

Antibody Staining and Embedding of Adult Eyes (From RON)

I. Dissect eye

Decapitate fly and then cut one eye off leaving the eye of interest attached to the proboscis.

II. Fix for **1 hour** in 0.8% glutaraldehyde in PBS.

Fix heads in dissection wells covered with filter paper at room temperature.

NOTE: We use Whatman Glass microfibre filters for this. At this point, it's worth taking extra care to see that the heads are under the meniscus and that there are no air bubbles trapped under the filter. If you don't do this right, the eyes will not get fixed properly and may have very bad morphology.

III. Continue dissection

In order for the antibody to penetrate the retina, it is necessary to dissect it out of the surrounding cuticle. Now that the tissue has fixed for an hour it will be easier to do this as the retina is more resilient and will not tear or shred as easily. I do this step in a drop of fix on top of a silgard plate. The plate doesn't have to be silgard but having plastic instead of glass sure does spare those fragile tips of dissecting forceps. I usually start by plucking out most of the brain so that I can see the retina more clearly. The retina is attached to the shell of lenses primarily at the edges of the eye. So, in order to remove the retina from that shell, I grab hold of the cuticle near the edge of the eye with two different forceps and then carefully rip them apart from each other. If you are lucky, the shearing force from this will pop the retina out of the cuticle. If you are unlucky it will only pull a small section of lenses off the retina or even only tear a chunk of head cuticle off. It helps to grab the cuticle right at the junction between the lenses and the head cuticle. You may damage a few of the outer ommatidia but that's ok because there are many more, and it's an effective way of getting rid of large chunks of cuticle. You can also work your way around the edge of the eye, damaging the retina enough to loosen that contact with the lenses. Continue dissecting until all of the cuticle is gone. If the retina happens to rip in half, or into smaller pieces, **don't worry**. Save those pieces and carry them through the staining reaction. You see, even if you are able to perfectly remove the cuticle, leaving the retina in one piece, you will have problems with penetration of your antibody because the retina is so thick. So, if you do have an entire retina you will have to take the edge of a fine needle and use it to slice into your retina to increase the permeability (like slicing a pie)

If you don't do this you will only get strong staining at the edges of the retina and hardly any staining at the center. The trick is to slice into the retina often enough to get good staining all over, and yet not too much so that you will still have big enough groups of undamaged ommatidia between the slices to take pictures of.

After this dissection I transfer the retinas/retina fragments into a basket made out of an eppendorf tube, which is sitting in fixative (in one of the wells of a 24 well tissue culture plate) and allow them to fix for another 30 min.

If you haven't used baskets like this I would highly recommend it. They allow you to transfer lots of tissue from soln to soln without damaging anything. Furthermore, the wells in those plates hold about 2 mls of solution which is about the right amount for all of the washes.

IV. Rinse **3X10 minutes** in PBS.

V. Incubate in primary antibody overnight at 4 degrees C.

I do this in PBS with 0.4% NP-40 and 4% Goat serum when staining with Rabbit gal. Of course, **you will need to change both the fix and the detergent** to things that are compatible with the antibody that you are using. Gluteraldehyde is a nice, strong fix, but it tends to destroy a lot of epitopes. If your antibody is sensitive then you should probably use something like PLP, which is far more gentle.

VI. Rinse **3X10 minutes** in PBS with detergent and goat serum.

Just use the same solution that you diluted the antibody in for these washes.

However, its important to make these PBS solutions fresh each day. Do not use day old PBS.

VII. Incubate in secondary antibody for **2 hours** at room temperature.

Dilute the secondary in PBS with detergent and serum just as you did the primary. I have only done this with a secondary conjugated to HRP.

VIII. Rinse **3X10 minutes** in PBS (this time **without** detergent or serum).

IX. Do the color reaction.

X. Rinse **10 minutes** in PBS.

XI. Dehydrate in alcohol solutions (EtOH)

50% for **10 minutes**

70% for **10 minutes**

90% for **10 minutes**

100% for **10 minutes**

100% for **10 minutes**

After this step you will have to move the retinas from the baskets into actual eppendorf tubes (they will just settle to the bottom). If you don't do this and try instead to do the propylene oxide incubation in the 24 well plates, you will find that propylene oxide dissolves the plastic in those plates, making a sticky mess around your retinas. Propylene oxide will not dissolve the eppendorfs however. So, to transfer them, I just invert the basket over an eppendorf and then squirt 100% EtOH through the basket into the eppendorf with a pipet. This will flush the tissue down

into the eppendorf. Then, after the tissue has settled to the bottom, I carefully draw off the 100% EtOH and add the propylene oxide.

V. Clear in 100% propylene oxide for **2X10 minutes**.

VI. Infiltrate in 1:1 propylene oxide/plastic mixture for **overnight**.

This is still in the eppendorf tube. Just make sure to close the cap, otherwise the propylene oxide will evaporate overnight and you will have a hard time getting your retinae out.

VII. Dessicate in pure plastic **3 hours**.

Make this plastic fresh.

VIII. Embed as above.

Make this plastic fresh. Without the cuticle surrounding the retinae, it is much easier to position them tangentially to the plane of sectioning. We just use beam capsules: