

Samples handling and fixation SOP for histopathology

1. Samples for paraffin embedding

1.1. Fixative: formalin

Tissue samples for routine histology should be fixed with 10% Buffered Formalin Phosphate (Fisher Scientific #SF100). Fresh unsqueezed tissues should be cut in a half (brain) and placed in fixative immediately after they have been removed from an animal. The fixative should be at least 10 times the volume of the samples and the size of the container appropriate to the specimen (i.e. no tissue squashing). Samples must be fixed 48H @ room temperature for half mouse brain (“hemibrain”), 4 days for full brain, if another type of tissue follow ratio 0.5mm/hour, but not less than 24 h. Check for clarity of formalin solution day to day. If solution is cloudy, change out the old solution with fresh, especially if tissues will not be processed immediately. Then samples should be rinsed in tap water for 20 min and thereafter stored in 70% ethanol at 4°C. This step will halt formalin fixation and acclimatize tissue to the first stage of the dehydrating process. However, prolonged exposure to 70% alcohol may have a drying effect on small specimens. You can either leave the samples @RT in formalin (as they will be processed within 14 days after delivery or alternatively transfer them to 70% and store them @4C. Do not over fix, as it can impair the downstream experiments.

1.2. Fixative: Carnoy's solution

100% ethanol	60 ml
Chloroform (Sigma #C2432)	30 ml
Glacial acetic acid (Fisher Scientific #351270212)	10 ml

Prepare just before use!

Fresh unsqueezed tissues should be cut in a half (brain) and placed in fixative immediately after they have been removed from an animal. The fixative should be at least 10 times the volume of the samples and the size of the container appropriated to the specimen (i.e. no tissue squashing). Samples must be fixed 2-4 hours (depending on size) at room temperature, 12H for full brain. Then Carnoy's solution is removed and replaced by 70% ethanol. Store at 4°C.

1.3. Fixative: Methacarn

100% methanol	60 ml
Chloroform (Sigma #C2432)	30 ml
Glacial acetic acid (Fisher Scientific #351270212)	10 ml

Prepare just before use!

Same procedure as for Carnoy fixative

1.4. Sample storage

Bring the infected samples in refrigerator (if samples in 70% EtOH) or under the hood (for samples in formalin) in room B21, non-infected samples from the clean side of the facility, in refrigerator (if samples in 70% EtOH) or room 214 on Hristina's shelf or on Hristina's bench (for samples in formalin).

Samples are processed twice a month.

2. Snap-freezing of tissue samples for histoblot

Fresh unsqueezed tissue is quickly rinsed with PBS (to remove blood), cut in a half (usually in a coronal plane), then put on dry ice until frozen. Snap-frozen tissue should be stored in container appropriated to the specimen (e.g. the plastic tube) in -80°C freezer (room B29). Please send an email to Hristina (gapeshin@ualberta.ca) to inform her that you have put new samples into the freezer.

3. Samples identification

Each sample should be labeled with the animal ID, type of fixative, type of tissue, orientation of cut (sagittal or coronal plane), initials of owner.

Put in a single tube the samples that you want embedded together (no more than 3 pieces).

NEW: Provide electronic document (“sample processing request form”, available on the CPPFD website) with relevant information concerning the samples (animal ID, type of tissue, fixative, your name, your PI), send it to Hristina_ (gapeshin@ualberta.ca) or Nathalie (daude@ualberta.ca).

Samples without proper ID will NOT be processed in a timely manner

5. Guideline for filling out sample processing request

-Animal type: mouse, hamster, rat...

-Tissue type: brain, spleen, heart, etc...

If you want different tissues embedded in the same block, please put them together in the same tube. If you required dedicated blocks per tissue sample, then please put each in separate tubes.

-Fixation: formalin, carnoy, methacarn, frozen, frozen-4%PFA....

-Orientation: sagittal, coronal, semi-coronal, horizontal

-Typically sagittal brain samples are mounted one half-brain per block (“hemi-brains”); for coronal sections, provide the full brain and specify how many thick sections you want made before further processing and microtome sectioning (we typically do 2 or 5 thick sections mounted in several blocks). Please let us know if you need anything processed differently.

-Frozen sections should be already cut

6. Services available (as of October 2013)

6.1 Staining:

-Hematoxylin and Eosin Routine staining to observe morphologic changes

- Nissl* (to observe Nissl substances in cell bodies of neurons and glia)
- Luxol Fast Blue* (to observe myelin sheaths; white matter tracts)
- Congo red* (to reveal fibrillar amyloid plaques)
- Thioflavine S* (fluorescent dye to visualize fibrillar amyloid plaques in formalin fixed tissue)
- Campbell-Switzer* (stains the broadest range of amyloid plaques in formalin fixed tissue)
- Orange G* (to differentiate cells in formalin fixed pituitary gland)
- Hoechst or DAPI* (routine fluorescent staining of nuclei)

5.2. Immunohistochemistry

- BrdU* (Thermo # MS-1058-B0)
- Calbindin* (Sigma D28K #C9848 in formalin and Carnoy's solution fixed tissue, Neuronal marker)
- NeuN* (Millipore # MAB377B) Neuronal marker
- GFAP* (BD #55630, Sigma #G3893 in formalin-fixed tissue, Inflammation marker)
- PrP^C* (SHA31 (Medicorp), SAF83 (Cayman #189765) in formalin and Carnoy's solution fixed tissue)
- PrP^C for histoblot* (2D6 (from H.Rezaei), SAF83 Cayman #189765)
- PrP^{Sc} for histoblot* (SAF83 Cayman #189765)
- Petblot* (PrPSc SAF83 Cayman #189765)
- PrP^{Sc}* (SAF83 (Cayman #189765 for mouse brain, 3F4 (Covance #SIG-39600) for hamster brain, 8G8 (Cayman A03220) and Bar224 (Cayman # A03211) mouse and deer Tissues, all in formalin fixed tissues)
- Tau* (AT8 Thermo # MN1020; AT180 Thermo # MN1040; CP13, PHF1 from Peter Davies)
- TH* (Tyrosine hydroxylase, Abcam [1b5] #49640 in formalin fixed tissue, Marker for dopaminergic and noradrenergic neurons)
- PRL* (Prolactin, National Hormone and Peptide Program AFP 879151 in formalin fixed tissue)
- GH* (Growth hormone, National Hormone and Peptide Program AFP5672099 in formalin fixed tissue)
- Shadoo* (06SH1 (DW lab), only in Carnoy's fixed tissue)

7. Filling out the Histopathology request form

Please fill the form with:

- The date of submission
- Your name
- Your email
- Your phone number
- The PI name

-The procedure:

One procedure, one antibody per request form

For example, if you need PrP^{Sc} with SAF83, and 8G8, and H&E staining: These are 3 requests.

-The path

To find the path# corresponding to your samples, go to the histology database, username:histo; password:histo

Enter your animal ID, “perform find” and you’ll find the path #.

If you do not find it, it means that your tissue hasn’t been processed yet. Try again one week later, as the tissues are embedded around twice a month.

Do not submit a request without the path number.

Maximum number of samples per request: 18

Please identify one sample for positive control and one sample for negative control where appropriate

If you need a procedure which is not listed in paragraph 4, or if you have any question, please contact Nathalie (daude@ualberta.ca)

Accession # is fill out by HG

Send the request to Nathalie (daude@ualberta.ca)

You will be informed by email when your experiment is ready