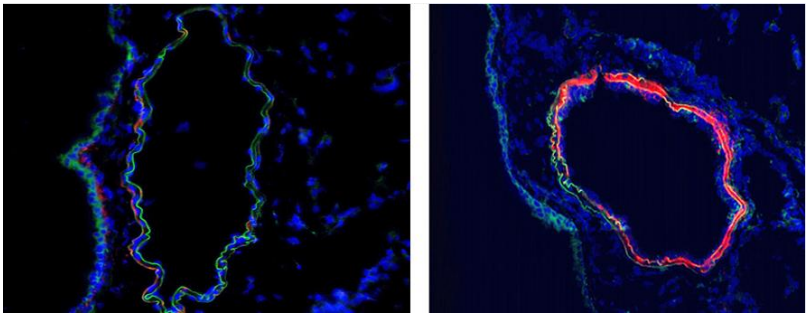
CERTIFICATE OF ANALYSIS – TECHNICAL DATA SHEET

Product name	C4, Mouse, clone 16D2		
Catalog number	HM1046-20UG		
Lot number	-	**Expiry date**	-
Volume	200 µl	**Amount**	20 µg
Formulation	0.2 µm filtered in PBS+0.1%BSA+0.02%NaN3	**Concentration**	100 µg/ml
Host Species	Rat IgG2a	**Conjugate**	None
Endotoxin	N.A.	**Purification**	Protein G
Storage	4°C		

Application notes

	IHC-F	IHC-P	IF	FC	FS	IA	IP	W
Reference #	7		2,5,6	8		2,3	1	1,4
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 mouse pulmonary artery of control (left) and mouse with hypoxia-induced pulmonary hypertension. Staining of C4 (red) with HM1046 in a 1:300 dilution. Green is autofluorescence of elastic lamellae defining vascular media, Blue is cell nuclei tabled 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.

- W: A non-reduced sample treatment and SDS-Page was used. The band size is 200 kDa (Ref.4).
- IA: Plates were coated overnight with rat anti-murine C4 mAb 16D2 in carbonate buffer and blocked with 5% dry milk in PBS and 0.01% Tween 20 (Ref.2).
- IF: Spleens were snap frozen and stored at -80°C until cryosections were cut. Sections (5 µm) were fixed for 4 min in ice-cold acetone. Sections were blocked with 2% BSA, 2% FCS, and PBS. Biotinylated 16D2 was visualized using avidin-PE (BD PharMingen, San Diego, CA) (Ref.2).
- IHC-F: OCT-embedded frozen sections were fixed in 10% buffered formalin solution for 10 min @ RT. Dilution of the primary mAb was 1:300, followed by Biotinylated secondary Abs and then by Streptavidin-Alexa 594.
- Positive control: T-cell regions of mouse spleen.

General Information

Description

The monoclonal antibody 16D2 recognizes mouse complement factor C4, formerly known as Gg protein, which consists of an alpha-, beta-, and gamma-chain. The classical pathway of complement and the Mannose binding lectin activation pathway converge at C4. C1s, MASP-1 and MASP-2 cleave C4 resulting in the formation of C4a and C4b. Subsequently, C4b can be cleaved to C4c and C4d by other serum enzymes. The monoclonal antibody 16D2 reacts with intact C4, C4b and C4d.
C4 is an acute phase protein that is produced by hepatocytes, monocytes and intestinal epithelial cells and can be used in experimental animals as a marker for activation of the classical complement pathway. Recent studies have demonstrated an association between graft rejection and C4d deposition in a mouse model for cardiac transplantation.

Immunogen

Thymocytes decorated with Thy-1 antibody and complement components

Aliases	Complement component 4
Cross reactivity	C4b: Yes; C4d: Yes

References

1. Kremmer, E et al; Monoclonal antibodies to complement components without the need of their prior purification. II. Antibodies to mouse C3 and C4. Hybridoma 1990, *9* : 309
2. Gadjeva, M et al; Macrophage-derived complement component C4 can restore humoral immunity in C4-deficient mice. J Immunol 2002, *169*: 5489
3. Paul E et al; Anti-DNA autoreactivity in C4-deficient mice. Eur J Immunol 2002, *32*: 2672
4. Murata, K et al; Synergistic deposition of C4d by complement-activating and non-activating antibodies in cardiac transplants. Am J Transplant 2007, *7*: 2605
5. Huugen D et al; Inhibition of complement factor C5 protects against anti-myeloperoxidase antibody-mediated glomerulonephritis in mice. Kidney International 2007 *71*: 646
6. Petitbarat, M et al; Critical Role and Therapeutic Control of the Lectin Pathway of Complement Activation in an Abortion-Prone Mouse Mating. J Immunol 2015, *195*: 5602
7. Kwak, J et al; Complement activation via a C3a receptor pathway alters CD4+ T lymphocytes and mediates lung cancer progression. Canc Res 2017, *78*:143
8. Katschke, K et al; Classical and alternative complement activation on photoreceptor outer segments drives monocyte-dependent retinal atrophy. Nature Scientific Reports 2018, *8*: 7348

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	Date
Brenda Teunissen	28/10/2020

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