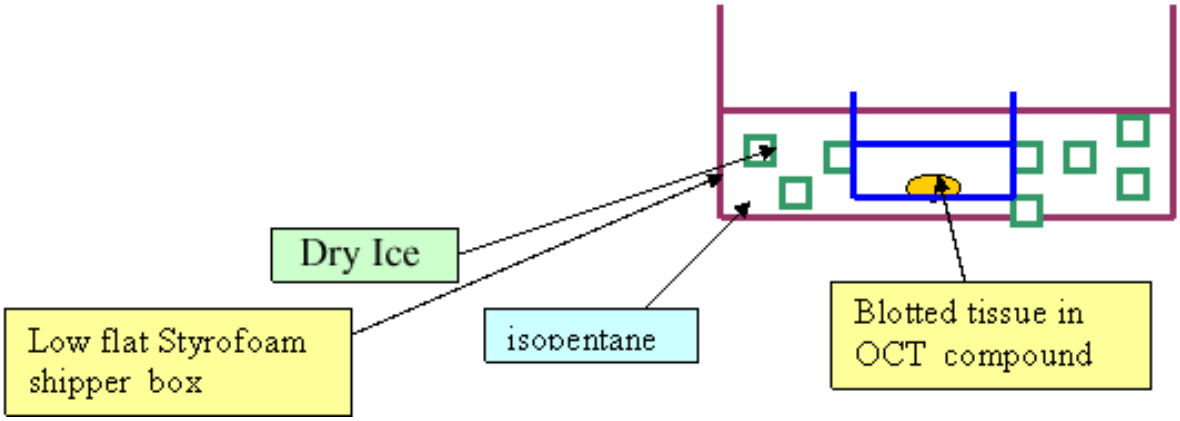
Freezing and Fixing Tissue

When tissues are removed from the body, the process of autolysis occurs rapidly. In order to preserve tissue morphology for histopathologic examination, it is important to immediately FIX (to process and embed in wax) OR FREEZE tissues promptly after removal from the body.

Freezing Tissues for Frozen Sectioning



1. **To inflate lungs before freezing**: Make 1:1 solution of OCT and PBS for tracheal infiltration to inflate lungs before freezing
2. Get bench area ready with absorbent material, Styrofoam board and dissection equipment
3. Make freezing mixture: In a small Styrofoam flat container, make a slurry with small bits of dry ice and 2 methyl butane. This will be the freezing mixture which will freeze the organs in OCT for frozen sections
4. Lay euthanized animal on dissecting board
5. To optimally examine brain, plan to perfuse animal with fixative, first with PBS, to deplete all of the blood, and then perfuse with freshly made 4% paraformaldehyde, to fix all the organs. If the fixed tissues are to be frozen for use in immunohistology assays, after fixation, they MUST be immersed in 30% sucrose in PBS at 4°C, until they sink, in order to cryoprotect and prevent freeze artefact and loss of tissue architecture.
6. Expose trachea and infiltrate lungs with OCT/PBS mixture. Remove entire block and place on paper towel to drain before freezing in vinyl mold surrounded with OCT.
7. Harvest tissues and place in sequence onto paper towels to dry off tissue fluids before placing into vinyl molds, FLAT ON THE BOTTOM OF THE MOLD to keep tissues all at the same level, and then fill with OCT.
    a. Spleen/thymus, lymph nodes. adrenals
    b. Liver, pancreas, kidney, heart
    c. OCT inflated and drained lungs
    d. GI (small intestine, colon, stomach) and GU (Bladder, prostate, testis, ovary, uterus)

   e. Skin, skeletal muscle, salivary glands, mammary glands (decalcified femur for blocks)
   f. Brain (dorsal surface down at bottom of mold)
8. Place plastic molds containing organs and OCT into dry ice/ isopentane slurry till OCT turns white
9. Remove plastic molds with frozen organs from dry ice/ 2 methyl butane and let them sit for a minute or so on paper towels, snap off the plastic molds and place frozen blocks into labeled bags for storage in labeled boxes in the second container of dry ice for storage at -70°C.

References

1. Rosene DL, Roy NJ, Davis BJ. A cryoprotection method that facilitates cutting frozen sections of whole monkey brains for histological and histochemical processing without freezing artifact. *J Histochem Cytochem* 1986 Oct; 34 (10): 1301-15. doi: 10.1177/34.10.3745909.
2. Williams JM, Duckworth CA, Vowell K, Burkitt MD, Pritchard DM. Intestinal Preparation Techniques for Histological Analysis in the Mouse. *Curr Protoc Mouse Biol*. 2016 Jun 1;6(2):148-168. doi: 10.1002/cpmo.2.
3. Morton J, Snider TA. Guidelines for collection and processing of lungs from aged mice for histological studies. *Pathobiol Aging Age Relat Dis*. 2017 Apr 21;7(1):1313676. doi: 10.1080/20010001.2017.1313676. eCollection 2017.

Supplies

1. OCT Compound: VWR cat. #: Cat No: 25608-930
2. Vinyl molds (cryomolds): Simport Scientific Catalog M475-3 or 80872-490
3. Frozen sample write-on bags: VWR Cat. #: 01-002-37
4. 2-methyl butane (isopentane): Fisher Cat #: 03551-4

Fixing Tissues for Processing into Paraffin Blocks

1. Place THINLY sliced organs into properly labeled plastic cassettes, snap shut, and immerse into 10 volumes of fixative (10% buffered formalin).
2. If using skin, spleen, pancreas, or thymus: flatten between two pieces of sponge before fixing to ensure the correct orientation when it is time to embed into paraffin wax and to ensure flat sections.
3. Let the organs fix for at least 24 hours (may go longer).
4. Use zinc-containing fixatives or alcohol if there is a need for immunohistochemistry. INFORM THE CORE LAB OF THE DIFFERENT FIXATIVE THAT WAS USED or else the tissue will become too hard for sectioning if it stays in alcohol for days.
5. Deliver to core lab for processing into paraffin blocks.