GFAP Immunohistochemistry Protocol

Day 1: (NOTE: Antibody has GFP conjugated to it, do all washes, etc, with light blocking foil)

1. Wash tissue 3 x 5 minutes 0.01M (1X) PBS at room temperature
2. Block wash: Rinse tissue in blocking solution (10% donkey serum, PBT [0.25% Triton-X in 1X PBS]) for 2 hours at room temp.
3. Incubate slide overnight at 4°C with primary antibody solution (10% donkey serum, PBT [0.25% Triton-X in 1X PBS], and 1:1000 anti-GFAP (Alexa Fluor 488, Millipore, MAB3402X)

**Note: KEEP IN DARK DURING AND AFTER GFAP ANTIBODY IS ADDED**

Day 2:

1. Wash slides 3X 10 minutes 0.01M PBS at room temperature
2. Remove from PBS, mount on slides, apply coverslips with mounting media (NOTE: do in dark or low light)
3. Lay slides flat at room temperature 22C for 24 hours, then transfer to dark slide box and keep at -20°C.

Lot number and specifics (tested by Julia 7/21/17)

Millipore (AB5804): 488 conjugated lot# 2293843 (original/first GFAP staining used)

Looks good 1:1000 or 1:2000!

Abcam (ab49874): Cy3 conjugated (lot?

Looks good 1:1000 or 1:2000!