C4d (Abcam Ab183311) – Manual Chromogen (AEC) Protocol

Run Information

Samples:	
Prepared by:	
Date of Run:	
Stainer Used: N/A	
Ready for Scanning (Date/Time): Date of Scanning:	
Scanned by:	
Scanned by: Telepathology Control Case ID:	
Results/Summary	
Satisfactory Staining (Yes/No):	Initials:
Comments/Corrective Action:	
Samples to be Repeated (if applicable):	
Samples to be Repeated (if applicable): Slide ID	Reason
	Reason

C4d Paraffin

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

PROTOCOL

- Deparaffinize slides and bring to Dh2o Steam slides in pH 9 sol'n /60'
 Cool 30 minutes to room temperature Wash PBS
 Quench with 3% H2O2 5 minutes
 Wash PBST
 Block w Avidin Block @ rt 15 minutes
 Wash PBST

- 3. 4. 5. 6. 7. 8. 9. 10. 11. 12.
- Block w Biotin Block @ rt 15 minutes Wash PBST

- Wash PBST bath

 Apply C4d primary (1:100 in PBS) incubate 2 HR RT or 4°C overnight
 Wash PBST bath
- 2º link antibody (Biotin-Goat Anti-Rabbit (1:3:200 in PBS) incubate @ rt 30 minutes in Wash in PBST bath 3X
- wash and 1851 total 3A Apply ABC incubate @ rt in humidity chamber 30 minutes (VECTASTAIN ELITE ABC HRP KIT PK6100) Wash PBST 3X
- Apply AEC incubate @ rt 10-15 minutes (AEC Chromogen/Substrate Kit ACG500) Rinse DH2O
- 14. 15. 16. 17. 18. 19. 20. 21. Counterstain Aqueous Hematoxylin- 30 sec. (rinse in Tap H2O)Blue in Scott's Tap H2O Crystal mount- air dry /Coverslip

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