

## Protocol: CD11b Fluorescent Immunostaining

**Citation:** Ryu JK, Rafalski VA, Meyer-Franke A, Adams RA, Poda SB, Rios Coronado PE, Pedersen LO, Menon V, Baeten KM, Sikorski SL, Bedard C, Hanspers K, Bardehle S, Mendiola AS, Davalos D, Machado MR, Chan JP, Plastira I, Petersen MA, Pfaff SJ, Ang KK, Hallenbeck KK, Syme C, Hakozaki H, Ellisman MH, Swanson RA, Zamvil SS, Arkin MR, Zorn SH, Pico AR, Mucke L, Freedman SB, Stavenhagen JB, Nelson RB, Akassoglou K. Fibrin-targeting immunotherapy protects against neuroinflammation and neurodegeneration. **Nat Immunol** 2018, 19(11):1212-1223.

Sections should be 30 um thick

Sections stored long term at -20C in Cyroprotectant medium (30% Glycerol , 30% Ethylene-glycol in PBS)

**\*\*\*Brains should not be postfixed in PFA for more than 4 hrs after perfusion with PFA.** 30% sucrose cryopreservation should be done prior to cutting sections.

Can be adapted for cryosections

### Protocol

1. Wash sections 3 x 5' PBS (to remove cryoprotectant medium), shaking  
Note: Wash in netwells
2. **Block** for 60 min at room temp, shaking  
Dilute in blocking solution:
  - 5% Normal Donkey Serum (can be goat serum, depending on secondary)
  - 3% BSA
  - 0.3% Triton X
  - Up to volume in 1X PBSNote: Can block in netwells if serum/BSA is not limiting
3. **Primary antibody incubation** (overnight, shaking at 4C, shaking)  
Rat anti-CD11b 1:150; eBioscience clone M1/70 (#14-0112-85)  
Dilute in antibody diluent:
  - 1% Normal Donkey Serum (can be goat serum, depending on secondary)
  - 1% BSA
  - 0.3% Triton X
  - Up to volume in 1X PBSNote: incubate in smaller volumes (300 ul in 96 well or 500 ul in 24 well) to save antibody
4. Wash sections 3 x 5' PBS, shaking  
Note: Wash in netwells
5. **Secondary antibody incubation** (1-3 hrs room temp, shaking)  
Jackson Immunoresearch Donkey anti-rat Cy3 (1:500)  
Dilute in antibody diluent (see above for recipe)  
Note: Typical dilution is 1:500 for secondary but can be adjusted as necessary.
6. Wash sections 3 x 5' PBS, shaking  
Note: Wash in netwells
7. Mount sections, let dry on slide for ~10 min, then coverslip with anti-fade reagent (with DAPI if desired)