Immunohistochemistry Protocol
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I. Rehydration & Antigen Retrieval
   a. Bring 100 mL beaker containing ddH2O to a boil.
   b. Commercial rehydration and antigen retrieval solution comes as 10X
      i. 4 mL of this in a 50 ml Falcon tube 10X.
      ii. Bring total volume to 40 mL total with ddH2O.
   c. Put tube in boiling water for 30 minutes.
   d. Remove Falcon tube from water and leave on bench for 10-15 minutes to cool.
   e. Wash with PBT (0.1% Triton-X in PBS) 3 times, 5’ each.

II. Blocking
   a. 5% sheep serum
      i. Use 1 mL frozen aliquots.
      ii. Dilute (bring 1 mL up to total volume of 20 ml with PBT).
   b. Add 500 ul per slide and incubate for 30 minutes at RT.

III. Primary Antibody
   a. Dilute with blocking solution
   b. Add 400 ul per slide, cover with parafilm, and incubate at 4 °C overnight (18 hours).
   c. Wash slides by placing all of them in a large beaker containing 400 ml PBT for 15 minutes.

IV. Secondary Antibody
   a. Anti-primary antibody animal = 1:500
      i. Dilute with blocking solution
   b. Add 400-500 ul per slide and incubate at RT for 30 minutes.
   c. Wash slides by placing all of them in a large beaker containing 400 ml PBT for 15 minutes.

V. Counterstain
   a. Mount slides with mounting media containing DAPI.
   b. Store slides at 4 °C overnight.