

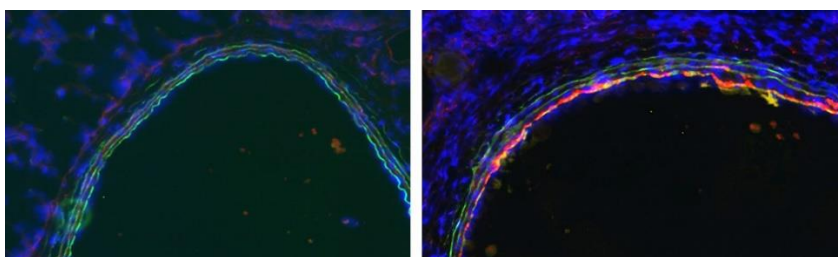
CERTIFICATE OF ANALYSIS – TECHNICAL DATA SHEET

Product name	C4d, Rat, pAb			Expiry date	-
Catalog number	HP8034			Amount	100 µg
Lot number	-	Concentration	100 µg/ml	Conjugate	None
Volume	1 ml	Purification	Affinity	Host species	Rabbit IgG
Formulation	0.2 µm filtered in PBS+0.1%BSA+0.02%NaN3			Endotoxin	N.A.
Storage	4°C				

Application notes

	IHC-F	IHC-P	IF	FC	FS	IA	IP	W
Reference #	1,7	1,2,3,4	5,8	1,2,3,6,8				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: Frozen section of rat lung tissue of control (left) and rat with hypoxia-induced pulmonary hypertension. Staining of C4d (red) with HP8034 in a 1:200 dilution. Green is autofluorescence of elastic lamellae defining vascular media, Blue is cell nuclei stained with DAPI. Pictures are kindly provided by Maria Frid, Pediatrics, University Colorado Denver.

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.

- IHC-F: Frozen lung sections were fixed in cold (-20°C) AcetoneMethanol (1:1) for 10 min, and primary Ab dilution was 1:200, followed by Biotinylated secondary Abs, and then by Streptavidin-Alexa594.
- IHC-P: Full cross sections of cardiac grafts obtained at the time of sacrifice were fixed in acidic methanol (60% methanol, 10% acetic acid, 30% water), embedded in paraffin and sectioned at seven microns (Ref.2).

General Information

Description	The polyclonal antibody recognizes rat 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, 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.
Immunogen	A synthesized 14-mer peptide (STPAPRNPSEPVPQ) corresponding to amino acids 1223-1236 of rat C4, conjugated to keyhole limpet hemocyanin at the C-terminal part (for this a cysteine was added). (Ref.1)

Cross reactivity Mouse C4 (alpha chain): Yes

- References**
1. Minami, K et al; C4d deposition and clearance in cardiac transplants correlates with alloantibody levels and rejection in rats. Am J Transplant 2006, 6: 923
 2. Qian, Z et al; Antibody and complement mediated injury in transplants following sensitization by allogeneic blood transfusion. Transplant 2006, 82: 857
 3. Asano, H, et al; Treatment with riboflavin and ultraviolet light prevents alloimmunization to platelet transfusions and cardiac transplants. Transplant 2007, 84: 1174
 4. Murata, K, et al; C4d deposition and cellular infiltrates as markers of acute rejection in rat models of orthotopic lung transplantation. Transplant 2008, 86: 123
 5. Seifert, M et al; Detrimental effects of rat mesenchymal stromal cell pre-treatment in a model of acute kidney rejection. Front Immunol 2012 3:1
 6. Huang, G et al; Characterization of Transfusion-Elicited Acute Antibody-Mediated Rejection in a Rat Model of Kidney Transplantation. Am J Trans 2014, 14:1061
 7. Yamanaka, K et al; Associated with the Progress of Acute T-Cell Mediated Rejection. PlosONE 2016, 11: e0148881
 8. Liao, T et al; Noninvasive and quantitative measurement of C4d deposition for the diagnosis of antibody-mediated cardiac allograft rejection. EBioMedicine 2018, 37:236

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.

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
01/02/2019

Do you have any questions or comments regarding this product? Please contact us via support@hycultbiotech.com.