ULAM IN-VIVO ANIMAL CORE
UNIVERSITY OF MICHIGAN

https://animalcare.umich.edu/business-services/vivo-animal-core

Fixation Tips

1. *Prompt fixation*- Tissues should be placed in fixative immediately upon collection.
    - Collect the tissues most sensitive to degradation first: ex. GI tract
    - If collecting from large groups of animals – euthanize them one at a time for tissue collection (do not euthanize the whole group first and then collect)

2. *Proper tissue collection size* – Tissue MUST be trimmed to appropriate size
    - 3-5 mm in width (~ thickness of a nickel)
        - Formalin only penetrates ~0.5 mm/hr. Large tissues will degrade before fixing.
    - No larger than 2 cm (postage stamp sized)
    - Tissue too large will degrade centrally and microscopic analysis will not be possible
    - Tissue too large will unevenly fix and affect downstream assays (i.e. IHC)

3. *Fixative Volume:* Use at least a fixative : tissue volume ratio of 20 : 1
    - Too little formalin – tissues will degrade and microscopic analysis will not be possible
    - Fix in WIDE-MOUTHED, FLAT-BOTTOMED jars: narrow neck vials make it hard to remove tissue, in cone-bottomed tubes the tissue will deform to the shape of tube
    - Pre-filled 10% neutral buffered formalin biopsy jars are available (ex. Fisher Scientific)

4. *Fixation Duration:* For immersion fixation in formalin, fix tissues for at least 24 hours
    - For standard histology, tissues can be left in formalin indefinitely
    - For immunohistochemistry, tissues should be switched to 70% EtOH after 48 hours

5. *Organ-specific tips*
    - GI-tract: Remove first (rapid degradation) and either infuse with formalin using a needle OR open the intestinal tract to allow formalin to penetrate.
    - Lung: Insufflate with formalin using a needle within the trachea (not the same needle use for the GI!). Fill to approximately the volume of the chest cavity (do not overfill).
    - Liver: Do not put an entire rodent liver in fixative (center will not fix). Separate the lobes for fixation and/or cut sections from larger lobes.
    - For mice found dead: DO NOT FREEZE mouse carcasses. Either necropsy immediately, place in 4°C for necropsy within 24 hrs, or at minimum open the thorax and abdomen to allow fixative penetration and place in a jar of fixative large enough to accommodate 15-20:1 volume ratio of fixative:tissue.

Which Fixative Should I Use?

For most routine histology we recommend ***fixation in 10% neutral buffered formalin for a minimum of 24 hours of fixation at 20:1 volume: tissue ratio.*** *10% NBF is the most commonly used cross-linking fixative and provides excellent tissue morphology. For histology the tissue can remain in fixative almost indefinitely before processing. For immunohistochemistry, transfer to 70% ethanol is recommended after 24-48 hours. Certain immunohistochemistry reactions are sensitive to fixation and must use alternative fixatives or fresh frozen tissues – this depends on the antibody. Please email us with your specific antibody target if you have questions.*

Commonly used fixatives for routine histology

*10% Neutral Buffered Formalin (NBF)**	*4% Paraformaldehyde***	*Others:*
COMMON USES: • Most common general use fixative for paraffin embedded tissues • Suitable for most routine immunohistochemistry with appropriate retrieval PROS: • Can be ordered as working solution and stored at RT CONS: • Degrades with age and produces formic acid	COMMON USES: • Short fixation or cryo-embedded tissues, in-situ hybridization • Certain neuroscience and immunohistochemical applications PROS: • Lacks methanol for methanol-sensitive procedures CONS: • Has to be freshly prepared • More $$ than 10% NBF	Glutaraldehyde: used for electron microscopy; NOT suitable for routine light microscopy (makes tissues dry and brittle) Davidson's fixative: suitable for eye fixation (special procedures apply) 70% ethanol: A precipitating (not cross-linking) fixative; gives inferior morphology for tissue evaluation; for immunohistochemistry tissues are often transferred to 70% ethanol after 24-48 hrs to avoid over-fixation

**Formalin is an aqueous solution of formaldehyde (which is a gas at room temperature). The traditional original stock solution of formalin was made at 37.5% concentration.*

"10% neutral buffered formalin" refers to a 1:10 dilution of this stock solution, so 10% NBF really equals 3.75% formalin! Nowadays 10% NBF is sold pre-made at the appropriate working concentration (3.75% formalin) so a) you don't need to dilute it and b) it is essentially the SAME concentration of formaldehyde as 4% paraformaldehyde!

***4% paraformaldehyde is made from polymerized (solid) formaldehyde that is dissolved with heat into aqueous solution. While it has approximately the SAME concentration of formaldehyde as 10% NBF (see above) it does not contain methanol. Methanol is typically added to 10% NBF as a stabilizing agent – formaldehyde breaks down over time to methanol and adding methanol at the beginning slows down this process. The lack of methanol in 4% PF makes it more suitable for certain applications but decreases its stability.*