



F4/80

Biolegend (Cat #123102); or Novus (Cat # NBP2-12506)  
Immunohistochemistry Protocol For Formalin Fixed Frozen Embedded Tissue  
Cut sections at 7 microns and stored in -80°C

Day 1

1. Thaw the slides at room temperature in a humid chamber for 10 minutes.
2. Rinse the slides with 3 changes of PBS for 10 minutes each.
3. Outline the tissues with a **PAP pen**.
4. Rinse the slides with PBST (0.2% TritonX diluted in PBS) once for 10 minutes.
5. Incubate with **5% normal donkey serum** diluted in PBST (0.2% TritonX diluted in PBS) at room temperature in a humid chamber for **30 minutes**.
6. Rinse the slides with 3 changes of PBS for 10 minutes each.
7. Prepare primary antibody (1. *RatF4/80, Biolegend 123102*; or 2. *RabbitF4/80, Novus NBP2-12506*) in **1% normal donkey serum** diluted in PBS.
8. Incubate the slides with a **1:50** dilution of F4/80 primary antibody in a humid chamber at **4°C overnight**.

Day 2

9. Rinse the slides with 3 changes of PBS for 10 minutes each.
10. Prepare secondary antibody (1. *Goat $\alpha$ Rat-Fluro488, Abcam ab150165*; or 2. *Goat $\alpha$ Rabbit-Fluro488, Abcam ab150077*) in **1% normal donkey serum** diluted in PBS.
11. Incubate the slides with a **1:500** dilution of secondary antibody in a humid chamber at **room temperature for 1 hour**.
12. Rinse the slides with 3 changes of PBS for 10 minutes each.
13. Mount with **mounting medium containing Dapi** (*Vectashield H-1200*).

