

WORKING PROTOCOL

Anti human C4d Antibody (C4dpAb)

Cat. no.: BI- RC4D

For Research Use Only!

Immunohistochemical staining of paraffin sections using the C4d Polyclonal Antibody

This protocol has been developed for staining of paraffin embedded tissue sections (tissue fixed with 4.5% neutrally buffered formalin)

1. Deparaffinize and rehydrate tissue section
2. For antigen retrieval, pressure-cook slides for 5 minutes at +125°C in TRIS/EDTA buffer (pH 8.5) (in Pascal Pressurized Heating Chamber, Dako or equivalent) and cool down to room temperature (20 minutes)
TRIS/EDTA Buffer: 10 ml 1M TRIS (pH 9) + 20 ml 0.05M EDTA (pH 7.5) + 970 ml H₂O bidest.
3. Wash sections in PBS
4. Block endogenous peroxidase with 3% H₂O₂ in PBS for 10 minutes
5. Wash sections in PBS
6. Apply 5 minutes UltraVision Block (LabVision)
7. Wash sections in PBS
8. Apply anti C4d antibody 1:30-1:50 in PBS including 1% BSA for 60 minutes at room temperature
9. Wash in PBS
10. Apply Value Primary Antibody Enhancer (LabVision) 20 minutes at room temperature
11. Wash in PBS
12. Apply Value HRP Polymer (LabVision) 30 minutes at room temperature (dark chamber)
13. Wash in PBS
14. Apply DAB or AEC chromogen (adjust time according to positive control)
15. Wash sections in tap water for 10 minutes
16. Counter stain sections with haemalaun acc. Mayer for 1 minute
17. Coverslip sections using an aqueous mounting media

Indirect immunofluorescence on frozen sections using the C4d Polyclonal Antibody.

1. Frozen sections are incubated with rabbit anti-C4d pAb in a dilution of 1:20-1:30 in phosphate buffered saline (PBS) for 30 minutes at room temperature.
2. Wash 4 times with PBS
3. After washing with PBS, bound antibody is detected by incubation with an appropriate FITC-labeled secondary anti-rabbit antibody (dilution according to the respective supplier's protocol).
4. Wash 4 times with PBS
5. Coverslip sections using an aqueous mounting media

Additional reagents required:

Detection System: LabVision Corporation, Cat. No.: TL-060-HLS or TL-125-HLS
UltraVision LP Value Large volume detection system HRP Polymer (ready to use)
<http://www.labvision.com/>

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