Wax Embedding Protocol

Using Paraplast X-tra from Fisher Scientific

1.Following sacrifice of the animals, fix the tissue in 4% paraformaldehyde in 0.1M sodium cacodylate buffer (pH 7.4) for 24 hrs at 4ºC. Can fix for as little as 2 hrs if epitope is fixation sensitive. (Can also use PBS buffer). For larger eyes, remove the anterior structures (e.g. cornea and lens) prior to fixation. For smaller eyes, a slit in the cornea is usually adequate for penetration of the fixative.

2. Following fixation, cut eye into quadrants (for larger eyes such as the cat). For smaller eyes either cut off the anterior structures and embed the eyecup, or bisect the eye in half so that the wax and solutions will penetrate the globe. We process our tissue in small Wheaton glass vials with snap caps (from Fisher Scientific).

3. Rinse tissue 2x20 min in 0.137M $NaPO_4$ buffer, keeping the vials at 4ºC during the rinses.

4. After rinsing, dehydrate the tissue in a graded ethanol/water series: 15%,30%, 50%, 70%, 85%, and 95% for 10 minutes each then place in 100% ethanol for 2 changes of 10 minutes each (all solutions should be 4ºC).

5. Replace ethanol with 100% toluene for 2 changes, each 15 min at room temp. At this time, make a solution of 2:1 toluene:paraplast to be used in the next step, keeping in mind that any step requiring Paraplast must be done at 60ºC to keep the wax from hardening.

6. Remove 100% toluene and add 2:1 toluene:paraplast for 30 min at 60ºC. During this incubation make 1:2 toluene:paraplast.

7. Remove the 2:1 mixture and add 1:2 toluene:paraplast for 30 min at 60ºC.

8. Remove toluene:paraplast mixture and add 100% melted paraplast overnight at 60ºC.

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9. Remove the Paraplast and add fresh 100% paraplast for 2 hrs at 60ºC.

10.Transfer tissue to small aluminum pans (labeled appropriately) filled with 100% paraplast. Place pans on a warming plate to keep the wax from melting until the tissue is oriented correctly. Once the tissue is in the pan and oriented, set it aside to harden undisturbed for at least 30 min. At this time place the pans in an ice bath for at least one hr.

11.Cut out blocks of wax containing the tissue and mount on wood dowels that fit into microtome chucks. Section at 4 micron (or more).