

# **Anti C4d antibody (BI-RC4D)**

## **Frequently Asked Questions (FAQ) in C4d immunohistochemistry**

### ***What should I use as positive control for the C4d pab antibody?***

The best positive controls are cases of antibody-mediated rejection (ABMR). Evidence for ABMR would ideally come from positive C4 staining on frozen sections from the same sample. Otherwise cases with typical clinical presentation of ABMR and the presence donor specific antibodies (DSA) proven by Luminex technology or conventional crossmatch testing could also do fine.

In case there is no unequivocal case of humoral rejection available for use as positive control, you might also employ membranous GN (native kidney or TX) sections. C4d should yield a granular staining pattern along the GBM (Fig 1A), almost identical to the one obtained with IgG (Fig 1B). It might be necessary to test several cases since deposition of classical pathway complement-components might be variable in MGN.

### ***What are the diagnostically relevant staining patterns for C4d?***

The only diagnostically relevant staining pattern for ABMR is a linear C4d deposition along peritubular capillaries (PTC) (Fig 2A) (1, 2). A discontinuous coarsely granular and typically focal staining pattern in PTC (Fig 2B) was found to be not associated with ABMR in a recent study (1).

### ***What is the significance of C4d depositions in other locations?***

C4d depositions can be found in different compartments of the kidney (1). In general non-PTC C4d deposits have no diagnostic relevance for ABMR (except for glomerular endothelial cell staining (Fig 3A)). It is not uncommon to see variable amounts of C4d in the walls of arteries and arterioles (Fig 2C, arrows and triangles). One can for example expect to find glomerular C4d deposits in many cases of immune complex mediated glomerulonephritis (IC-GN) matching the distribution and pattern of other complement components (Fig 2D, arrows and triangles). Furthermore glomerular C4d deposits are also typically found in cases of chronic transplant glomerulopathy (cg). The C4d staining in cg typically appears to affect most of, or even the entire capillary wall (in contrast to the lumenally oriented endothelial cell staining pattern found in some cases of ABMR (Fig 3A)). The glomerular capillary wall staining pattern of cg is frequently reminiscent of that observed in IC-GN with subendothelial deposits (Fig 3B). In many cases immunohistochemistry for other complement fragments and immunoglobulins is required for a reliable differential diagnosis.

### ***Is C4d pab suitable for immunofluorescence on frozen sections?***

Yes, C4d pab can be used on frozen sections and yields staining results identical to the pattern observed with monoclonal anti-C4d antibodies which however are not suitable for paraffin embedded tissue (3).

***What is the ideal staining protocol?***

The ideal staining protocol has to be determined in each lab individually. It is reasonable to start from the protocol provided with the C4d antibody by the vendor. Heat induced antigen retrieval was found to be essential in most labs. Some institutions found pressure-cooking being superior to microwave treatment since the latter might result in reduced sensitivity. But this and other details of the staining protocol remain a matter of testing and adjustment to lab conditions.

***Is sensitivity of IHC on paraffin sections equal to IF on frozen sections?***

From the few studies specifically addressing this issue it can be concluded that IF on frozen is generally more sensitive than IHC on paraffin sections (4). Taking this fact into account, the Banff classification suggested lower thresholds for the grading of C4d staining on paraffin section than on frozen sections (2).

***For customers, who do not have the ability to pressure-cook the slides, is there an alternative procedure to pressure-cooking?***

An alternative would be to use a micro-wave for heating the slides. Since we do not have a validated protocol for this, the customer then should try several dilutions of the antibody to check the unspecific binding.

***Are the references on the protocol ones that used the antibody?***

The references cited did use that antibody. Please see the Link Literature References.

***What is the IgG concentration?***

0.2mg/ml, pure IgG, purified via Protein G, no carrier proteins added.

***Is the PBS buffer listed in the Working Protocol just plain PBS buffer or does it contain BSA or some protein in it?***

The buffer is just plain 10mM PBS / 140mM NaCl pH 7.

***Is the antibody cross-reacting with dog?***

In general the C4d activation is very conserved across mammalian species, so there is a good chance that it will also be found in dogs. At present nothing is known about the cross reactivity to dogs.

***Is the H<sub>2</sub>O<sub>2</sub> block from the superstain kit used or another one.***

We use Fluka hydrogen peroxide 30%, diluted 1:10 in methanol (end concentration 3%) for 10 minutes at room temperature.

***The protocol specifies 20mM PBS – What is the recipe?***

20mM PBS solution prepare 200mM stock solution:

A: NaH<sub>2</sub>PO<sub>4</sub>x1H<sub>2</sub>O 5,244g in ca. 190ml aqua bidest pH to 7,4

B: Na<sub>2</sub>HPO<sub>4</sub>x2H<sub>2</sub>O 28,83g in ca. 810ml aqua bidest pH 7,4

Mix A and B, pH control (7,4), fill up to 1l Working solution: dilute stock 1:10 in 0,9% NaCl.

**Will counterstaining with Harris hematoxylin cause any problems?**

Counterstaining with hematoxylin should not give any problems, as the reaction product is stable, you will have to optimise the counterstaining time accordingly (use your usual paraffin protocol).

**Is it possible to use a 5 min / wash time?**

Yes, but 5 minutes washing time is the minimum: You should change the PBS solution 4 times.

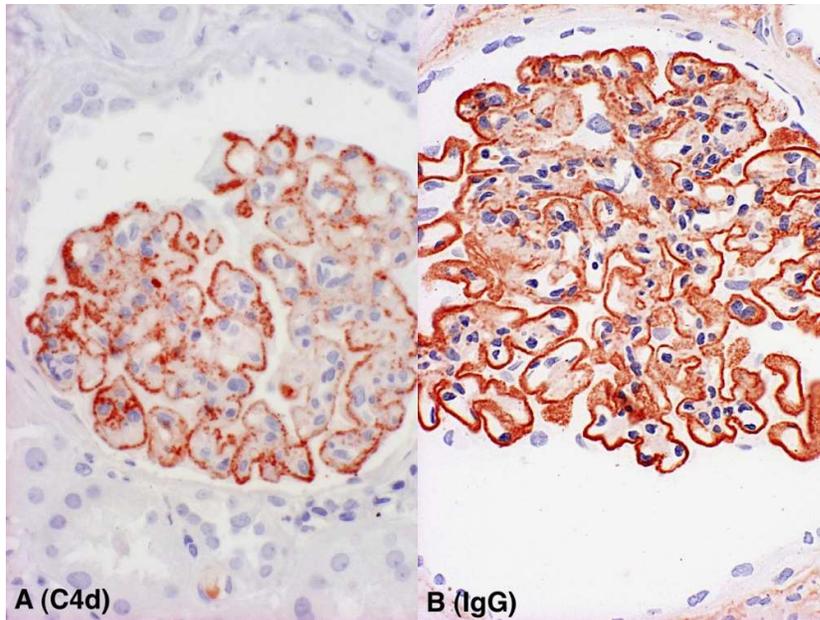


Fig. 1.

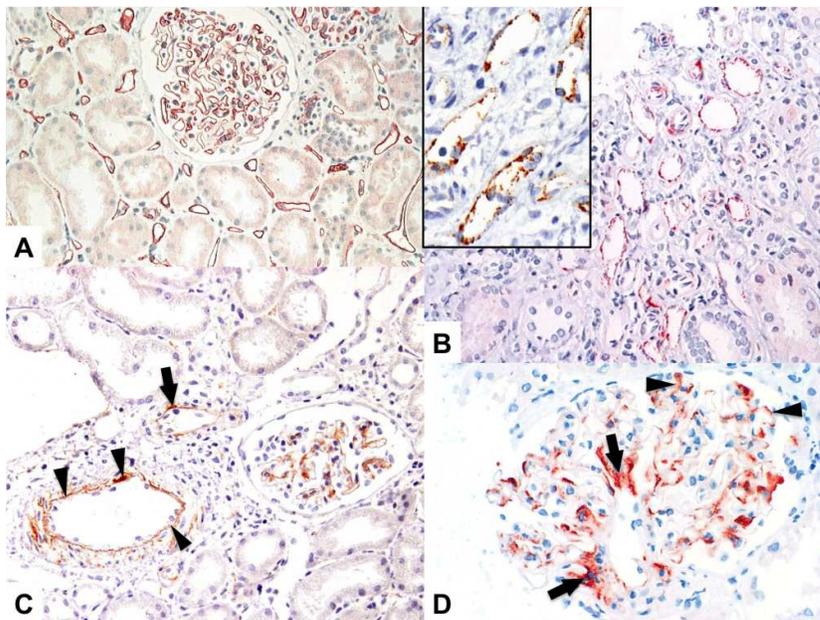
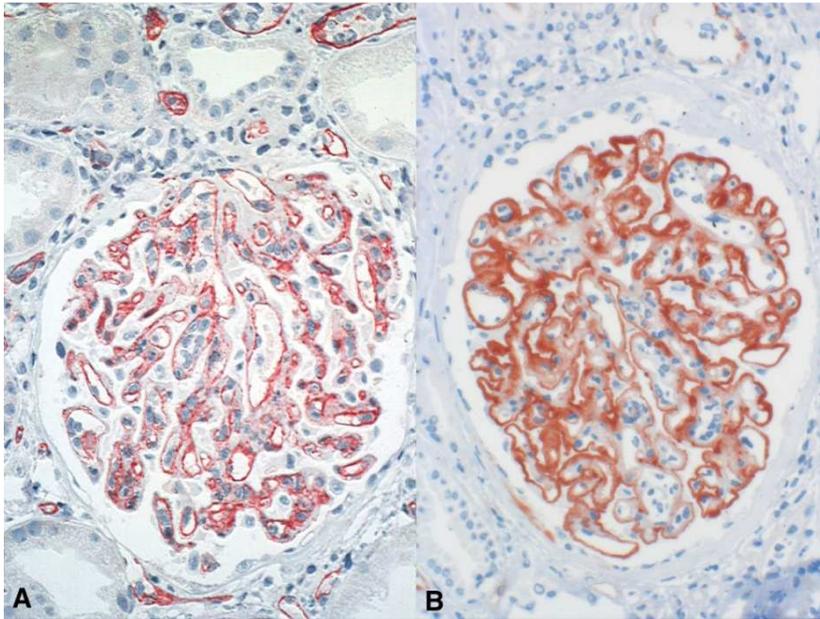


Fig. 2.



**Fig. 3.**

### **Publications:**

1. Kikic Z, Regele H, Nordmeyer V, Wahrmann M, Kletzmayer J, Bartel G, Bohmig GA (2011) Significance of peritubular capillary, glomerular, and arteriolar c4d staining patterns in paraffin sections of early kidney transplant biopsies. *Transplantation*. 91(4):440-6.
2. Solez K, Colvin RB, Racusen LC, Haas M, Sis B, Mengel M, Halloran PF, Baldwin W, Banfi G, Collins AB, Cosio F, David DS, Drachenberg C, Einecke G, Fogo AB, Gibson IW, Glotz D, Iskandar SS, Kraus E, Lerut E, Mannon RB, Mihatsch M, Nankivell BJ, Nickeleit V, Papadimitriou JC, Randhawa P, Regele H, Renaudin K, Roberts I, Seron D, Smith RN, Valente M (2008) Banff 07 classification of renal allograft pathology: updates and future directions. *Am J Transplant*. 8(4):753-60.
3. Regele H, Exner M, Watschinger B, Wenter C, Wahrmann M, Osterreicher C, Saemann MD, Mersich N, Horl WH, Zlabinger GJ, Bohmig GA (2001) Endothelial C4d deposition is associated with inferior kidney allograft outcome independently of cellular rejection. *Nephrol Dial Transplant*. 16(10):2058-66.
4. Seemayer CA, Gaspert A, Nickeleit V, Mihatsch MJ (2007) C4d staining of renal allograft biopsies: a comparative analysis of different staining techniques. *Nephrol Dial Transplant*. 22(2):568-76.