

Part 1 - Histology Tissue Preparation Protocols

A. Fixed Tissues

TISSUE SIZE (for optimal fixation):

Embryos from ES 15.5 to P1 cut in half sagittally at midline (vertical cut dividing the brain into equal right and left halves.) **OR** Alternatively, cut the head off at the neck and then cut the body in two sections (transversal / horizontal) paying attention to cut below the diaphragm. The development of the skin and the development of the cartilage in the skull prevent efficient penetration with processing solutions. **Therefore, embryos > ES 15.5 should be cut prior to processing.**

Tubes: Tubes such as aorta intended to be embedded on end showing the lumen can be processed uncut to embedding size. At the embedding station, they can be cut to approx. 3mm in length and embedded on end. For example: an aorta 1.2cm in length would be cut into 4 pieces, 3mm long and embedded close together in a square on end like this (: :).

Other: Tissue **maximum thickness is 3mm** and length is 2.5cm. Tissues of approximately <= 1cm square are optimal for good sectioning. **At least one dimension should be no thicker than 3mm.**

Small Tissues: Wrap small tissues and small embryos in lens paper folded four times.

VOLUME: 20 times as much fixative solution as tissue. 1 cm³ = 1 ml, therefore this size tissue requires 20ml of fixative solution.

FIXATIVES: Do not expect to be able to do all procedures on tissues fixed in just one particular solution. In fact, for immunohistochemistry each antibody usually requires its own special fixation protocol. **There is no universal fixative!** Select the fixative appropriate for the application. The following is a list of common ones:

4% ParaFormaldehyde in PBS pH 7.4 (NFPA 3,1,0) - Time: 24 hrs at 4°C for routine Histology and IHC's

DdH ₂ O	850 ml	(alternate times dependent upon
ParaFormaldehyde	40 gm	size and application use)
10 N NaOH – Sodium Hydroxide (fw 40)	0.1 ml	
10X PBS stock	100 ml	

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.

Methyl Carnoy's fixative (NFPA 3,3,0) - Time: 3 – max 4 hrs room temp Use only glass or polypropylene tubes, **NOT polystyrene. WARNING! Fixative dissolves Histology Marking Pen ink on cassettes.**

Methanol	60 ml	(for regular Carnoy's, use absolute ethanol in place of methanol)
Chloroform	30 ml	
Glacial acetic acid	10 ml	

Bouin's Solution (NFPA 3,1,0) - Time: 6 - 12 hrs at 4°C

Saturated Picric Acid	75 ml
Formaldehyde, 40%	25 ml
Glacial Acetic Acid	5 ml

Red blood cells are lysed and the picric acid in the fixative demineralizes adult mouse bones in about 5-7 days and baby mouse bones in 2 days, so often a separate step of demineralizing tissue is NOT necessary. Remove Bouin's from tissues by washing them in plastic cassettes in several changes of 70% ethanol until no more yellow exudes from the tissue then store in 70% alcohol until processed.

FIXATION PROCEDURE: Fix tissues in appropriate fixative with occasional swirling. With the exception of Bouin's Solution (see above), the tissues should be washed with water or PBS and transferred to 70% ethanol upon completion of fixation. Store them at 4°C and process into paraffin blocks as soon as possible.

B. Frozen Tissue - If tissues are fixed, wash them in changes of 20% Sucrose in sodium phosphate buffer (PBS) at 4°C until the tissue sinks (usually overnight). Freeze tissue in OCT compound and section or store at -70°C.

Reference:

1. Carson, Freida, Histotechnology, A Self-Instructional Text, pg 19-24.
2. <http://wheat.pw.usda.gov/~lazo/methods/au/contents.html>
3. [www.hms.harvard.edu/pathol/ tsai/spg2004path211/BronsonNotes.doc](http://www.hms.harvard.edu/pathol/tsai/spg2004path211/BronsonNotes.doc), 9-19-05.
4. <http://www.bristol.ac.uk/vetpath/cpl/histfix.htm>, 9-19-05.

Part 2 - Histology Core Submission Instructions

Obtain a a) 'Histology Core Request Form' and follow the
b) 'Histology Tissue Preparation Protocols'.

Identification / Labeling: (ID numbers in this section are examples only.)

Each lab maintains a lab database or record system to give an identifying number to the sample to be submitted. Samples are submitted labeled with the initials of the investigator and the ID# of the sample.

For example: Mouse from Lindner lab database number 9999 would be labeled as ID# "vI9999"
Details such as age, genes, Tg, Mut, WT, fixatives used, etc. are all maintained in the lab database associated with the ID#. (The face of the blocks is reserved for the ID#. Additional information can be added to the slides / blocks when returned to the submitting lab.)

Request forms, containers, cassettes, slides and blocks labeled as example "vI9999".

If samples have multiple parts (i.e. kidney, liver, lung, etc. all from the same mouse), they can be referenced back to the lab database via an alphabetical character.

For example: from given example above

<u>Block/Slide label</u>	<u>Lab database reference</u>
vI9999-A	#9999-A liver
vI9999-B	#9999-B lung
vI9999-C	#9999-C heart
ID# - body part letter – block#	
vI9991-A1	#9991-A1 liver fixed in 4% ParaFormaldehyde
vI9991-A2	#9991-A2 liver fixed in Methyl Carnoy

IMPORTANT: Must use a Histology Solvent-Resistant Marking Pen (Ex. Surgipath 01880) to label cassettes because there is a risk of the processor washing off the labeling.

Scheduling:

Arrange with core tech. Samples dropped off when tech is unavailable should be placed in the walk-in refrigerator in the "Histology Specimens" bin for protection. Leave the request forms in the Histology room on the counter by the scope.

Tissue submission:

Submitting lab performs dissection and grossing. Fixed specimens, unless otherwise noted, are assumed to be at their end-point of fixation. Submit tissue in blocks, slides or cassettes labeled as described above. If cassettes are submitted, please note the container solution (Ex. fixative, alcohol, melted paraffin-in oven) and the preferred processing protocol (15 min, 30 min or 1 hr per station).

Size: Tissue maximum thickness and length is 3mm by 25mm. Tissues of approximately <= 1cm square are optimal for good sectioning. A good guide for maximum size is based on the size of the cassette itself: 1. The tissue should not touch the bottom and the cover of the cassette at the same time. 2. The tissue should not be longer than the width of the cassette.

Tubes: Tubes such as aorta intended to be embedded on end showing the lumen can be processed uncut to final embedding size. At the embedding station, they can be cut to approx. 3mm in length and embedded on end. For example: an aorta 1.2cm in length would be cut into 4 pieces, 3mm long and embedded close together in a square on end like this (: :).

Histology Core Request Form:

Fill in all Sample ID#, Contact Information, Tissue Submitted and check off which Principle Investigator Lab it came from. Then as appropriate, fill in the remaining embedding, sectioning and staining areas and note any special instructions. IHC's will be announced as they become available. **IMPORTANT:** Have PI sign it and indicate the account number to be charged. No work will be performed without prior approval and an account number.

NOTE: For trial staining that is not fully developed or functioning well, number of sections per block will be limited to a minimum number of slides to test the procedure with. After the tissue has demonstrated appropriate staining, further slides can be sectioned from the block. This will be mandated to reduce waste of sectioning time and materials.

Questions: Contact Core Manager: Kathleen Carrier, HT/MLT (ASCP) 885-8151, carrik@mmc.org
or Core Director: Volkhard Lindner, M.D., Ph.D. 885-8143, lindnv@mmc.org