

WORKING PROTOCOL – Anti human C4d Antibody (C4dpAb)

Cat.no.: BI- RC4D

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 at least 10 minutes at 1 bar in mM citrate buffer (pH 6,0)
3. Wash sections in PBS-buffer
4. Block endogenous peroxidase with 3% H₂O₂ in PBS for 10 minutes
5. Wash sections in PBS buffer
6. Apply Avidin-Biotin Blocking Kit (Vector, Cat.Nr. SP-2001)
7. Apply Super Block (Super Stain System (HRP) ID Labs, UK, Cat.Nr. IDST-1007) and incubate for 7 minutes at room temperature to block nonspecific background staining
8. Wash section in PBS buffer
9. Apply anti C4d antibody 1:30 – 1:50 in 1% BSA in PBS buffer for 30 minutes at room temperature
10. Wash in PBS buffer
11. Apply Super Stain Anti-Polyvalent (yellow solution) and incubate for 10 minutes at room temperature
12. Wash in PBS buffer
13. Apply Super Stain HRP (red solution) and incubate for 10 minutes at room temperature
14. Wash in PBS buffer
15. Cover sections with the AEC-reagent for 5 minutes (adjust time according to positive control)
16. Wash sections in tap water for 10 minutes
17. Counter stain sections with haemalaun acc. Mayer.
18. Coverslip sections using an aqueous mounting media

Additional reagents required:

Vector Blocking Kit, Catalog Nr.: SP-2001

Vector Laboratories Inc. 30, Ingold Road, Burlingame, CA 94010 USA,
<http://www.vectorlabs.com/>

Super Stain System Kit (HRP), Catalog Nr.: IDST 1007

ID Labs, PO Box 1145 Station B, N6A 5K2 London, ON Canada,
<http://www.idlabs.com/>

