Immunocytochemistry for Confocal Microscopy
I. Agarose Embedding

1. Rinse the tissue. Entire mouse eyecups can be embedded and sectioned. Alternatively, half of an eyecup or a 3-4 mm piece of retina from larger animals can be used. Rinse the tissue in cold (4°C) 1xPBS (pH = 7.4) if the tissue was stored in fixative.
   - 3 15min rinses
   - 1 1hr. rinse.

2. Melt the Agarose: Place the solid agarose (that is in a plastic 50 ml tube) in a beaker full of water, with the tube lid loose. Put the beaker in the microwave for 2 min (or until melted).

3. Insert a thermometer directly into the melted agarose and transfer the agarose tube to a second beaker filled with room temperature water to slowly cool the agarose. Stir agarose.

4. Allow the agarose to cool to 44-45°C (any lower and the agarose will harden and hotter temperatures may cause tissue autofluorescence).

5. Embed the tissue in an agarose block by pouring the agarose into a small plastic weigh boat, then transfer the tissue from PBS to liquid agarose and orient it (as shown on the next page in final form) using a spatula.

   (Note: Ideally the tissue should be put in liquid agarose at 40-42°C.
   If you pour at 44-45°C, a few seconds of cooling in the boat is all that is needed.)

   When the tissue is on the spatula use a Kimwipe and CAREFULLY wipe around the tissue to remove excess buffer. It's a good idea to swirl/stir the agarose immediately after placing the tissue into the agarose to mix in any buffer that might still be on the tissue. By doing this you get a better adhesion between the tissue and the agarose. Also, when you first put the tissue in agarose, watch it for 1-2min to make sure the tissue does not move. If it does, reposition it quickly with a spatula.

6. Harden the agarose. Place the tissue-agarose block in a Petri dish on a wet paper towel making sure there is ample water for the block to keep hydrated. Cover the dish with plastic wrap and store at 4°C for 30 min to 1-hour minimum to harden (it can be kept overnight or longer as long as the block is well hydrated).

7. When ready to section, remove the hardened block of agarose from the plastic cup (by sticking a razor blade between block and plastic cup). The block should come out easily (see picture below).