

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.