Histology: Tissue Submission Procedure

- Obtain and fill out a Histology Core Request Form
- Follow Histology tissue preparation protocols (below)
- For formalin fixed tissue; submit cassettes in a labeled container of 70% alcohol and place in the Histology Core refrigerator (RM20350)

Labeling and Identification
- Label cassettes with the identifier of the sample tissue to be submitted
- Use Histology marking pens. Solvent resistant pens are available through the Histology Core. Other marking pens will wash off during processing and identification will be lost.

Filling out the Request Form, important information
- Sample ID
- Contact information
- Identify tissue source and type of tissue
- Indicate Principal Investigator/ Lab
- Fill out embedding, sectioning, and staining areas, and note any special instructions.
- PI signature and indicate the account number to be charged. No work will be performed without prior approval for use of account number.

External Client Tissue Submission

- Obtain and fill out a Histology Core Request Form
- Follow Histology tissue preparation protocols (below)
- Follow proper shipping guidelines. If tissue cannot be shipped in a cassette, arrangements can be made for Histology Core staff to prepare the samples as needed.

Filling out the Request Form, important information:
- Sample ID
- Contact information
- Identify tissue source and type of tissue
- Indicate Principal Investigator/ Lab
- Fill out embedding, sectioning, and staining areas, and note any special instructions.
- Provide proper billing information
- Provide return shipping billing information, or courier account number

For questions Contact:
Core Director: Volkhard Lindner M.D.,Ph.D (207)396-8143 volkhard.Lindner@mainehealth.org
Core Manager: Grazina Armie Mangoba HTL (ASCP) (207)396-8151 Grazina.Mangoba@mainehealth.org
Fixation Procedure

4% ParaFormaldehyde in PBS pH 7.4 (NFPA 3,1,0)  

<table>
<thead>
<tr>
<th></th>
<th>1000 ml</th>
<th>4000 ml</th>
<th>6000 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>DdH2O</td>
<td>870 ml</td>
<td>3480 ml</td>
<td>5350 ml</td>
</tr>
<tr>
<td>ParaFormaldehyde</td>
<td>40 gm</td>
<td>160 gm</td>
<td>240 gm</td>
</tr>
<tr>
<td>10 N NaOH - Sodium Hydroxide (fw 40)</td>
<td>0.1 ml</td>
<td>0.4 ml</td>
<td>0.6 ml</td>
</tr>
<tr>
<td>10X PBS stock</td>
<td>100 ml</td>
<td>400 ml</td>
<td>600 ml</td>
</tr>
</tbody>
</table>

ParaFormaldehyde is toxic. Use only under the hood with gloves, mask and lab coat. In a flask, first heat distilled water to 60°C maximum. While stirring, weigh out ParaFormaldehyde and pour it into the flask. Let almost completely dissolve while maintaining temperature. PFA breaks down at temps above 70°C. Add 2 drops of 10N NaOH per litre of PFA (or 0.04 gms of pellets/L). Cover and stir until completely dissolved. Remove from heat; add 10X PBS and pH to 7.4 with HCl. Q.S. to total volume with DdH2O.

10% Neutral Buffered Formalin pH 7.4 (NFPA 3,1,0)  

<table>
<thead>
<tr>
<th></th>
<th>100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>DdH2O</td>
<td>900 ml</td>
</tr>
<tr>
<td>Sodium Phosphate, monobasic (NaH₂PO₄*H₂O, fw-138)</td>
<td>4 gm</td>
</tr>
<tr>
<td>Sodium Phosphate, diabasic (Na₂HPO₄, anhydrous, fw-142.07)</td>
<td>6.5 gm (12.26 g of Na₂H PO₄*7H₂O)</td>
</tr>
</tbody>
</table>

pH should be 7.4 (adjust with 0.1M solutions of the salts, add monobasic for lower pH)

If 36% of Formaldehyde is used, the mix is adjusted to 111 ml of Formaldehyde and 889 ml of DdH2O.

NOTE: 10% Neutral Buffered Formalin is available through the Histology Core. Alternative fixative solutions and protocols are also available, contact Histology Core for questions.

Procedure:

1. Place freshly dissected tissue in fixative, 20X the volume of the tissue or greater
2. Fixation time depends on tissue size, smaller tissues (ex. embryonic kidney) requires only a few (4-6) hours of fixation. Larger tissue (4mm thick or more) may be left overnight in fixative. A minimum of 8-12hrs is recommended. Thick, fatty tissue requires even longer fixation times.
3. After appropriate fixation, rinse in water and submit to the Histology Core in 70% alcohol

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

Frozen Tissue

1. Embed fresh tissue in OCT compound and freeze in dry ice or at -20°C; Store at -70°C
2. If tissue is fixed, wash them in changes of 20% sucrose in PBS at 4°C until tissue sinks, usually overnight, embed in OCT.
3. Indicate to Histology staff if post fixation is desired
Decalcification of Bone for Histology Processing

**Chelator – EDTA (for IHC or Enzyme histochemistry, change solution every 2-3 days)**
Binds ionized calcium on the outer layer of the apatite crystal and the crystal is reduced in size as the process continues. Adjustment of the pH is essential. If the pH is too low it works only as a too-weak acid. If the pH is too high (above 8) decalcification is accelerated but alkalinity can be damaging to tissues.

- 5% EDTA at pH 7.0
- 10% EDTA at pH 7.0 – 7.4

**NOTE:** Acid decalcification protocols are also an option but not recommended for IHC. Procedures and recipes are available through the Histology Core

**Decal End Point Determination**
Manipulation – Probe with a needle and/or bend tissue to determine if tissue is soft enough to section. Over-decalcification will cause tissue or cells to lose affinity for certain stains.

**NOTE:** Make sure that solution is at least 20X the volume of the tissue to ensure proper decalcification

**Decal solution options**

**10% EDTA pH 7.2 – 7.4 Suggestion for Adult Mouse bones**
- EDTA Disodium Salt (Sigma E5134, mw 372.24) 50 g
- DdH2O 500 ml
- pH to 7.2-7.4 using 10 N NaOH

**10% Buffered EDTA pH 7.2 – 7.4**
- 1x PBS solution made with Na phosphates.
  - Na phosphate dibasic 6 g / L of DdH2O
  - Na phosphate monobasic 4.5 g / L of DdH2O
  - pH to 7.2-7.4
- EDTA Disodium Salt (Sigma E5134, mw 372.24) 100 g

To 700 ml of PBS, add 100 g EDTA and begin stirring. Adjust pH as needed to 7.2-7.4 by cautiously adding drops of 10N NaOH (or pellets). Cover and stir until completely dissolved. If you overshoot the pH (more alkaline that 7.4), adjust with Acetic Acid. After the EDTA is completely dissolved, QS to one liter with DdH2O.

**Procedure:**
Specimens can be decalcified in this solution over several days up to several weeks in a refrigerator at 4°C, depending on degree of mineralization and size of specimen.

1. Dissect bone and remove as much soft tissue as possible.
2. **After appropriate fixation,** wash tissue in distilled H2O.
3. Place tissue in 10%-15% EDTA solution at 4°C. This may take one week or more depending on tissue size. Use enough solution to saturate tissue.
4. Periodically check bone for adequate decalcification. Refresh EDTA solution after each check.
5. **Decalcification is complete when bone is soft and pliable. Check with Histology Core if unsure.**
6. Rinse with distilled H2O.
7. Submit to Histology Core in 70% alcohol.