Autofluorescence

Many mammalian tissues contain endogenous substances, such as lipofuscin in the retina, that auto-fluoresce. Additionally, aldehyde-based fixatives (formalin, paraformaldehyde, and glutaraldehyde) may alter tissues, such as brain tissue, and induce auto-fluorescence. For more details, see the following reference:

It may be useful to perform a preliminary examination of control animal tissues for auto-fluorescence. The tissues need to be processed (fresh frozen, fixed frozen, fixed paraffin-embedded) and examined via fluorescent microscopy (as would be done with experimental tissues) but without using any antibodies or fluorochromes; examining the tissues using multiple wavelengths of light is also recommended (see example below). Any detected auto-fluorescence indicates the need to either remove or reduce the auto-fluorescence or use different fluorochromes.

For example, formalin-fixed brain tissue (or collagen) will auto-fluoresce when exposed to light at a wavelength of 488 nm. In light of this, a good option would be to use a fluorochrome excited at a higher (or lower) wavelength rather than one excited at 488 nm.

Alternatively, if Glial fibrillary acidic protein (GFAP) production is being used as a tissue marker in formalin-fixed brain tissues, one could use an anti-GFAP antibody with a secondary antibody using a fluorochrome excited at a different wavelength of light (such as 555 nm), thereby avoiding the confounding effects of auto-fluorescence at 488 nm.

Below is one protocol to reduce auto-fluorescence in fixed brain tissue; other references are also listed below.

Sodium Borohydride Protocol

Following perfusion fixation with paraformaldehyde, perfusion with sucrose, and storage in sucrose (see reference listed below for protocol details, which may require modification to optimize results for different tissues or conditions):

1) Brain tissues were frozen and cryo-sectioned.
2) Free-floating tissue sections were immersed in Sodium Borohydride (NaBH₄, 0.1-2.0% in 0.1M phosphate buffer solution at a pH 7.4 (PBS) from Sigma) for 3 minutes to 4 hours. (Immersion in NaBH₄ was carried out in reduced light conditions, on a shaker table, under a fume hood. NOTE: NaBH₄ solutions produce hydrogen gas, which may be explosive.) In some cases, the NaBH₄ solution was replaced with fresh solution after an initial 10-20 minute soak.
3) Tissue sections were then rinsed several times in PBS.
4) Tissue sections were briefly rinsed in dilute (1mM) phosphate buffer and then cold PBS.
5) Tissue sections were mounted onto gelatin coated slides, dehydrated and cleared.

Review of methods to reduce autofluorescence:


Sudan black has also been used to reduce autofluorescence, but some report a black outline in tissues:


A simpler method has been proposed to reduce brain and liver autofluorescence: