

C4d Paraffin

Reagent	Name	Vendor	Catalog #
Primary	C4d (1:100) <i>rabbit mono</i>	Abcam	Ab183311
Secondary	Biotinylated Goat Anti-Rabbit H+L (1:3:200)	Vector	BA-1000

PROTOCOL

1. Deparaffinize slides and bring to Dh2o
2. Steam slides in pH 9 sol'n /60'
3. Cool 30 minutes to room temperature
4. Wash PBS
5. Quench with 3% H2O2 5 minutes
6. Wash PBST
7. Block w Avidin Block @ rt 15 minutes
8. Wash PBST
9. Block w Biotin Block @ rt 15 minutes
10. Wash PBST
11. Block w/ Protein block @ rt 10 min.
12. Apply C4d primary (1:100 in PBS) incubate 2 HR RT or 4°C overnight
13. Wash PBST bath
14. 2° link antibody (Biotin-Goat Anti-Rabbit (1:3:200 in PBS) incubate @ rt 30 minutes in
15. Wash in PBST bath 3X
16. Apply ABC incubate @ rt in humidity chamber 30 minutes – (VECTASTAIN ELITE ABC HRP KIT – PK6100)
17. Wash PBST 3X
18. Apply AEC incubate @ rt 10-15 minutes – (AEC Chromogen/Substrate Kit – ACG500)
19. Rinse DH2O
20. Counterstain Aqueous Hematoxylin- 30 sec. (rinse in Tap H2O)Blue in Scott's Tap H2O
21. Crystal mount- air dry /Coverslip

Links to products:
 VECTASTAIN ELITE ABC HRP KIT - <https://vectorlabs.com/vectastain-elite-abc-kit-standard.html>