**Protocol for DAB staining (floating sections)**

* Enclose brain in PBS + 4% agarose

→ Use microwave to boil the mix PBS + agarose

→ Let it cool a little bit

→ Enclose the brain

* Cut 30-40 μm sections on a vibratome

→ Use glue to fix the agarose block on the platform

→ Use PBS and ice eventually

* Collect sections into eppendorf tubes (or multi-well plates) containing cryobuffer

Cryobuffer (500 ml):

250 ml PBS

150 ml ethylen glycol

100 ml glycerol

0.5 g sodium azide

* Store sections at 4°C (the cryobuffer is important if you want to store sections for long storage. If you want to use them in the following days, PBS is sufficient)
* Wash your sections in PBS (to remove glycerol, etc.)
* Deactivate endogenous peroxidases by incubating with PBS + 3% H2O2 for 15 min with gentle agitation
* Wash (3 x 10-15 min) in PBS + 0.2% Triton with gentle agitation
* Incubate the sections in blocking buffer (PBS + 0.2% Triton + 5% serum) for at least 1h at RT with gentle agitation
* Incubate the sections with the primary antibody at 4°C overnight with gentle agitation (or 2h at RT if needed)
* Wash (3 x 10-15 min) in PBS + 0.2% Triton with gentle agitation
* Incubate the sections with the secondary antibody biotinylated (anti-mouse biotinylated if primary antibody is a mouse) between 1 or 2h at RT
* Wash (3 x 10-15 min) in PBS with gentle agitation
* Use the ABC kit ([**https://vectorlabs.com/vectastain-abc-kit-standard.html**)](https://vectorlabs.com/vectastain-abc-kit-standard.html))
* Wash (3 x 10-15 min) in PBS with gentle agitation
* Use the DAB kit ([**https://www.seracare.com/products/kpl-immunoassay-reagents-and-kits/immunohistochemistry/dab-reagent-set/**](https://www.seracare.com/products/kpl-immunoassay-reagents-and-kits/immunohistochemistry/dab-reagent-set/))
* Stop the reaction with by incubating the sections in H2O
* Mount the sections on slides ([**https://www.fishersci.com/shop/products/fisherbrand-superfrost-plus-microscope-slides-2/p-45174**](https://www.fishersci.com/shop/products/fisherbrand-superfrost-plus-microscope-slides-2/p-45174)) in PB (or PBS but the slides will be more clean without salt)
* Let the slides dry (a few hours under the hood or overnight in a drawer)
* Dehydrate the sections with ethanol series + xylene

Dehydratation steps:

→ EtOH 50% → EtOH 70% → EtOH 95% → EtOH 100% → EtOH 100% → Xylene → Xylene

2-3 min each step

* Cover with mounting medium and a thin glass coverslip ([**https://www.fishersci.com/shop/products/fisherbrand-cover-glasses-squares-8/p-45512**](https://www.fishersci.com/shop/products/fisherbrand-cover-glasses-squares-8/p-45512))

Comments:

- Read the datasheet for the dilution of the primary antibody (1/500-1/1000 for the AT8)

- Usually secondary antibodies are used at 1/2000 (for fluorescence as DAB)