GFAP, Iba1, and Hoechst IHC

1 DAY 1

1. Defrost sealed slide box 30 min @ RT, dry slides 15-30min at 65°C, edge tissue with hydrophobic marker
2. 3 x 10 min washes 0.1M PBS (all washes shaking at ~1)
3. 15 min citric acid pH 6.0 @ 85°C (Warm CA first)
4. 3 x 10 min washes 0.1M PBS
5. Block 30min in 2% BSA blocking buffer (pipette 300ml onto slides)
   Note: 2% BSA Blocking Buffer = 0.1g BSA + 100ul 10% TritonX100 in 5 mL 0.1M PBS
6. (1:500) 1′ rabbit α-Iba1 and (1:500) 1′ Cy 3 mouse pre-conjugated α-GFAP in 2% BSA Blocking Buffer overnight @ RT DARK
   2 µl Iba1 + 2 µl GFAP in 1 mL 2% BSA Blocking Buffer

2 DAY 2 (ALL DARK)

1. 3 x 10 min washes 0.1M PBS
2. (1:500) 2′ Cy3 α-mouse and (1:400) 2′ Cy5 α-rabbit in 0.1M PBS + for 4 hours @ RT, DARK
   2 µl Cy3 + 2.5 µl Cy5 + 20µl 10% TX-100 in 1 mL 0.1M PBS
3. (1:20000) Hoechst for 15 minutes (5 µl into 100 ml in pink container, shaking)
4. 3 x 10 min washes 0.1M PBS
5. Coverslip with FluoromountG (150 µl) and edge with nailpolish