Immunofluorescence: General Animal Brain Tissue Protocol

Animal surgery and tissue processing:

1. Inject colchicine into the lateral ventricle (mouse 5 ml ~ 30mg; rat 15 ml ~ 90mg).
2. Transcardially perfuse animals 24 hours after surgery (4% paraformaldehyde in 0.1M Phosphate Buffer, pH 7.4).
3. Dehydrate brains in 10% sucrose (>48 hours) and snap freeze.
4. Cut 16mm thick sections on a cryo-microtome and thaw mount onto gelatin-coated glass slides. Store tissue sections (slides) at -20°C until ready to use.

General staining procedure*:

1. Remove tissue sections (slides) from the freezer and keep at room temperature for at least 30 minutes.
2. Pre-rinse tissue sections in PBS (Phosphate Buffered Saline, pH 7.4) for 30 minutes.
3. Incubate the tissue section with the primary antibody (16-24 hours at 4°C). (Other labeling antibodies can be incubated during this step as long as these are raised in different species, e.g. Mouse Anti-GFAP.)**
4. Wash the tissue section twice for 30 minutes in TBST (Tris Buffered Saline, pH 7.4 with 0.05% Tween 20).
5. Incubate the tissue section with the blocking buffer for 30 minutes.
6. Incubate the tissue section with a peroxidase conjugated secondary antibody for 60 minutes in the blocking buffer or according to the manufacturer's instructions.
7. Incubate the tissue section with Hoechst stain for 30 minutes in wash buffer.
8. Wash the tissue section once for 30 minutes.
9. Incubate the tissue section with a fluorescein Tyramide Signal Amplification (TSA™) reagent for 15 minutes or according to the manufacturer's instructions.
10. Wash the tissue section twice for 30 minutes.
11. Incubate the tissue section with any additional fluorescent conjugated antibodies (e.g. Mouse Anti-GFAP) or fluorescence affinity reagents according to the manufacturer's instructions. Wash the tissue section for 30 minutes and continue with the staining protocol.
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, 2007; Mulder et al, 2009):

Mulder J, Bjorling E, Jonasson K, Wernerus H, Hober S, Hokfelt T, Uhlen M (2009) Tissue profiling of the mammalian central nervous system using human antibody-based proteomics. Mol Cell Proteomics. 2009 Jul;8(7):1612-22.

Mulder J, Wernerus H, Shi TJ, Ponten F, Hober S, Uhlen M, Hokflet T (2007) Systematically generated antibodies against human gene products: High throughput screening on sections from the rat nervous system. Neuroscience. 2007 Jun 8;146(4):1689-703.

** For mouse primary antibodies, please see protocol step number 11.