

## Preparation performed by investigator prior to sample submission to the Core Facility

### *Primary Fixation of Tissue Samples*

#### General guidelines

1. Tissues can be fixed in aldehydes by **immersion fixation** method using 2.5 % glutaraldehyde, or by **cardiovascular perfusion** with 2 % paraformaldehyde and 2.5 % glutaraldehyde. In both methods solutions are prepared in 0.1 M buffer (PIPES, PBS or cacodylate pH 7.2 - 7.4).
  2. 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 mm<sup>3</sup>).
  3. 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. or overnight at 4 °C.
  4. Rinse 3 x 5 min with PIPES or PBS buffer.
  5. Submit the samples to the Core facility in PBS and identify with permanent labels.
- **Brain:** Perform tissue fixation of fragments (1 - 2 mm<sup>3</sup>) or slices in a solution containing 2.5% paraformaldehyde and 2 % glutaraldehyde in 0.1M PIPES or PBS buffer pH 7.4 for 1 to 2 hrs. or overnight at 4 °C, and transfer to PIPES or PBS buffer.
  - **Heart:** Perform tissue fixation of stretched heart tissue fragments (1 - 2 mm) in a solution containing 2.5 - 3 % glutaraldehyde in 0.1M PIPES or PBS buffer pH 7.2 for 1 to 2 hrs. at 4 °C and transfer to PBS buffer.
  - **Skeletal muscle:** Perform tissue fixation of skeletal muscle tissue stretched in a cork or a sylgard dish with a solution containing 3.7 % paraformaldehyde, 3 % glutaraldehyde and 0.2 % tannic acid in 0.1 M PBS buffer pH 7.2 for 1 to 2 hrs. at 4 °C and transfer to PBS buffer.
  - **Kidney, Liver:** Perform tissue fixation of kidney or liver tissue fragments (1 - 2 mm) in a solution containing 3 % glutaraldehyde in 0.1M PIPES or PBS buffer pH 7.2 for 1 to 2 hrs. or overnight at 4 °C and transfer to PBS buffer.
  - **Eye:** After the enucleation, section the central cornea with a surgical blade and perform the fixation in a solution containing 2.5% paraformaldehyde and 2 % glutaraldehyde in 0.1M PIPES or PBS buffer pH 7.2 - 7.4 overnight at 4 °C and transfer to PIPES or PBS buffer.