

Imaging Cores – Electron

Guidelines for TEM of Tissue Samples

Sample Preparation performed by investigator prior submission to the Core

Primary Fixation of Tissue Samples

- Tissues should be removed from the animal as quickly as possible postmortem and immersed in the primary fixative during dissection into small pieces (1 – 2 mm³).
- Fixation with glutaraldehyde 2.5 - 3 % (EM grade) freshly prepared in 0.1 M PIPES or PBS (pH 7.2 – 7.4) for 1 to 2 hrs.
- For brain tissue, use as fixative a mix of 2 % paraformaldehyde and glutaraldehyde 2.5 % in cacodylate, PIPES or PBS (pH 7.2 – 7.4) for 2 hrs, overnight or 24 hrs.
- Rinse 3 x 5 min with PIPES or PBS buffer.
- Submit the samples to the Core facility identified with permanent labels.

Procedure

- **Necropsy:** During necropsy, quickly cut off a small piece of fresh tissue several mm in thickness and hold them in clamps or carefully pin them in a cork or sylgard dish prior to primary fixation. Slice the tissue sample with a razor blade into pieces 1 mm. Determine if a long dimension is needed for sample orientation. Prepare 3 fragments per tissue sample. Pick up the 1-mm-thin slices of tissue by touching them with an applicator stick, and then place them into a 4 mL vials containing 1–2 ml of fixative.
- **Skeletal Muscle.** Cut long, thin superficial slices from muscles and hold them in clamps or carefully pin them in a cork or sylgard dish prior to primary fixation. Each sample should be flat embedded for longitudinal sections and/or transverse sections.
- **Heart Muscle.** The wall of a heart can be oriented as described for skeletal muscle. Attaching the sample to pins prior to primary fixation will prevent contraction. Orientation should be performed to obtain longitudinal and transverse sections.
- **Brain.** For Peripheral Nerves, orient long sections of tissue for transverse sections and for Central Nervous System, random orientation is usually adequate.
- **Kidney.**
- **Eyes.** For best fixation of eye tissue, the eye is incised on 1 side, top to bottom, midway between the cornea and optic nerve. The entire eye should then be quickly placed into a vial with 10X more fixative than the eye volume. Specific areas of interest should be dissected out after primary fixation, but before further processing since tissue blackening after osmication makes identification of specific areas in the eye difficult.