

**Immunohistochemistry Protocol for Whole-Mount or Vibratome-Sectioned Tissue**  
**Notch 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) [or whichever species your secondary antibody is made in]
  - 2 hours
  - room temperature
2. Incubate with rabbit anti-human Cleaved Notch 1 (Val1744) antibody (Cell Signaling Technology), diluted 1:250-1:500 (depending on tissue) in blocking buffer/ OR rabbit anti-rat 5261-P Notch 1 ECD antibody diluted 1:1000 in blocking buffer
  - 12-15 hours (overnight)
  - 4°C
3. Wash with 1X PBS
  - 1 hour
  - room temperature
4. Incubate with Cy3 (or FITC) –labeled donkey secondary anti-rabbit IgG antibody (Jackson ImmunoResearch, Catalog # 711-165-152), diluted 1:200 in blocking buffer
  - 3-4 hours
  - 4°C
5. Wash with 1X PBS
  - 2 hours-overnight (best if overnight)
  - 4°C