Molecular Detection Core
Tissue Processing

The keys to successful immunohistochemical detection are tissue fixation, specific antibodies and sensitive signal amplification. The antibody manufacturer will usually recommend a type of fixation and labeling protocol.

FIXATION

Perfusion and/or immersion fixation

In experimental adult animals, fixation is optimally accomplished by perfusing anesthetized rats or mice with heparinized saline followed by 4% paraformaldehyde. Perfusion with Bouins is difficult because of its acidity; however, it can be used as a post-fix following 4% paraformaldehyde perfusion of rodent brains.

Alternatively, small samples, such as mouse embryos, or rodent brains can be cut into multiple pieces (5 mm being the maximum thickness), rinsed quickly in saline solution then immersion fixed for 12-24 hours at 4°C in Bouins solution or 4% paraformaldehyde. Use 10-20 parts cold fixative to 1 part tissue and swirl the container several times after collecting the tissue to avoid sticking of the tissue to the side of the container.

Prolonged fixation may destroy the antigenicity of some antigens. Try to avoid over-fixation of the tissue.

The Molecular Detection Core is available to assist with a trial run to check for adequate fixation and processing before experimental tissue is collected.

We have found immersion fixation in cold Bouins gives the best results for our ISH protocols (no perfusion). Alternative fixatives, such as Methacarn or Carnoy's fixative may be preferable to aldehyde fixation in some applications.

PARAFFIN PROCESSING

Cassette preparation and processing

Label the cassette with a sharp No. 2 lead pencil or special solvent-proof histology marking pen (such as Secureline MarkerII/Superfrost or Shur/Mark). Ink from 'Solvent resistant' markers and Sharpies will dissolve during processing and make the samples unidentifiable. Only a 3-7 character identification label should be written on the front of the cassette, extra information for your use can be written on the sides of the cassette.

Tissue processing

After the appropriate time in cold fixative, the tissue is washed with several changes of 70% ethanol and trimmed to fit in the cassette. Five (5) millimeters (thick as a nickel) is the preferred maximum thickness. Tissues should be kept wet and the cassettes containing them should be stored immersed in 70% ethanol until processed.

See http://main.uab.edu/sites/ComparativePathology/105514/ for information on trimming tissue. *[Tip: It may be beneficial to include a small piece of positive control tissue in the cassette. Also a tissue array or the same tissue fixed in several fixatives (such as bouins and 4% paraformaldehyde can be embedded together for antibody screening.]*

The tissue processor dehydrates the tissue through multiple changes of graded alcohols, xylenes and paraffin. The cassettes are opened and the tissue is transferred to a mold, and filled with melted paraffin. The solidified paraffin block is cut as 3-5 micron thick tissue sections and tissue is picked up on plus coated borosilicate glass slides. [*Tip: Most manufactures of plus coated slides recommend using plain warm water in the flotation bath.*]

The slides are air dried and baked overnight. [*Tip: 6*0°C *for 1 hr if staining them immediately; 50 -* 55°C *for 3 - 6 hrs if staining them on the same day; 50°C if left overnight.]*

Tissue histology can be demonstrated by a routine Hematoxylin and eosin stain.

Protocol for routine H&E (hematoxylin nuclear) and eosin (cytoplasmic) staining:

1. Deparaffinize slides and hydrate to water.
2. Filter the Hematoxylin through a coarse filter paper to remove precipitates and the shiny film that forms on the surface. We use Harris modified Hematoxylin with Acetic Acid, mercury free (Fisher SH26-500D)
3. Place in Hematoxylin for 1 minute.
4. Wash well with tap water until rinse runs clear.
5. Differentiate in acid alcohol (0.5% HCl in 70% ethanol) for about 20 seconds.
6. Check under the microscope to see the cytoplasm has cleared. If the cytoplasm appears grey, repeat the acid alcohol step. The nuclei should remain colored.
7. Wash in tap water.
8. Blue by soaking the slides in ammonia water (1.5 ml NH_4OH in 500 ml distilled water) 2-5 minutes. This will turn hematoxylin the bright blue.
9. Wash well in running water. If washing is inadequate eosin will not stain evenly. (Note: Keep eosin glassware separate from IHC glassware since it will contaminate fluorescently detected slides.)
10. Stain with eosin for 30 seconds, agitating lightly.
11. Dehydrate in graded ethanols (95% and 100%) until excess eosin is removed (2 minutes each). Check under the microscope, repeat as necessary.
12. Wash with two changes of 100% ethanol, 3 minutes each.
13. Finish with two changes of xylene 5 minutes each.
14. Mount the cover glass using Cytoseal60, Permount or similar media.
15. Let air dry (1 hour or more).

Nuclei: blue with some metchromasia
Cytoplasm: various shades of pink identifying different tissue components.

Common fixatives for paraffin embedded tissue.

- 4% paraformaldehyde (in 0.01 M Phosphate Buffered Saline, PBS, pH=7.2),
- Bouins solution (15 parts saturated picric acid, 5 parts formalin, 1 part glacial acetic acid)
- Methacarn (6 parts Methanol: 3 parts Chloroform: 1 part Glacial Acetic Acid).

PROCESSING AND SECTIONING OF FROZEN SECTIONS:

Fixation of fresh frozen tissue:

- Precipitating fixatives:

  Acetone, methanol or ethanol: Used on fresh frozen sections after sectioning and air drying. Cryosections (5-10 micron) are placed in the fixative at -20°C for 10-20 minutes followed by air drying.

- Penetrating fixatives: Cold 4% paraformaldehyde or Bouins fixed (overnight at 4°C) and cryoprotected (30% sucrose in PBS for 24-48 hours at 4°C followed by sectioning in a cryostat at 5-10 microns.

[Tip: Prolonged freezer storage of frozen sections is not recommended because of tissue desiccation.]

See http://main.uab.edu/sites/ComparativePathology/105946/ for information on freezing tissues for cryosectioning.

See Cryotechniques for Light Microscopy © Woods and Ellis 2000 for more information.

Thank you for using the UAB Neuroscience Molecular Detection Core. Please give appropriate acknowledgement to P30 NS47466 in your papers.