GUIDELINES FOR FFPE TISSUE FIXATION

-22/09/2022-

Fixative

4% paraformaldehyde (PFA) or 10% neutral-buffered formalin (NBF). Fixative solution slowly degrades over time; thus **freshly prepared PFA or NBF is preferred**. Alternatively, commercial ready-to-use NBF can be used provided it is not too old/expired. Check pH in case of doubt; when PFA or NBF degrades, its pH will drop below 7.0.

Fixation

If possible, flush out blood cells from the circulation by cardiac perfusion with PBS.

If possible, perfuse the animal with fixative prior to tissue removal.

Harvest the tissue of interest immediately after euthanasia to prevent autolysis. Changes occur in tissues within minutes of death. If an animal dies and you cannot perform tissue collection right away, put the carcass in the refridgerator and collect the tissue as soon as you are able to.
Do not place tissue samples on an absorbent paper surface ! Dried-out tissue edges can create staining artifacts !

Wash tissue with PBS to remove any blood on the outside.
Intestine should be flushed with PBS using a syringe and blunt needle to remove fecal content.

Cut large tissue samples to a proper size using a sharp razor blade.
Diffusion of formalin into tissue is relatively slow (+/- 1 mm/hr);
FOR PROPER FIXATION, TISSUE THICKNESS SHOULD BE MAXIMUM 5 MM !
Most mouse organs except liver can be collected whole without having to cut them into smaller pieces.
If a tissue sample is too thick, the interior may not become fully fixed and autolysis may occur before the fixative diffuses to the interior of the tissue, potentially resulting in heterogenous staining !
Lung should be inflated with fixative.

Transfer tissue to a flat bottomed container with fixative and **FIX DURING +/- 24 HRS AT ROOM TEMPERATURE**. Avoid using 15/50 mL conical tubes for tissue fixation; if a tissue sits at the bottom of a conical tube without agitation during fixation, it will not have adequate fixative exposure.
VOLUME OF FIXATIVE SHOULD BE AT LEAST 20 TIMES THE TISSUE VOLUME.
If possible, apply **gentle agitation** during fixation (platform shaker or tube roller/rotator).
Avoid **overfixation** (> 72 hrs) as this could potentially lead to false-negative staining.

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After fixation

Quick rinse tissue in water.

Bone or mineralized tissue must be decalcified prior to paraffin embedding !

Transfer tissue to a **tissue cassette of the appropriate size**. Avoid squeezing the tissue into a too small tissue cassette to prevent pressure artifact !
Tissues can be combined in the same cassette but should be of the same density (f.i. heart, liver, spleen, kidney; not decalcified bone and lung). Avoid overloading of cassettes !
For small tissues, use cassettes with (very) small holes or use biopsy sponges or lens/biopsy paper to avoid sample loss during processing.
Cassettes with 4-6 separate compartments may be helpful to process different samples in one cassette while retaining their identification.

Label the tissue cassette with **pencil** or **solvent-resistant marker**.
Preferably, **use a unique number** (linked to a lab sample database) **to label your tissue cassette(s)**.

Transfer the tissue casette to a container with 70% ethanol.

Store at 4°C until paraffin embedding.

Drop-off samples for paraffin embedding with **Emmy (D032) or Ann (D261)**.

Never let tissue samples dry out during any step of the procedure !