Immunofluorescence Staining SOP

1. Dewax the slides through two buckets of dewaxing Xylene, for 5 minutes each
2. Rehydrate the tissue through an ethanol series (100%, 100%, 90%, 70%), for 5 minutes each. Place in dH2O for 5 minutes.
3. Perform an appropriate antigen retrieval, then cool in an ice bucket until it’s at RT. Perform a dH2O wash for 5 minutes, followed by a 1xPBS wash for 5 minutes
4. Draw a hydrophobic barrier around the tissue, then apply a 10% animal serum solution diluted in Primary Diluent (from the animal the secondary antibody is made from). Then incubate for an hour at room temperature.
5. Apply the primary antibody, at the appropriate concentration diluted with 10% animal serum solution, to the tissue. Then incubate overnight at 4°C
6. Wash the slides in 1xPBS for 5 minutes, repeat twice.
7. Apply the secondary antibody at a concentration of 1:1000 in 1xPBS, then incubate the slides for an hour in the dark
8. Wash the slides in 1xPBS for 5 minutes in the dark, repeat twice.
9. Apply enough Sudan Black (1% in 70% ethanol) to cover the tissue, incubate the slides at room temperate in the dark for 10 minutes
10. Wash the slides in a bucket of dH2O for 5 minutes in the dark, repeat twice.
11. Mount the slides with ANTIFADE w DAPI, then allow the slides to dry for 10 minutes in the dark. Apply clear nail varnish to seal the coverslip, store at 4°C in a dry box in the dark.

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| Antibody | Antigen Retrieval | Primary Antibody Concentration | Secondary Antibody Wavelength |
| GFAP | Citrate buffer in a steamer for 20 minutes | 1:1000 – Rabbit (Dako) | 647 wavelength – far redRabbit made in Goat |
| Iba1 | Citrate buffer in a steamer for 20 minutes | 1:1000 – Rabbit (Dako) | 568 wavelength – redRabbit made in Goat |
| Aβ | Citrate buffer in a steamer for 20 minutes | 1:1500 – Mouse (Dako) | 555 wavelength - orangeMouse made in Goat |
| Tau | Citrate buffer in a steamer for 20 minutes | 1:200 – Mouse (Dako) | 555 wavelength - orangeMouse made in Goat |
| CD31 | Citrate buffer in a steamer for 20 minutes | 1:250 – Mouse (Dako) | 555 wavelength - orangeMouse made in Goat |
| HLA | Citrate buffer in a steamer for 20 minutes | 1:500 – Mouse (Dako) | 555 wavelength - orangeMouse made in Goat |
| TSPO | Citrate buffer in a steamer for 20 minutes | 1:2000 – Rabbit (Abcam) | 568 wavelength - redRabbit made in Goat |
| PLP | Citrate buffer in a steamer for 20 minutes | 1:500 – Mouse (BioRad) | 647 wavelength – far redMouse made in Donkey |
| S100B | Citrate buffer in a steamer for 20 minutes | 1:200 – Rabbit (Novus) | 568 wavelength - redRabbit made in Goat |