WORKING PROTOCOL Anti human C4d Antibody (C4dpAb)

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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
- 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

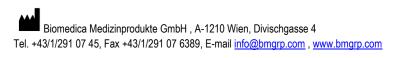
Indirect immunofluorescence on frozen sections using the C4d Polyclonal Antibody.

- 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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