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Freezing and Formalin Fixation of Tissue V.3

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1 Works for me dx.doi.org/10.17504/protocols.io.br4fm8tn

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ABSTRACT

Scope:
To describe the procedure for freezing fresh tissue procured from the Cooperative Human Tissue Network.

Expected Outcome:
Frozen tissue should have minimal structural damage from freezing.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

DMG Anderson, W Lambert, DJ Calkins, Z Ablonczy, RK Crouch, RM Caprioli, and KL Schey. "Imaging MS of Rodent Ocular Tissues and the Optic Nerve." Methods in Molecular Biology. 2017, 1618, 15-27.

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PROTOCOL CITATION

Jamie Allen, Maya Brewer, Elizabeth Neumann, Carrie Romer, Danielle Gutierrez, Mark deCaestecker, Jeff Spraggins 2021. Freezing and Formalin Fixation of Tissue. **protocols.io** https://dx.doi.org/10.17504/protocols.io.br4fm8tn
Version created by Jamie Allen

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

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GUIDELINES

Definitions:

1. Isopentane is 2-Methylbutane
2. CMC is carboxymethylcellulose

MATERIALS TEXT

Reagents:

1. Dry Ice
2. Isopentane, Fisher O3551-4
3. Carboxymethylcellulose, Fisher C9481
4. Richard-Allan Scientific Neutral Buffered Formalin (10%), Fisher 22-050

Equipment:

1. Styrofoam box with lid or solvent compatible ice bucket
2. Ruler
3. Forceps
4. Tissue Cassettes
5. Plastic jar for formalin fixation
6. Aluminum Foil, Fisher 01-213-100
7. Aluminum Weigh Dishes, Fisher 08-732-103
8. Thermo Scientific Peel-A-Way Disposable Embedding Molds, 20mm x 40mm, Fisher 18-41

Reagent Preparation:

2.6% Carboxymethylcellulose:
Add 500mL water to 500mL bottle
Microwave for 2 minutes
Place bottle on heated stir plate ~70^{o}
Slowly add 13g to warmed water
Stir on low hotplate overnight, occasionally tightening lid and shaking bottle
Store solution in 4^{o} refrigerator
Before each use, pour solution into 125mL bottle and sonicate for 10 minutes
(the smaller bottle is easier to use with the transfer pipettes)

SAFETY WARNINGS

Health and Safety:

1. Safety glasses or goggles, proper gloves, and a lab coat required. The area should be adequately vented and a lab mat placed underneath all solutions and tissue.
2. Warning: Isopentane: Harmful by inhalation, in contact with skin and if swallowed. Keep container tightly closed and insure adequate ventilation. Flammable liquid and vapor.

Documentation and Preparation

1 Place a layer of dry ice in to box and add enough isopentane to make a slurry.

2 Prepare a container of 10% Formalin for preparing FFPE samples.

3 Remove sample container from ice and document on sample sheet any information that is written on the lid.

4 Label embedding molds and add a small amount of CMC to each one (barely cover the bottom).

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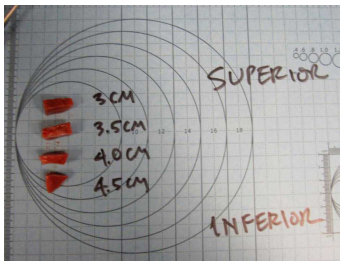
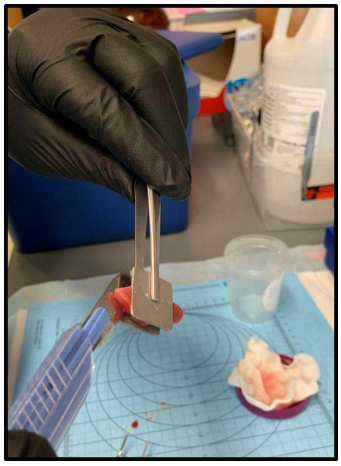
5 Place sample on dissection board. Cut sample into 3-5 pieces. These will need to be small enough to fit into the embedding mold and allow the sample to be completely covered with CMC.



Place these smaller pieces in reference to the original position and take a picture.

Tissue for Fixation

6 Carefully slice a small section of tissue (preferably having both cortex and medulla). Do this in a way that you create two "bookends." The section for fixation should be no thicker than a nickel.



7 Carefully place the piece of tissue bookend down into an embedding cassette. Place in 10% formalin container. After freezing the remaining sample, continue with Step 17 for fixation.

8 Measure and document the length, width, and height. Place sample on metal weigh boat and document weight. Document any other information or conditions (attachment).

Accession and Freezing Documentation.docx

Freezing of Tissue

9 Place metal weigh boat with sample on top of slurry. Cover box with lid and allow tissue to completely freeze.

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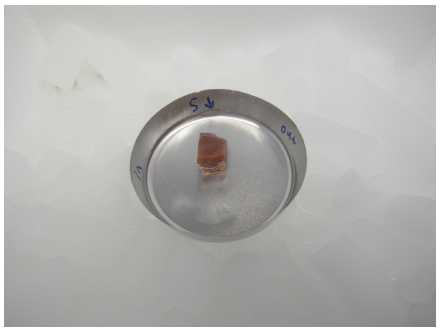
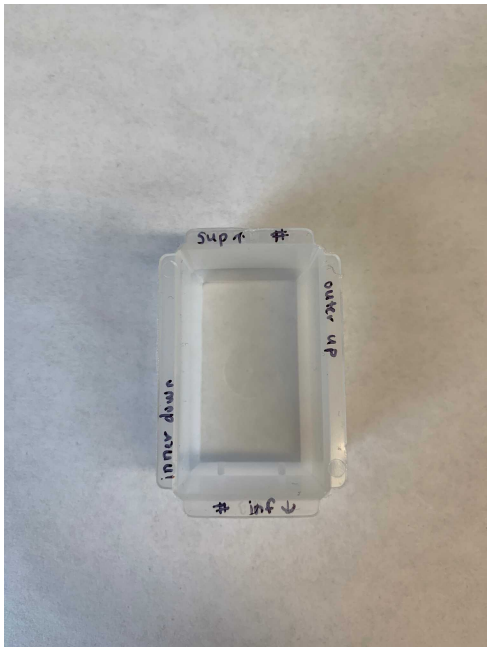


10 Write sample number and orientation on the edges of the molds (indicating inner/outer, superior/inferior).



Place embedding mold with CMC on top of the slurry.

11 Check the mold in the slurry to see if it is starting to freeze. Add tissue when the CMC starts to become opaque. Orient the tissue so that the side that is to be cut is face down.

12 Slowly add more CMC on top of the tissue until it is covered. Keep the lid closed over the slurry as much as possible.

13 Freezing Pic.jpg

14 When completely frozen, wrap in foil and label sample number with sharpie.

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15 Place all samples from one patient in a labeled box and place in the **-80 °C** .

16 Place disposable equipment in the Biohazard trash. Wipe all forceps, rulers, bottles with appropriate cleansing wipes.

Formalin Fixing and Embedding Tissue

17 Allow tissue to “fix” overnight at room temperature for at least 12 hours but typically 24 hours.

18 Move tissue cassettes into 70% ethanol for 35 minutes.

19 Submerge the tissue into 90% ethanol for 35 minutes.

20 Submerge the tissue into 95% ethanol for 35 minutes.

21 Submerge the tissue into three sequential containers of 100% ethanol, each for 35 minutes.

22 Submerge the tissue into 100% xylenes for 35 minutes, three times.

23 Submerge the tissue into paraffin (was) for 60 minutes, three times.

24 Tissue is then embedded in more paraffin to make a block.

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