

Basic Sample Preparation for automated paraffin processing and embedding in the Experimental Pathology (Exp Path) Research Laboratory:

Before bringing your tissue samples to the Exp Lab, we recommend that you:

- Rinse at least 3 times for 20 minutes (15-20x volumes for all washes) in PBS after any formaldehyde fixation. Bouin's fixed tissues require more extensive washes.
- Dehydrate first in 50% ethanol for 30 minutes. Phosphate in PBS will sometimes precipitate in 70% EtOH, so this step is a safety measure. It is often skipped, but there is some risk.
- Dehydrate then in 70% ethanol for 30 minutes
- Bring tissues to Exp Path in 70% EtOH in processing cassettes in a sealed container (cassettes and screw-top containers are available at cost from the Resource Center [Cat. Info. below]). We can perform all of the above in Exp Path for a fee, if desired.
 - The ideal tissue storage solution after fixation -- but prior to paraffin processing -- is controversial. We currently recommend that tissues are stored in 70% EtOH at 4°, ideally in an explosion-proof refrigerator. The samples should be brought to the Exp Path lab for processing within one week, if possible. Extended periods of time in 70% ethanol may cause excessive "drying" of mouse tissues and may be detrimental for subsequent immunostaining for a limited number of antibodies. However, many research labs store tissues in 70% EtOH at 4° for months. In our experience, storage in 70% EtOH is less problematic than either over-fixing in formaldehyde solutions or excessive time in 95-100% EtOH or other organic solvents, such as xylene. For longer storage periods, the Loomis lab stores samples in PBS with a drop or two of PFA to prevent bacterial growth. This is not published but works well for rodent tissues, which can become excessively dry if stored in 70% EtOH for long periods.

IMPORTANT NOTES:

- REMEMBER LABELING OF CASSETTES MUST BE DONE WITH EITHER A #2 pencil or Superfrost marker, SO THAT THE LABEL DOESN'T WASH OFF IN ETOH AND XYLENE. SHARPIES WILL NOT WORK!!!!
- **Make sure that tissue samples are not so large that they become compressed in the cassettes**, as this will prevent adequate dehydration and paraffin infiltration. The tissues should be <4mm in thickness and able to move freely within the cassette.
- It is *critical* that tissues are fixed thoroughly before bringing to Exp Path for processing. Insufficient fixation leads to water retention in the tissue and incomplete paraffin infiltration (please see [Fixation Recommendations](#)). This cannot be undone.
- Screw-top specimen containers are available from Fisher in various sizes.
 - Fisher – 22-026-314
- Cassettes come in various sizes for different sized samples. We can provide at cost if you do not have in your lab.
 - Standard – Tissue-Tek 4119-01
 - Small – Fisher 15182702
 - Mega – Tissue-Tek 4173