

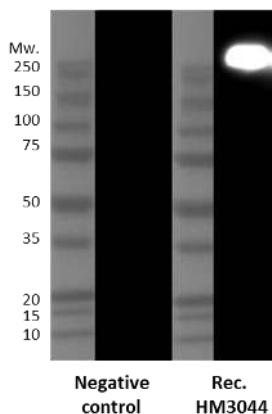
CERTIFICATE OF ANALYSIS – TECHNICAL DATA SHEET

Product name	C5, Rat, mAb 4G2		
Catalog number	HM3044-100UG		
Lot number	xxxxxXxxxx-X	Expiry date	MMM YYYY
Volume	1 ml	Amount	100 µg
Formulation	0.2 µm filtered in PBS +0.1% BSA	Concentration	100 µg/ml
Host Species	Mouse, recombinantly produced in CHO cells	Conjugate	None
Endotoxin	<24 EU/mg	Purification	Protein A
Storage	4°C		

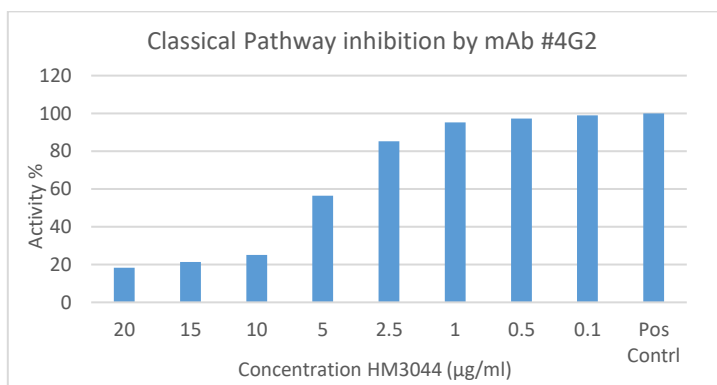
Application notes

	IHC-F	IHC-P	IF	FC	FS	IA	IP	W
Reference #					1	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



W: Western blot analysis human C5 protein was resolved on 4–20% PAGE gels under non-reducing conditions and detected with antibody HM3044 at 2 µg/ml . Band size is 250 kDa.



*This is an example, a batch specific figure will be added to the CoA

FS: HM3044 inhibits membrane attack complex formation as determined in rat classical complement pathway ELISA assay HIT410.

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 250 kDa.
- FS: Antibody 4G2 functions as an inhibiting antibody.(Ref.1) The 50% complement inhibitory dose is dependent on the type of assay (e.g. haemolysis assay or pathway ELISA) and should be determined per institute in combination with a proper reference control.

General Information

Description

Monoclonal antibody clone 4G2 recognizes human, rabbit and rat complement C5. The complement system plays important roles in both innate and adaptive immune response and can produce an inflammatory and protective reaction to challenges from pathogens. Complement C5 is a central molecule in all three pathways and after cleavage by its convertases, it initiates the terminal pathway in order to generate the cytolytic MAC. C5 is mainly synthesised in the liver as a single polypeptide chain and is present in serum in a concentration of 50–80 µm/ml. Besides, local synthesis of C5 is also supported by other cell types including monocytes/macrophages, neutrophils, fibroblasts, and astrocytes. Before secretion the molecule is glycosylated and secreted into plasma as a 190 kDa glycoprotein consisting of a disulphide linked alpha-chain (111 kDa) and beta-chain (75 kDa). The complement has become an interesting therapeutic target. Especially after the success of Eculizumab, a monoclonal antibody against C5, in the treatment for aHUS and PNH and the application in clinical trials for many other diseases. Monoclonal antibody 4G2 acts as an equivalent of Eculizumab and recognizes human and rabbit C5, however the affinity for rat C5 is even stronger. This cross reactivity makes it a powerful tool in translational animal

studies. Besides C5 the antibody also recognizes C5b6, but not C6. The antibody recognizes intact alpha chain and binds to an epitope distinct from Eculizumab. Using haemolytic assays the antibody shows efficient inhibition of AP as well as CP, in all mentioned models.

Clone 4G2 shows strong binding to C5 with a relatively slow off rate in rat. Suggesting to be functional in in vivo studies. Indeed, 4G2 was applied in a rat prophylactic Myasthenia gravis model. In this study the animals were protected from disease and for destruction of the muscle endplates.

Antibody 4G2 has been tested in western blotting, ELISA, IHC and functional studies (Ref. 1)

Immunogen	Rat C5
Aliases	-
Gene	Gene name Complement C5 Entrez Gene ID 362119 Uniprot P08650
Cross reactivity	Cross reaction with human, rabbit, guinea pig and mouse (ref 1)
References	1. Zelek, W.M., et al; Development and characterization of novel anti-C5 monoclonal antibodies capable of inhibiting complement in multiple species, 2019. Immunology, 157 , 283-295 (DOI: 10.1111/imm.13083)
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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Approved by Manager of QC

Date

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