MASSACHUSETTS GENERAL HOSPITAL

Center for Skeletal Research

Histology & Histomorphometry Core

EMAIL: ENDOCRINEHISTOCORE@PARTNERS.ORG

Paraffin Tissue Submission Procedure

Follow Histology tissue preparation protocols below.

Fill out a Histocore Requisition Form online.

Use pencil to label cassettes with the identifier of the tissue sample. Other markers will be washed off during processing and your sample identification will be lost.

Rinse tissue with 70% ethanol. If your tissue has blood or anything else that discolors the ethanol, rinse repeatedly in 70% ethanol until clear. Since any particles will clog the processing machine, any sample submitted in 70%ethanol that is not clear will be returned to investigators for further washing.

Submit your tissue cassettes in a labeled container of 70% ethanol. Ethanol is mixed in distilled H2O. **Not** PBS…it will clog the processing machine.

Place container in the Histocore refrigerator on Their 11, along with a print out of the online requisition form. This refrigerator is to the R in the bathroom hallway opposite the elevators.

FIXATION OF SPECIMENS

This is the most important procedure in histology!

1. Always dissect tissue, including bone, in ice cold buffer (Saline or PBS) to prevent drying and preserve tissue morphology.
Place freshly dissected tissue in 4°C fixative in an ice bucket, ASAP after harvesting. Use a fixative volume at least 20X the volume of the tissue.
Tissue may be fixed in formalin (Mix10 ml formaldehyde (37-40%) in 90 ml of PBS and store in 4°C.) or paraformaldehyde (Warm up 440 ml distilled H2O to 60°C on a heated stir plate, don't let it get up to 65°C. If it does cool it to 60°C . Weigh 20 g paraformaldehyde (PFA) in a hood and stir in the prewarmed water on a heated hot plate in the hood. Don't let it get up to 65°C. If it does discard in appropriate chemical waste container and start over. Add ~50 ul 10N NaOH which will help the PFA dissolve. 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 overnight or freeze at -20 for up to 2 weeks.)

2. Fixation time depends on tissue size, smaller tissues (eg. E10.5 mouse embryo) require 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 conditions are needed for tissues such as lung.

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

MASSACHUSETTS GENERAL HOSPITAL

Center for Skeletal Research

Histology & Histomorphometry Core

EMAIL: ENDOCRINEHISTOCORE@PARTNERS.ORG

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 poor tissue morphology.
Do not try to fit large pieces of tissue into small cassettes, use larger cassettes.