C4D-FITC – Manual Protocol

Run Information

Samples: _____
Prepared by: _____
Date of Run: _____

Stainer Used: N/A

Ready for Scanning (Date/Time): _____

Date of Scanning: _____
Scanned by: _____
Telepathology Control Case ID (if applicable): _____
Filter Configuration: _____

Exposure Times:

Fluorophore	Exposure Time (ms)	Light Intensity

Results/Summary

Satisfactory Staining (Yes/No): _____ Initials: _____

Comments/Corrective Action:

_____
_____
_____
_____
_____
_____

Plan for Next Run (if applicable):

_____
_____
_____
_____
_____
_____

IMMUNOHISTOCHEMICAL STAINING PROTOCOL
C4d Frozen (C4d *mAb*)

OBJECTIVE: Detection of precipitates indicative of antibody-mediated rejection.

Recommended Control: Allograft Kidney with Serum Confirmation of DSA

ID OF CONTROL USED: _____ Type of Tissue: _____

Reagent	Name	Vendor	Catalog #	Was this a new Bottle (Y/N)? If Yes, check lot log.
Primary	C4d (1:500) *mouse*	QUIDEL	A213	
Secondary	Biotinylated Horse Anti-Mouse H+L (1:200)	Vector	BA-2000	
Tertiary	FITC-Avidin D (1:500)	Vector	A-2001	
Fixative	Cold Acetone			
Blocks	Avidin/Biotin	Vector	SP 2001	
Protein Block	Serum-free Protein Block	Dako	X0909	
Nuclear Counterstain	DAPI (1:1000)	ThermoScientific	62248	

PROTOCOL

1. Cut slides and air dry under hood minimum 30 min to overnight
2. Fix slides in cold Acetone 10 minutes
3. Air dry 30 minutes to room temperature
4. Fix slides again in Acetone 5 minutes
5. Air dry slides
6. Wash PBS 3X
7. Avidin Block 2 drops 15 minutes
8. Wash PBS 3X
9. Biotin Block 2 drops 15 minutes
10. Wash PBS 3X
11. Block with Protein block @ RT, 10 min. Tap off protein block, do not wash. – apply primary directly to slide)
12. Apply primary antibody dilution directly to slide. Incubate with C4d primary (1:500 diluted in Protein Block) 1hr @ RT
13. Wash PBS 3X
14. Apply 2° antibody, Biotin-Horse Anti-Mouse (1:3:200 (anti mouse : horse serum : PBS). Incubate @ RT, 30 minutes.
15. Wash in PBS 3X
16. During washes, spin FITC @ 6800 RPM for 3 minutes.
17. Apply FITC-Avidin D (1:500 PBS) @ RT, 1hr
18. Wash PBS 3X
19. Nuclear stain with DAPI (1:1000 in PBS), 20 minutes @ RT
20. Wash PBS 3X then place in PBST
21. Coverslip with gelvatol
22. Ready for scanning

EXPECTED RESULTS: Specimens with ABMR will have C4d staining in capillaries.

CASE IDS/SLIDE NUMBERS: