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Histology and Histomorphometry Core

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4% PFA and 10% formalin fixative protocol

Warm up distilled H2O, 440 ml to 60°C, don't let it get up to 65°C. If it does DISCARD and start over. Dissolve 20 g paraformaldehyde in the water, add ~50 ul 10N NaOH.

After dissolving PFA, add 50 ml 10X PBS, pH7.4.

Check final pH, it should be 7.4 and total volume should be 500 ml. Store in 4°C.

Fixation Procedure:

This is the most important procedure in histology!!!

1. Place freshly dissected tissue in 4°C fixative; place fixative on an ice bucket, ASAP after harvesting, 20X the volume of the tissue or greater is required. Always dissect tissue, including bone in either buffer (ex. PBS) or fixative in 4°C condition to prevent drying and preserve tissue morphology.

2. Fixation time depends on tissue size, smaller tissues (ex. E10.5 mouse embryo) requires less time. Larger tissue (3 mm thick or more) needs to be fixed overnight or even longer. Fixation is best carried out in 4°C with shaking or agitation; sometimes, vacuum condition is needed for difficulty tissue.

3. After appropriate fixation, rinse in distilled H2O and submit to the Histocore in 70% ethanol (mix in distilled H2O).

Caution: Over-fixation will cause tissue hardening, and poor tissue sections, as well as loss of nuclear staining and damage antigenicity.

Under fixation will cause tissue to be too soft to cut and deteriorating tissue morphology.

-For formalin fixation use above procedure, but prepare formalin as follows: Mix10 ml formaldehyde (37-40%) in 90 ml of PBS and store in 4°C.