

Standard Protocol for Formalin-Fixed Paraffin Embedded Tissue Samples

1. Harvest tissue and rinse in PBS to remove blood
2. Place tissue in at least 10 volumes of buffered formalin (3.7% formaldehyde:10mM phosphate buffer, ph-7.4) or buffered paraformaldehyde (3.7% paraformaldehyde: 10mM phosphate buffer, ph-7.4)
 - incubate for necessary fixation time
 - 1-2mm slices – 2-3h RT
 - 5-10mm thick – 5h RT
 - >10mm thick – 2-3h RT + 4°C O/N
3. Rinse tissue 1-2X with PBS, Store at 4°C in 70% ETOH in H₂O
4. Prepare tissue processing histology cassettes for samples



- Label PI name in pencil on side

- Label Date/Sample ID on front



5. Prepare transport/storage bucket with 70% ETOH in H₂O
6. Trim and transfer individual samples into histology cassette, clamp closed and immerse in the 70% ETOH in the storage bucket.

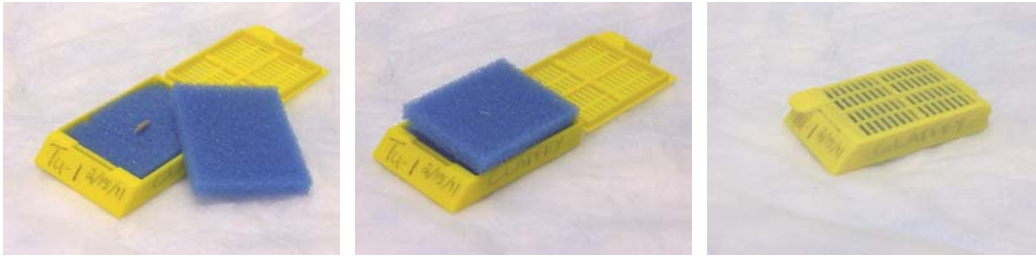
Notes:

- Samples should be placed in the cassettes in the same orientation that they need to be embedded. (i.e. the face of the tissue that needs to be cut first)



should be placed towards the bottom of the cassette)

- Do not allow individual samples to dry at all – one-at-a-time
- Multiple tissues can fit into one cassette
- Tissues should not be crushed, trim to fit in cassette used (multiple depth cassettes are available).
- Small tissue pieces < 1-2mm should be placed between blue sponges within cassette



7. Cover all cassettes with excess 70% ETOH and store at 4°C until submitted to core facility

8. Drop off samples at core facility (L-1017) and complete intake form:

Information required – PI, FRS/Grant Coding to bill to, contact person, contact Phone, email, processing, slides and stain request, specific requests (eg. We will embed tissues ourselves to assure specific orientation).



< Leave Forms Here

< Leave Samples Here

9. Histology Core Personnel will contact you when samples are ready or if PI does not have account available on Research Store Portal.

Materials and Purchasing Information:

<u>Item</u>	<u>Catalogue No.</u>	<u>Vendor</u>	<u>Price</u>
Standard Histology Cassettes (white)	22-272-416	Fisher	Case of 1000 for \$119.30
Histology Sponges (blue)	NC9518995	Fisher	Case of 100 for \$21.95
Formalin (5gal)	23-245685	Fisher	Each for \$123.68
Paraformaldehyde, 40%, 100ml	NC9810518	Fisher	Each for \$42.88
Tissue/Sample Containers (500ml)	02-544-226	Fisher	Case of 25 for \$112.59

RESEARCH HISTOLOGY WORK REQUEST

Date: _____

PI: _____

Name: _____

Phone Number: _____

FRS Number: _____

Email: _____

Services Requested:

Paraffin Processing and Embedding: Number of Samples _____

Paraffin Sectioning: Number of Slides per Block _____

 Number of Sections per Slide _____

H&E Staining: Number of H&E per Sample/Block _____

 Which Slide to Stain (first, last, etc...) _____

Special Staining: Type of Special Stain _____

 Which Slide to Stain (first, last, etc...) _____

How the cassettes are labeled: _____

Special Instructions or Comments-*please provide any additional details below:*

Any questions should be directed to the Histology Core Director:

Dr. Kevin Claffey

Phone: 860-679-8713

Email: claffey@nso2.uhc.edu