
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 μ l Iba1 + 2 μ l 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 μ l Cy3 + 2.5 μ l Cy5 + 20 μ l 10% TX-100 in 1 mL 0.1M PBS
3. (1:20000) Hoechst for 15 minutes (5 μ l into 100 ml in pink container, shaking)
4. 3 x 10 min washes 0.1M PBS
5. Coverslip with FluoromountG (150 μ l) and edge with nailpolish