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OCT-Embedded Tissue Preparation

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ABSTRACT

This protocol describes embedding of tissue samples in optimal cutting temperature (OCT) compound (Lin & Lin, 2020). Tissue embedded in OCT can then undergo frozen sectioning on a microtome-cryostat. The advantage of OCT embedding is it requires minimal tissue processing and tissue does not need to undergo fixation prior to snap freezing. OCT blocks should be stored in -80°C freezer and can yield thin tissue sections (< 5µm). Formation of bubbles in the OCT can distort and disrupt tissue. To minimize bubble formation, we recommend storing OCT upside down in a small bucket between uses. When ready for use, uncap OCT tube and dispense slowly to prevent bubbles from forming. Once tissue is in cassette surrounded by OCT, snap freezing is done. Ensure you have a portable liquid nitrogen dewar partially filled with liquid nitrogen. Place a piece of Styrofoam atop the liquid nitrogen to prevent direct contact of the embedding molds with the cold liquid.

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MATERIALS TEXT

- Freshly dissected tissue block
- Dry ice
- Liquid nitrogen

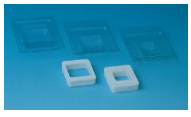
  Tissue-Tek OCT **Fisher**
- **Scientific Catalog #14-373-65**
- Access to -80°C ultra-low freezer
- Styrofoam (small piece)
- Aluminum foil

Portable liquid nitrogen dewar

Pope Scientific, Inc **8921**

Cryomolds

Tissue-Tek **4557**



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1 Label biopsy molds appropriately.

2 Add a small amount of OCT to the bottom of each mold, ensuring no bubbles are present.

3 Place tissue piece in the center of the mold on top of the OCT layer.

4 Pour OCT slowly into the mold, ensuring the tissue piece is completely covered.

5 Using a small piece of aluminum foil, tightly wrap the mold.

Tip: Label the outside of the aluminum for easy retrieval later.

6 Place foil-wrapped mold flat in a liquid nitrogen dewar on top of the Styrofoam.

7 After approximately one minute, move the frozen OCT mold to the dry ice container.

Note: OCT will become opaque when frozen.

8 Store molds at -80°C ultra-low freezer for Biobanking or until ready for downstream processing.

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