Cincinnati Children's

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General tissue processing guidelines

- Trim tissue pieces to about the thickness of a nickel. Specimens for frozen sectioning should also be trimmed down to improve fixation.
- For tissue that has a capsule or outer layer (such as kidney), nick the outer layer so that fixative can deeply and properly infiltrate.
- Often times the entire organ is not necessary for sectioning and visualization. It is recommended to provide only a representative portion of the tissue in order to improve infiltration of the fixative and processing reagents.
- Cassettes and slides should be marked using alcohol/xylene resistant pen. Sharpies and other "permanent" markers will wash off during processing.

Tissue Fixation Conditions

Paraffin embedding:

Generally, tissues for paraffin embedding are fixed in 10% neutral buffered formalin for 24-48 hours at room temperature. Tissues can be submitted to the Core for processing after being placed into formalin. Prolonged exposure to formalin, and fixation at temperatures other than room temperature can lead to suboptimal morphology and issues with downstream applications. When considering antibody staining, researchers should consult the technical data sheet for the antibody to be used for staining in order to identify the proper fixative and fixation time.

Tissues for frozen sectioning:

Should be fixed in 4% paraformaldehyde (in 1X PBS) and stored at 4°C for 24 hours. The tissues can then be moved to 30% sucrose (in 1X PBS) and stored at 4°C. The tissues can be submitted to the Core in the sucrose solution. If snap frozen tissues are needed for your project, they should be frozen in OCT embedding medium immediately upon removal and submitted to the Core on dry ice. Please inform the Core staff in advance if you need assistance with this.

Tissues provided for Transmission Electron Microscopy:

Should be fixed in 3% glutaraldehyde in cacodylate buffer as soon as possible. It is recommended that the tissue pieces be cut to approximately one cubic millimeter for optimal results. And submitted to electron microscopy core for processing.