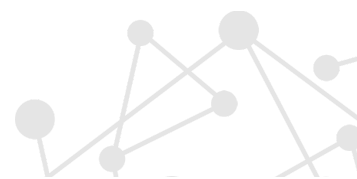


A. Tissue Preparation and cryopreservation with sucrose for Frozen/OCT Processing

- Perform all steps at 4°C, remove tissue from host and wash in cold phosphate-buffered saline (PBS).
- Tissue samples should be placed in fixative as soon as possible. Fresh 4% paraformaldehyde (4% PFA) at 4°C could be used for most routine paraffin processing at a ratio of 20:1, i.e. 20 mL to 1 cm³ of tissue at necropsy. Some tissue types such as lungs and muscle may require different fixatives and/or special processing (refer to Table 1).
- Large tissues should be trimmed no larger than 3-5 mm thick immediately after necropsy for proper penetration of fixative and placed in fixative for 24-48 hours at room temperature.
- Once tissue is fixed, transfer to PBS prior to shipment at 4°C. Cover sample containers with parafilm prior to prevent leakage. If tissue is being shipped in fixative, place samples in parafilm containers in Styrofoam or secondary containment for shipping.
- Tissue Trimming will be performed by Reveal Biosciences upon receipt of samples (as needed).
 - Standard trimming of rodent tissues will be performed by our histologists based on RITA and NACAD guidelines for organ sampling and trimming in rats and mice.
(https://www.niehs.nih.gov/research/atniehs/labs/assets/docs/q_z/revised_guides_for_organ_sampling_and_trimming_in_rats_and_mice_508.pdf)
 - Custom trimming can also be performed by our histologists. Please contact Reveal Biosciences if you have special trimming instructions.
- Cryopreservation with sucrose will be performed by Reveal Biosciences for fixed tissues prior to OCT embedding. The tissue is placed in 15% sucrose in PBS until tissue sinks, then transferred to 30% sucrose in PBS until tissue sinks, and followed by embedding tissue in OCT.

Table 1. Fixatives and/or special processing for some tissues.

Tissue	Special Processing Prior to Tissue Fixation	Fixative
Lungs	Inflate lungs for fixed and cryopreserve with sucrose for frozen/OCT processing (refer to section B)	4% PFA at 4°C, transfer to PBS to ship at 4°C
Lungs	Inflate lungs for unfixed, frozen/OCT processing (refer to section C)	
Muscle	Unfixed, frozen/OCT processing (refer to section D)	



B. Lung Inflation and Fixation for Frozen/OCT Processing

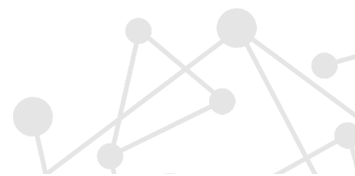
- Lung inflation is performed for best morphology, since it is challenging to obtain good frozen sections when sectioning collapsed lung tissue. Perform all steps at 4°C, perfuse the lungs gently through the trachea with fresh 4% paraformaldehyde (4% PFA) until the lungs are fully expanded to a normal level as expected to fill the chest cavity.
- Place sample in fresh 4% paraformaldehyde (4% PFA) at 4°C for 24-48 hours at room temperature.
- Once tissue is fixed, transfer to PBS prior to shipment at 4°C. Cover sample containers with parafilm prior to shipment to Reveal.
- Please contact Reveal Biosciences if you would like a consultation regarding sample prep.

C. Lung Inflation for Unfixed, Frozen/OCT Processing

- Lung inflation is performed for best morphology, since it is challenging to obtain good frozen sections when sectioning collapsed lung tissue. Perform all steps at 4°C, perfuse the lungs gently through the trachea with a mixture of 1:1 OCT/PBS until the lungs are fully expanded to a normal level as expected to fill the chest cavity.
- Place tissue flat on the bottom of the cryomold to keep tissues on the same plane.
- Gently fill the cryomold with OCT to avoid trapping in air bubbles especially near the tissue and completely to cover the entire tissue.
- Place the cryomold with tissue in OCT into a dry ice/isopentane slurry until OCT turns white.
- Remove excess moisture from frozen OCT block with paper towels.
- Frozen OCT blocks can be stored at -80°C. Ship in secondary containment on dry ice.
- Please contact Reveal Biosciences if you would like a consultation regarding sample prep.

D. Unfixed Muscle Preparation and Freezing for OCT Processing

- Remove excess moisture from muscle tissue sample with paper towels.
- Place tissue in the desired orientation in the cryomold.
- Fill the cryomold with OCT gently to avoid trapping in air bubbles especially near the tissue and completely to cover the entire tissue.
- Place the cryomold with tissue in OCT into a dry ice/isopentane slurry until OCT turns white.
- Remove excess moisture from frozen OCT block with paper towels.



- Frozen OCT blocks can be stored at -80°C . Ship in secondary containment on dry ice.
- Please contact Reveal Biosciences if you would like a consultation regarding sample prep.

