I. SCOPE AND PURPOSE

Fixation and heat involved in routine histologic processing can damage the sub-cellular structures that we study and many antigens within the tissue, therefore frozen sections must be used. This procedure is specific for the preparation of fixed and unfixed sections frozen tissue samples.

Please add your tissue type and brief explanation here

II. PROCEDURE

A. Safety Procedures

1. Manual instruction on Microtome and Tissue Sectioning should be followed for the guide for safe operation of the microtome.
2. Use appropriate knife safety procedures: remove blades after use and dispose of in sharps container. Put blade safety cover on when not sectioning.
3. Cryostat knifes are very sharp! Wear nitrile gloves at all times. Always lock cryostat handle before putting hands into the blade holder area. Always wear nitrile gloves and lab coat when handling blades that have been exposed to unfixed tissue.
4. Follow universal precautions: Wear gloves, mask, and gown when handling unfixed tissue.
5. Use caution when working with dry ice. Skin exposure can cause burns.
6. Ethanol is flammable. Store properly in flammable cabinet. Use gloves and lab coat when working with ethanol.
7. All spills should be cleaned up and material disposed of in accordance to local, state, and federal laws.

B. Specimen Embedding Information

1. Transfer tissue to mold, add OCT and place on dry ice for at least 60 minutes (up to 5 hours). Transfer specimens in storage bag label as biohazard to long-term storage in 20°C freezer. Do not allow tissues to thaw at any time.
2. Sample Preparation: Most samples should be frozen in OCT and stored in 20°C freezer. Some samples may already be in OCT.

C. Sectioning Frozen Tissue

1. Prior to initiation of cutting tissue, ensure the cryostat is clean. Between cuttings, ensure cryostat is free of debris and is wiped with 95% ethanol on gauze and dried with clean gauze.
2. Samples are received in BHSB Room 007 Histology. Slides and labels are created for each sample.

3. When ready to process samples, take on sample from the freezer and place into a small dry ice container.
4. Mount tissue on chuck (specimen holder) using very small amount of OCT.
5. Place new blade in holder and tighten down securely. Put chuck with tissue into holder and tighten down
6. Carefully advance knife to start taking sections. Do not face too far into the block.
7. Take 1-to-20-micron (as requested) sections.
8. Lock hand wheel and cover blade edge with roll plate. Remove chuck with tissue from the holder and place on dry ice.
9. All samples will be returned to -20^{o}C storage
10. Dispose of all blades, gauze, gloves, and cotton swabs. Clean cryostat and all instruments with 95% ethanol.

D. Transportation

1. A lab member will deliver the specimens to the Histology Core n the Block Health Science Building, Room 007 per their transportation requirements with no intermediate stops. Specimens must be transported in a leak-proof, secondary containment.
2. After sectioning, a staff member will retrieve the sections from the Histology Core. Slides will be placed inside a plastic slide holder box for storage in the -20^{o}C labeled “BSL2”, his/her name, and his/her phone number. The PI will maintain storage according to BSL2 guidelines until chemical fixation (which inactivates the sample) or disposal of slides and/or tissue as described in their protocol.