

# OCT Embedding of Tissue Samples for Cryosectioning Quick Guide

This guide outlines the three possible preparation of tissues in OCT immediately following tissue collection using Liquid Nitrogen, Dry Ice, or a  $-80^{\circ}\text{C}$  Freezer, depending on the facility's availability.

## IMPORTANT:

This protocol should occur **immediately** following tissue collection, such that a designated person should stand by with an appropriate container to transport samples directly to the laboratory right after collection for processing.

Tissue samples should be processed and stored at the appropriate temperatures as quickly as possible, ideally less than 15 minutes from collection. Short term storage of the tube containing the samples on wet ice should never pose a freezing danger to the samples.

Tissue should be fresh, not treated with formalin, and should be the appropriate size required for any future assays.

## WARNING! SAFETY CAUTION:

There are **TWO** major risks associated with the use and handling of Liquid Nitrogen ( $\text{LN}_2$ ) which presents potential hazards. Always follow your institution guidelines in the safety and handling of  $\text{LN}_2$ .

$\text{LN}_2$  is extremely cold ( $< -196^{\circ}\text{C}$ ). At normal atmospheric pressure  $\text{LN}_2$  vaporizes at  $-196^{\circ}\text{C}$ .  $\text{LN}_2$  itself, and every object in direct contact with it, will rapidly produce **frost burns and frostbite**.

During this vaporization process  $\text{LN}_2$  expands to produce a large volume of gaseous  $\text{N}_2$ . **Asphyxiation** may result from this large volume of gaseous  $\text{N}_2$  displacing the oxygen present in the atmosphere of any small unventilated room.

## ***A. Immediate Access to $\text{LN}_2$ Post-Tissue Collection [BEST OPTION]***

1. Fill a Styrofoam cooler halfway with  $\text{LN}_2$ . Place a Styrofoam float, with holes punched through, into the  $\text{LN}_2$ .
2. Lay cryomolds atop of the Styrofoam float allowing vapors to reach mold but not touching  $\text{LN}_2$ . DO NOT submerge in  $\text{LN}_2$  or use coolant spray.
3. Apply a thin layer of OCT medium in cryomold.
4. Orient the tissue sample in this thin OCT layer and allow to freeze slightly for about 1 minute. "Slight freeze" is complete when the medium is opaque.
5. Fill the cryomold completely with OCT medium. Allow the cryomolds to sit over  $\text{LN}_2$  vapors until frozen.
6. Once frozen, wrap labeled cryomold containing the OCT block in aluminum foil and transfer to an airtight bag.
7. Store in  $-80^{\circ}\text{C}$  freezer (at least overnight) until shipping.

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## ***B. Immediate Access to Dry Ice Post-Tissue Collection [GOOD OPTION]***

1. Fill a Styrofoam cooler with dry ice.
2. Wedge cryomolds in dry ice and/or adjust for media to freeze evenly in the bottom of the mold. **Do not submerge tissue sample in dry ice at this point!**
3. Apply a thin layer of OCT medium in cryomold.
4. Orient the tissue sample in this thin OCT layer and allow to freeze slightly for about 1 minute. "Slight freeze" is complete when media is opaque.
5. Fill the cryomold completely with OCT medium. Completely submerge cryomold in dry ice and allow to sit for 20 minutes.
6. Once, frozen, wrap labeled cryomold containing the OCT block in aluminum foil and transfer to an airtight bag.
7. Store in -80°C freezer (at least overnight) until shipping.

## ***C. Immediate Access to -80°C Freezer Post-Tissue Collection [ADEQUATE OPTION]***

1. Apply a thin layer of OCT medium in cryomold.
2. Orient the tissue sample in thin OCT layer and transfer to -80°C freezer allowing it to slightly freeze for about 1 minute. "Slight freeze" is completely when media is opaque.
3. Remove from freezer and fill the cryomold completely with OCT medium.
4. Return cryomolds to -80°C freezer and allow to sit for 20 minutes.
5. Once frozen, wrap labeled cryomold containing the OCT block in aluminum foil and transfer to an airtight bag.
6. Store in -80°C freezer (at least overnight) until shipping.

## ***D. Further Approach***

- Samples should be stored in -80°C freezer (no defrosting) until shipping.
- For shipping, please refer to Canopy Quick Guide – Shipping ChipCytometry Samples and Reagents to Canopy Hannover Site
- For cryosectioning and chip loading, please refer to Canopy Quick Guide – Tissue Cryosection Preparation for ZellSafe™ Tissue Chip Using AC/EtOH