Iba1 Immunohistochemistry Protocol

Mia Till, adapted from Hannah Reid's Protocol
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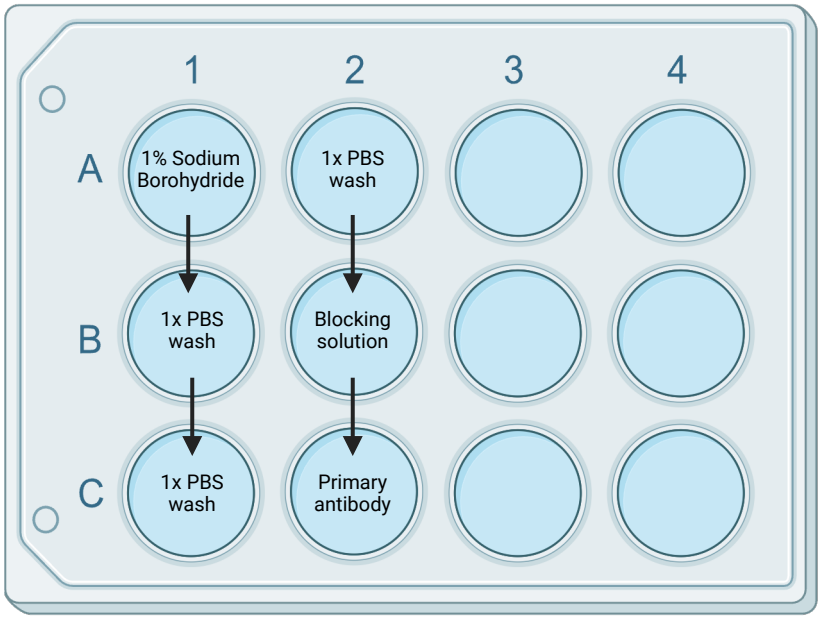
What is Iba1?
Iba1 (ionized calcium binding adaptor molecule 1) is a protein expressed in microglia, which are the resident immune cells of the brain. Iba1 is upregulated when microglia are activated, which can be used to examine disease states of the brain, as well as homeostatic microglial function.

Note: Iba1 can be used for either chromogenic or fluorescent staining. Day 1 of the protocol is the same but Day 2 differs.

Day 1



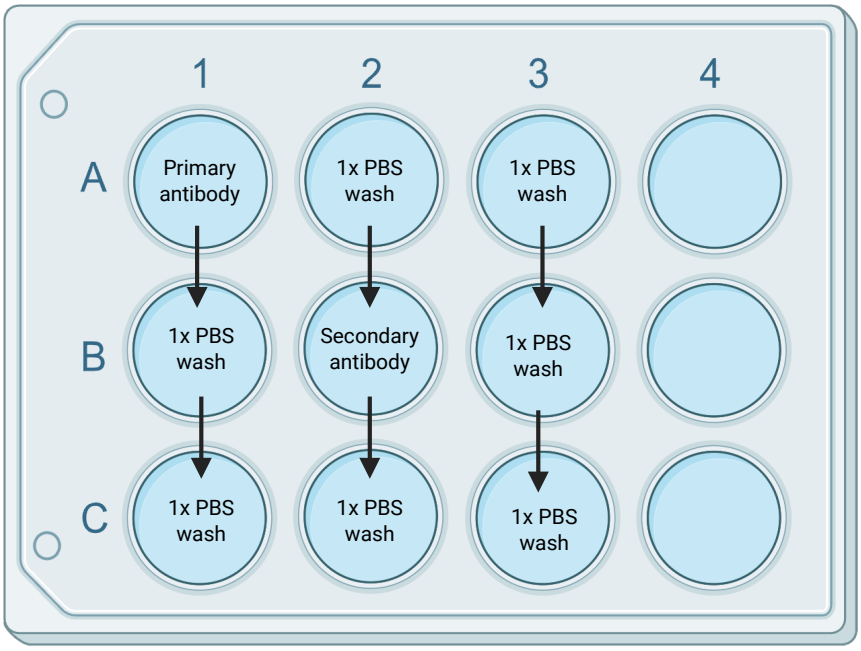
1. Antigen retrieval may be necessary for newer tissue. If needed, incubate the tissue in 1% sodium borohydride for 3min at RT while shaking.

2. Transfer the tissue into PBS and complete 3x 5min PBS washes at RT while shaking.

3. Prepare the blocking solution by combining 3% NGS in 0.5% Triton X-100 in PBS). Transfer the tissue to the blocking solution and incubate for 2hr at RT while shaking.

4. Dilute the primary antibody (Wako rabbit anti-Iba1) to a 1:1000 dilution in the blocking solution. Transfer the tissue into the diluted antibody and place it on a shaker at 4°C for 48hr.

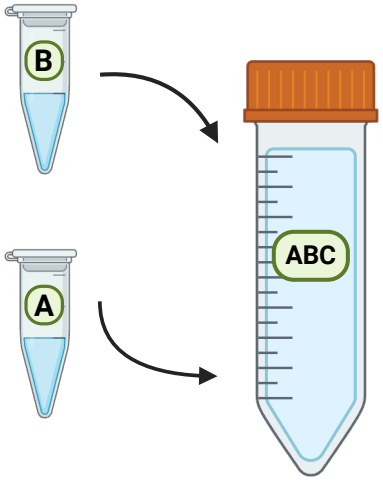
Day 2 - Chromogenic



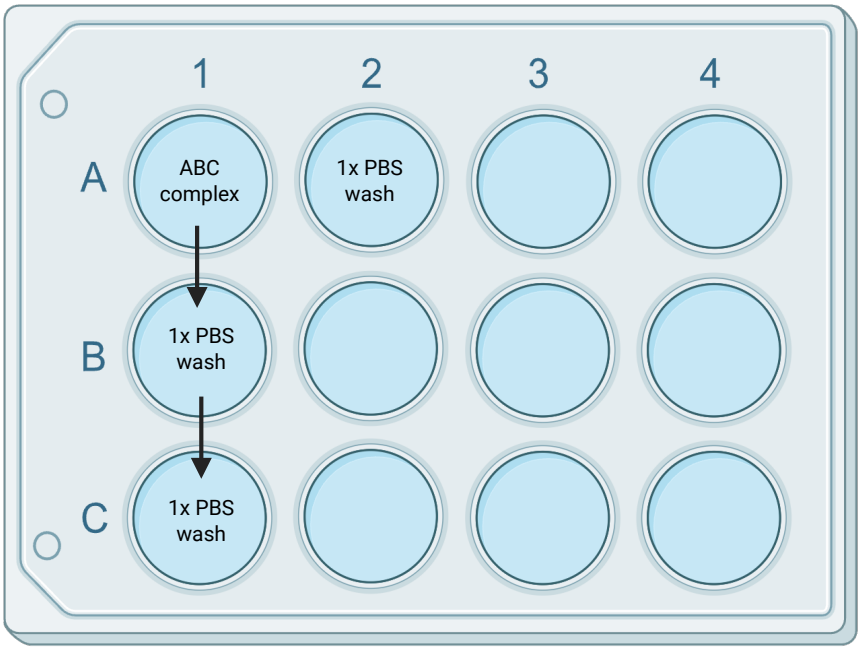
5. Transfer the tissue from the primary antibody into 3x 5min PBS washes at RT while shaking.

6. Dilute the secondary antibody (Thermofisher biotinylated goat anti-rabbit) to a 1:500 dilution in blocking solution. Incubate the tissue in the secondary antibody for 2hr at RT while shaking.

7. Transfer the tissue into 3x 10min PBS washes at RT while shaking.



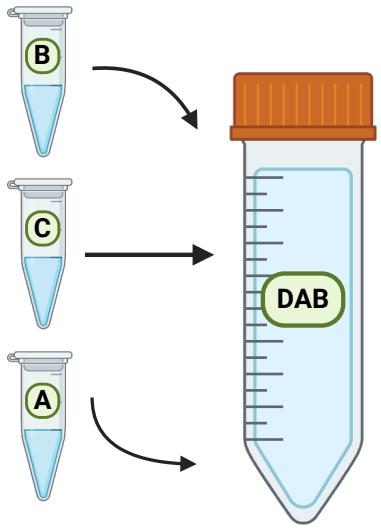
8. After putting the tissue in the first PBS wash, make the ABC complex (Vectastain kit) by combining 2 drops of Reagent A and 2 drops of Reagent B per 5ml of blocking solution. Allow the ABC complex to sit for 30min before use.



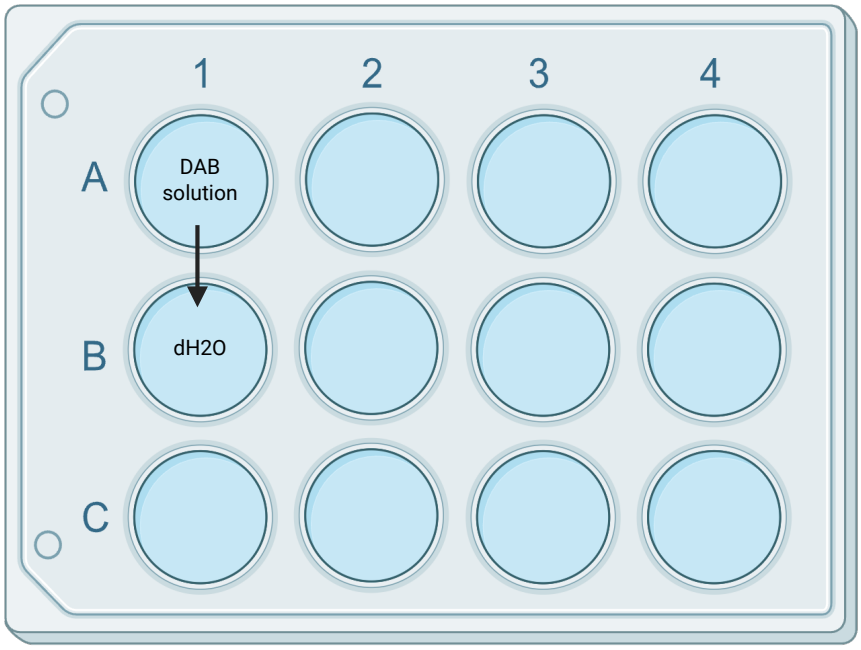
9. Transfer the tissue to ABC complex (1ml per well) and incubate the tissue for 30min at RT while shaking.

10. Transfer the tissue to PBS for 3x 10min washes at RT while shaking.

DAB is a mutagen and carcinogen. Use proper PPE and work in a fume hood while using it.

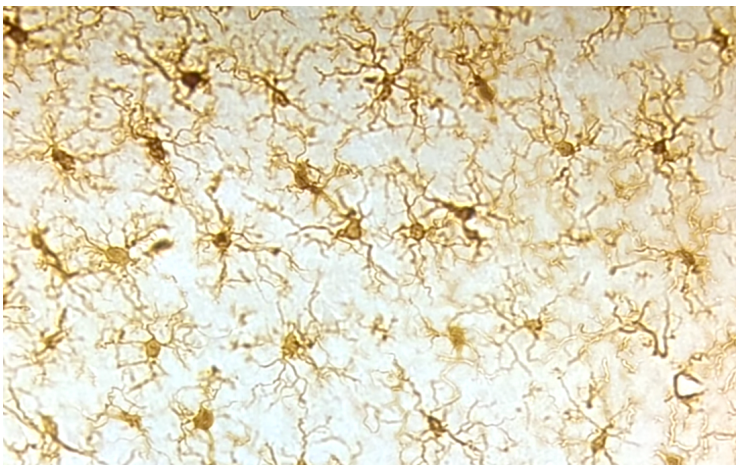


11. Prepare the DAB solution by adding 2 drops of Reagent A, 4 drops of Reagent B, and 2 drops of Reagent C per 5ml of dH2O.



12. Add 1ml of DAB solution per well. Wait 1-5min for the tissue to change colour to a toasty brown.

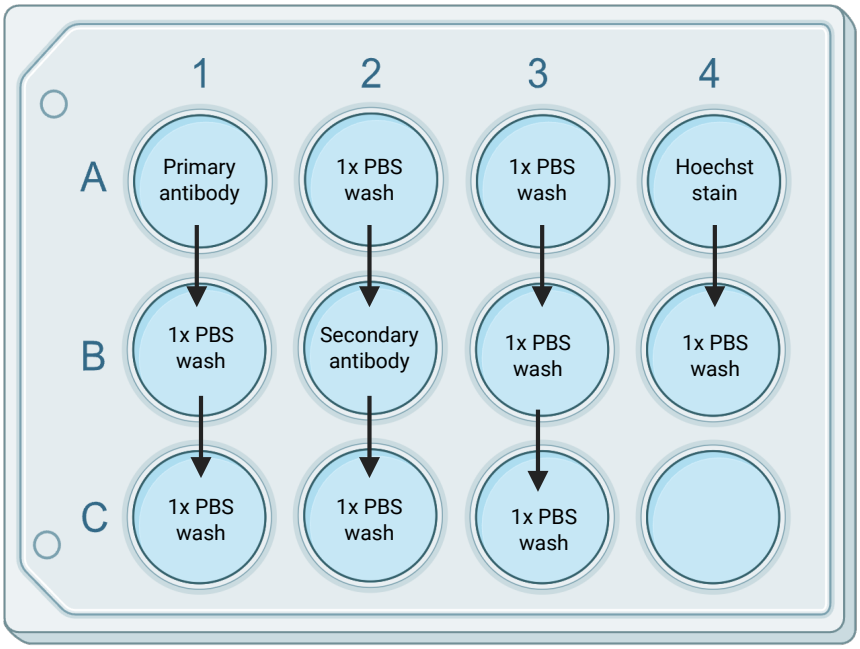
13. Immediately after development, transfer the tissue to dH2O and let it rest for 5min.

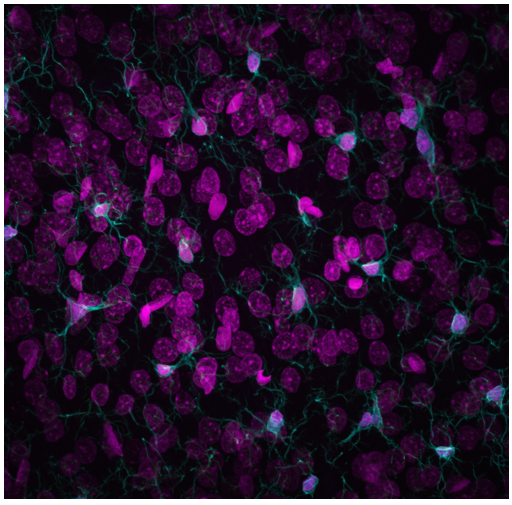


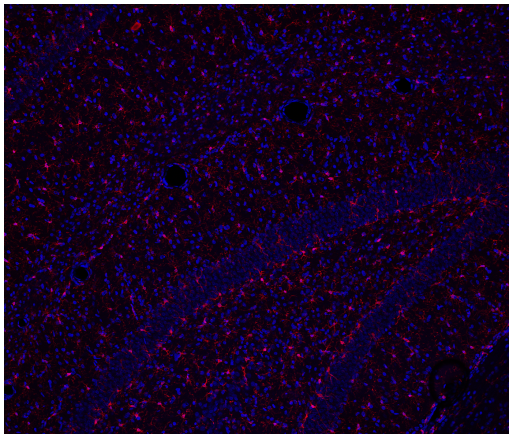
14. The tissue can now be mounted, cover slipped, and imaged under the brightfield microscope once dry.

Day 2 - Fluorescent

The secondary antibody used for fluorescence is light sensitive. You should work with the lights off and keep the tissue under tinfoil once the secondary is used.







5. Transfer the tissue from the primary antibody into 3x 5min PBS washes at RT while shaking.

6. Dilute the secondary antibody (anti-rabbit Alexa Fluor 488) to a 1:500 dilution in blocking solution. Incubate the tissue in the secondary antibody for 2hr at RT while shaking.

7. Transfer the tissue into 3x 10min PBS washes at RT while shaking.

8. Use Hoechst to stain the cell nuclei. The exact dilution and incubation time of the Hoechst will depend on the age of the original solution.

9. Transfer the tissue to one 5min PBS wash at RT while shaking.

10. Mount and cover slip the slides using fluoromount. Image the slides once dry using the confocal microscope. Note that you must be trained prior to using the confocal.