

Immunofluorescence: General FFPE Human Tissue Protocol

General staining procedure*:

1. Dewax and rehydrate formalin-fixed paraffin-embedded tissue sections.
2. Perform heat induced antigen retrieval at pH 9.5 (i.e., 0.1M Tris/HCL+ 5% Urea, pH 9.5).
3. Pre-rinse tissue sections in PBS (Phosphate Buffered Saline, pH 7.4) for 30 minutes.
4. Incubate the tissue section with the primary antibody (8 hours at room temperature). (Other labeling antibodies can be incubated during this step as long as these are raised in different species than the primary antibody, e.g. Mouse Anti-GFAP)**
5. Wash the tissue section twice for 30 minutes in TBST (Tris Buffered Saline, pH 7.4 with 0.05% Tween 20).
6. Incubate the tissue section with the blocking buffer for 30 minutes.
7. Incubate the tissue section with a fluorophore conjugated secondary antibody for 90 minutes in the blocking buffer or according to the manufacturer's instructions.
8. Wash the tissue section once for 30 minutes.
9. Incubate the tissue section with any additional fluorophore conjugated antibodies (such as Mouse Anti-GFAP) or fluorescence affinity reagents according to the manufacturer's instructions. Wash the tissue section once for 30 minutes and continue with the staining protocol.
10. Incubate the tissue section with Hoechst stain for 30 minutes in wash buffer.
11. Wash the tissue section once for 30 minutes.
12. Incubate the tissue section in 70% ethanol for 2 minutes.
13. Incubate the tissue section in Sudan Black (1% Sudan Black in 70% Ethanol) for 15 minutes.
14. Destain the tissue section in 70% ethanol until the desired staining is reached.
15. Coverslip the tissue section with the appropriate water-based mounting media.

*Information is courtesy of the Tissue Profiling group, SciLifeLab Stockholm. All experiments were performed according to standard protocol (Mulder et al, 2011).

Mulder J, Zilberter M, Pasquaré S, Alpár A, Schulte G, Ferreira S, Köfalvi A, Martín-Moreno A, Keimpema E, Tanila H, Watanabe M, Mackie K, Hortobágyi T, de Ceballos M, Harkany T (2011) Molecular reorganization of endocannabinoid signaling in Alzheimer's disease. *Brain*. 2011April;134(4):1041-1060.

** For mouse primary antibodies, please see protocol step number 9.