

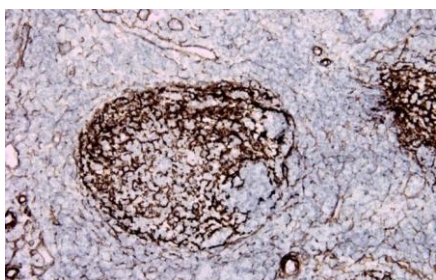
**CERTIFICATE OF ANALYSIS – TECHNICAL DATA SHEET**

<b>Product name</b>	C4d, Human, clone 12D11		
<b>Catalog number</b>	HM2229		
<b>Lot number</b>	-	<b>Expiry date</b>	-
<b>Volume</b>	1 ml	<b>Amount</b>	100 µg
<b>Formulation</b>	0.2 µm filtered in PBS+0.1%BSA+0.02%NaN3	<b>Concentration</b>	100 µg/ml
<b>Host Species</b>	Mouse IgG1	<b>Conjugate</b>	None
<b>Endotoxin</b>	N.A.	<b>Purification</b>	Protein G
<b>Storage</b>	4°C		

**Application notes**

	IHC-F	IHC-P	IF	FC	FS	IA	IP	W
Reference #	2,3,4			5				1
Yes	•			•		•		•
No								
N.D.		•	•		•		•	

N.D.= Not Determined; IHC = Immuno histochemistry; F = Frozen sections; P = Paraffin sections; IF = Immuno Fluorescence; FC = Flow Cytometry; FS = Functional Studies; IA = Immuno Assays; IP = Immuno Precipitation; W = Western blot



IHC-F: Immunohistochemical staining of C4d on Human tonsil frozen sections.

Dilutions to be used depend on detection system applied. It is recommended that users test the reagent and determine their own optimal dilutions. The typical starting working dilution is 1:50.

- F: Permeabilization was done in cold acetone with 0.5% hydrogen peroxidase for 10 min, after drying and washing, antibodies were incubated for 30 minutes.
- FC: Pneumococci were incubated with 25 µl of a 1:10 diluted 12D11, incubated for 30 minutes and washed (2x), followed by 30 minutes incubation on ice of a 1:100 diluted goat-anti-mouse IgG PE on ice in the dark and washed (2x) (Ref.5).

**General Information**
**Description**

The monoclonal antibody 12D11 (also known as clone M4d3) recognizes an epitope on the alpha 2 chain of human C4d. Complement factor C4 (MW 41 kDa), formerly known as Gg protein, consists of an alpha-, beta- and gamma-chain. The classical pathway of complement and the mannose binding lectin (MBL) activation pathway converge at C4. Activated C1, MASP-1 and MASP-2 cleave C4 resulting in the formation of C4a and C4b. The latter can be cleaved by factor I resulting in C4c and C4d, in which step all functional sites are lost. The C4d activation fragment of C4 is an excellent marker for classical complement pathway and MBL pathway activation. The thioester formed between the side chains of Cys1010 and Gln1013 within the C4d region of the α-chain mediates covalent attachment to the target surface bearing activated forms of C1s or MASP. Furthermore, C4d is highly homologous to C3d with over 35% shared amino acid sequence. In a number of diseases such as rheumatoid arthritis (RA), hereditary angioedema (HAE), systemic lupus erythematosus (SLE) and chronic urticaria with hypercomplementemia levels of C4d are significantly elevated in serum or plasma. C4d levels may also be elevated in plasma from patients with a variety of humoral autoimmune diseases in which complement activation is known to occur. Deposition of C4d in peritubular capillaries has been shown to be a sensitive marker for antibody-mediated (humoral) rejection in renal transplant biopsies. The monoclonal antibody 12D11 recognizes C4, C4b and C4d. Antibody 12D11 cross reacts with pig, bovine, baboon and rhesus monkey.

**Cross reactivity** Pig: Yes; Baboon: Yes; Bovine: Yes; Rhesus monkey: Yes.

- References**
1. Zwirner, J et al; Classical pathway of complement activation in normal and diseased human glomeruli. *Kidney Int* 1989, *36*: 1069.
  2. Zwirner, J et al; Classical pathway of complement activation in mammalian kidneys. *Immunology* 1993, *80*: 162.
  3. Feucht, H et al; Capillary deposition of C4d complement fragment and early renal graft loss. *Kidney Int* 1993, *43*: 1333.
  4. Rensen, S et al; Activation of the Complement System in Human Nonalcoholic Fatty Liver Disease. *Hepatology* 2009, *50*:1809
  5. Meijvis, S et al; Strategies to improve clinical management of community-acquired pneumonia. Thesis 2012.

**Storage&stability** Product should be stored at 4°C. Under recommended storage conditions, product is stable for at least one year.

**Precautions** For research use only. Not for use in or on humans or animals or for diagnostics. It is the responsibility of the user to comply with all local/state and federal rules in the use of this product. Hycult Biotech is not responsible for any patent infringements that might result from the use or derivation of this product.

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We hereby certify that the above-stated information is correct and that this product has been successfully tested by the Quality Control Department. This product was released for sale according to the existing specifications. This document has been produced electronically and is valid without a signature.

Approved by Manager of QC  
Robbert Zwinkels

Date  
19/03/2018

Do you have any questions or comments regarding this product? Please contact us via [support@hycultbiotech.com](mailto:support@hycultbiotech.com).