CERTIFICATE OF ANALYSIS – TECHNICAL DATA SHEET

Product name	C5a/C5a des-arg (neo-epitope), Human, clone 2952		
Catalog number	HM2079-100UG		
Lot number	-	**Expiry date**	-
Volume	1 ml	**Amount**	100 µg
Formulation	0.2 µm filtered in PBS+0.1%BSA	**Concentration**	100 µg/ml
Host Species	Mouse IgG1	**Conjugate**	None
Endotoxin	<24 EU/mg	**Purification**	Protein G
Storage	4°C		

Application notes

	IHC-F	IHC-P	IF	FC	FS	IA	IP	W
Reference #	4				2	1,2,3		
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

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. For functional studies, in vitro dilutions have to be optimized in user's experimental setting.

- IHC-P: No pretreatment was used on human tissue sections. Antibody was used at a concentration of 1 µg/ml. As positive control human granulocytes were used.
- IHC-F: Tissue sections were fixed in Histochoice MB (Amresco) and blocked with DakoCytomation Protein Block. Pretreatment with levamisole solution was performed to quench endogenous phosphatases. As positive control drusen eye sections was used and as negative control sections lacking the first antibody (Ref.4).
- FS: Antibody 2952 functions as a neutralizing antibody blocking the interaction between C5a and its receptor. The antibody was functionally tested by competitive binding studies using [125I]C5a and Bt2-cAMP differentiated U937 cells (Ref.2).The biological activity can be defined as the ability of antibody to displace C5a from U937 cell surface.
- Positive control Human Granulocytes.
- General comment: Please notice that under given conditions it is known that C5 can expose epitopes normally only found in the cleaved activation products (ref.5).

General Information

Description The monoclonal antibody 2952 recognizes a neo-epitope on human complement protein C5a/C5a des-Arg. C5 is involved in the activation of the lytic pathway within the complement system which is an important factor in innate immunity. The activation pathways lead via C3 to the cleavage of the fifth complement component C5. During complement activation, C5 is proteolytically cleaved and the anaphylatoxic peptide C5a is generated. C5a is a small polypeptide consisting of 74 amino acids (~11 kDa) and is derived from the N-terminus of the α-chain. C5a itself is very short-lived and in serum is cleaved rapidly into the more stable, though biologically still active C5a-desArg (also called acylation stimulating protein, ASP). C5a acts as a potent anaphylatoxin causing smooth muscle contraction, vasodilatation, increased vascular permeability, basophil and mast cell degranulation and lysosomal enzyme release. In addition, C5a is a potent chemotactic factor for neutrophils, eosinophils, basophils and monocytes. C5a is involved in inflammatory reactions seen in gram-negative bacterial sepsis, trauma, ischemic heart disease, post-dialysis syndrome and a variety of autoimmune diseases. Elevation of C5a is associated with increased cardiovascular risk in patients with advanced atherosclerosis. Also, C5a is closely associated with the capillary leak syndrome in leukemic children after bone marrow transplantation. C5a is also a marker in urine for predicting the onset of acute graft rejection after kidney transplantation. Monoclonal antibody 2952 is a subclone of clone 2925. Please be aware that under given conditions it is known that C5 can expose epitopes normally only found in the cleaved activation products (ref.5).

Immunogen ISHKDMQLG (C5a amino acid 65-73)

Cross reactivity Human C5: No

References

1. Hartmann, H et al; ;Rapid quantification of C3a and C5a using a combination of chromatographic and immunoassay procedures. J Immunol Meth 1993, *166*: 35

2. Kola, A et al; Epitope mapping of a C5a neutralizing mAb using a combined approach of phage display, synthetic peptides and site-directed mutagenesis. Immunotech 1996, *2*: 115
3. Stöve, S et al; Circulating complement proteins in patients with sepsis or systemic inflammatory response syndrome. Clin Diag Lab Immunol 1996, *3*: 175
4. Nozaki, M et al; Drusen complement components C3a and C5a promote choroidal neovascularization. Proc Natl Acad Sci USA 2006, *103*: 2328
5. Nilsson, P et al, A novel human whole blood model preventing fibrin formation reveals that thrombin does not cleave C5 under physiological conditions. Abstract Mol Imunol 2018, *102*: 194

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	02/12/2019

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