

**Immunohistochemistry Protocol for Whole-Mount or Vibratome-Sectioned Tissue**  
**PECAM Antibody Staining**  
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For use with mouse tissue (whole-mount or vibratome-sectioned), fixed with PFA (2-4%)

1. Block with 0.3% Triton/1X PBS solution containing 5% Normal Donkey Serum (Jackson ImmunoResearch, Catalog # 017-000-121)
  - 2 hours
  - room temperature
2. Incubate with rat anti-mouse CD31 antibody (Pharmingen, Catalog #553370), diluted 1:500 in blocking buffer
  - 12-15 hours (overnight)
  - 4° C
3. Wash with 1X PBS
  - 1 hour
  - room temperature
4. Incubate with Cy3-labeled donkey secondary anti-rat IgG antibody (Jackson ImmunoResearch, Catalog #712-165-153), diluted 1:200 in blocking buffer
  - 4-6 hours
  - 4° C
5. Wash with 1X PBS
  - 2 hours-overnight (best if overnight)
  - 4° C