and cell damage. Cryogenic vial contents can rise from -75°C to over -50°C in less than one minute if exposed to room temperature air.

1. Place 200 cc of crushed dry ice into the bottom of a Corning CoolBox. If using a Corning ice bucket or pan instead of a CoolBox, place enough crushed or caked dry ice in the pan to create a one-inch thick bed under the entire bottom surface of the CoolRack module.
2. Place the CoolRack module directly on the crushed or caked dry ice.

NOTE: A buzzing sound coming from the metal-ice contact is normal and safe.

3. Allow the CoolRack module to chill about 7 minutes to reach -78°C.
4. Place the cryogenic vial with the specimen into the CoolRack module at any time after the CoolRack reaches cryogenic temperature.
5. The sample will snap-freeze in 1 to 2 minutes and may be left in place while the remaining samples are being processed.

NOTE: All samples will remain at -78°C while the CoolRack module is in direct contact with the dry ice.

6. After all samples are frozen or the CoolRack is full, either remove samples for freezer archive or place the CoolRack with snap-frozen samples in place directly into a storage freezer.
7. Record the appropriate information about the tissue in your tissue repository.

NOTE: Records should include all of the following: tissue identity, date frozen, freezing medium used, and method and results of all quality control tests performed.

Snap-freezing on Liquid Nitrogen

1. Place the 15-well Corning CoolRack M15-PF directly in the CoolBox and fill with liquid nitrogen. The liquid nitrogen (LN_2) level should be kept halfway up the side of the CoolRack module (approximately 2 cm) to maintain cryogenic temperature. If using the Corning ice pan instead of the CoolBox, fill an ice pan with approximately 2 cm of LN_2 and place a CoolRack in the LN_2.
2. The CoolRack will cool to a snap-freezing temperature of -150°C in about 12 minutes. To preserve LN_2 and reduce the time it takes to reach the snap-freezing temperature, the CoolRack may be pre-chilled in a freezer.
3. With clean forceps, place the specimen to be frozen into an empty cryogenic vial, and place the cryogenic vial in the CoolRack.
4. The sample will quickly snap-freeze and may be left in place while the remaining samples are being processed.
5. After all the samples are frozen or the CoolRack is full, either remove the samples for storage or place the CoolRack into the freezer. If storing the samples in liquid nitrogen, it is recommended the samples be placed in the vapor phase of liquid nitrogen to prevent possible LN_2 aspiration into the cryogenic vial and a possible explosion when the cryogenic vial is returned to room temperature.

6. Record the appropriate information about the tissue in your tissue repository.

NOTE: Records should include all of the following: tissue identity, date frozen, freezing medium used, and method and results of all quality control tests performed.

Sample Warming

1. Place the Corning® CoolRack® module into a 37°C water bath. Alternatively, a Corning ThermalTray™ may be placed underneath the CoolRack to stabilize it.
2. Using appropriate safety equipment, remove the tube from its storage location and place it into a CoolRack.

NOTE: This set up will ensure consistent and rapid thawing of all cryogenic vials and aid in ensuring sterility, as the cryogenic vial will not be put into direct contact with the bath water. It might be necessary to offset the water bath by one degree (38°C) to maintain the ThermalTray and CoolRack modules at 37°C.

3. The tissue is now ready to be processed.

CAUTION: Dry ice/LN_2 or metal surfaces at dry ice/LN_2 temperature can freeze skin on contact and may cause serious burns. Never handle dry ice, LN_2, or cold metal with bare hands. Always use protective equipment including insulated gloves when handling dry ice or materials such as the CoolRack that has been in contact with dry ice or LN_2. This protocol is for orientation only and does not include, nor is intended to substitute for, proper laboratory safety procedures. Obtain training and certification for the safe handling of dry ice and LN_2 from your laboratory safety supervisor before attempting any procedures involving dry ice or LN_2.

Reference

Best Practices for Repositories I. Collection, Storage and Retrieval of Human Biological Materials for Research. International Society for Biological and Environmental Repositories (ISBER). www.isber.org

For more specific information on claims, visit the Certificates page at **www.corning.com/lifesciences.**

For additional product or technical information, visit **www.corning.com/lifesciences** or call 800.492.1110. Customers outside the United States, call +1.978.442.2200 or contact your local Corning sales office.

Corning Incorporated
Life Sciences
836 North St.
Building 300, Suite 3401
Tewksbury, MA 01876
t 800.492.1110
t 978.442.2200
f 978.442.2476
www.corning.com/lifesciences

Worldwide Support Offices

ASIA/PACIFIC

Australia/New Zealand
t 61 427286832

China
t 86 21 3338 4338
f 86 21 3338 4300

India
t 91 124 4604000
f 91 124 4604099

Japan
t 81 3-3586 1996
f 81 3-3586 1291

Korea
t 82 2-796-9500
f 82 2-796-9300

Singapore
t 65 6572-9740
f 65 6861-2913

Taiwan
t 886 2-2716-0338
f 886 2-2516-7500

EUROPE

France
t 0800 916 882
f 0800 918 636

Germany
t 0800 101 1153
f 0800 101 2427

The Netherlands
t 31 20 655 79 28
f 31 20 659 76 73

United Kingdom
t 0800 376 8660
f 0800 279 1117

All Other European Countries
t 31 (0) 20 659 60 51
f 31 (0) 20 659 76 73

LATIN AMERICA
grupoLA@corning.com

Brasil
t (55-11) 3089-7400

Mexico
t (52-81) 8158-8400

CORNING | FALCON AXYGEN GOSSELIN PYREX