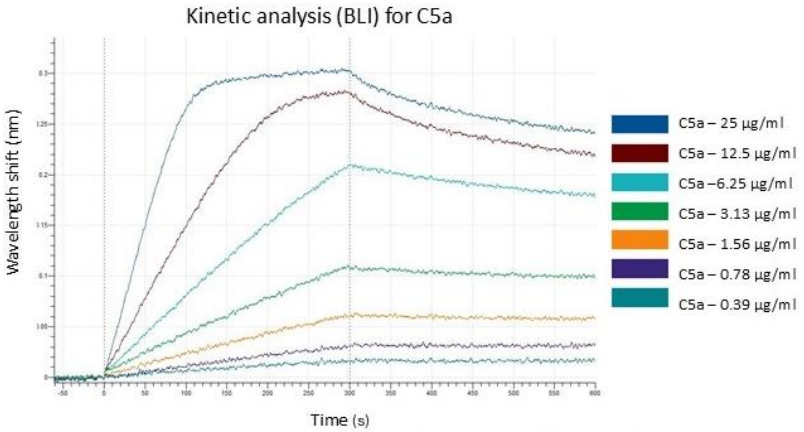
CERTIFICATE OF ANALYSIS – TECHNICAL DATA SHEET

Product name	C5a/C5a-desArg/C5, Human, mAb C17/5		
Catalog number	HM2445-100UG		
Lot number	-	**Expiry date**	-
Volume	1 ml	**Amount**	100 µg
Formulation	0.2 µm filtered in PBS+0.02%NaN3+0.1%BSA	**Concentration**	100 µg/ml
Host Species	Mouse IgG1	**Conjugate**	None
Endotoxin	x.x EU/mg	**Purification**	Protein G
Storage	4°C		

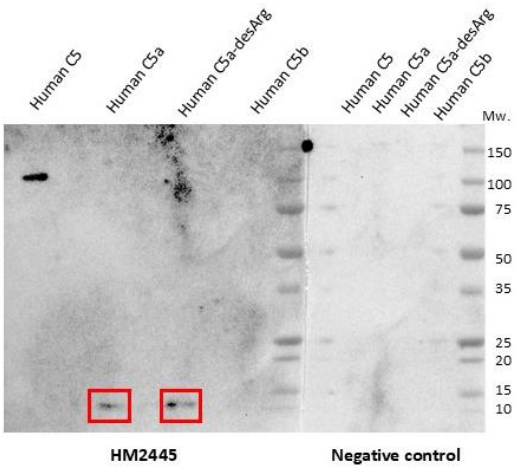
Application notes

	IHC-F	IHC-P	IF	FC	FS	IA	IP	BLI	W
Reference #					1	1-4			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; BLI = Bio-layer Interferometry, W = Western blot



Protein	ka (1/Ms)	kdis (1/s)	KD (M)	R^2
C5	2.8E05	2.0E-04	7.1E-10	0.9994
C5a	2.4E05	4.7E-04	2.0E-09	0.9992
C5a-desArg	6.2E05	9.7E-04	1.6E-09	0.9785



BLI: The kinetic parameters of antibody C17/5 were determined for native human C5, C5a and C5ades-Arg using BLI (Octet R8). The C17/5 antibody was immobilized on the sensors and kinetic parameters were measured against the proteins in solution. Curve fitting and calculations of kinetic parameters were executed in a 1:1 model using the Octet Analysis Studio Software version 13.0.2.46. The graph represents the association-dissociation curves for C17/5 against C5a.

W: Western blot analysis performed with reduced Human C5, C5a, C5a-desArg and C5b with antibody C17/5 at 2 µg/ml.

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.

- FS: Antibody C17/5 can inhibit C5a activity in vitro when C5a was absorbed in a 9x-fold molar excess of C17/5 prior to functional testing (Ref. 1).
- IA: Antibody C17/5 can be used as capture antibody in ELISA (Ref. 1 – 4).
- W: A reduced sample treatment and SDS-Page was used to visualize C5a/C5a-desArg. The expected band size is 11 kDa (Ref. 1).

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. 1, 5).

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General Information

Description

Mouse monoclonal antibody clone C17/5 recognizes human complement protein C5a/C5a-desArg/C5.
The complement system is a key component of innate immunity and consists of more than 30 plasma and membrane-associated proteins that cooperate to detect and eliminate pathogens, damaged cells, and immune complexes. Activation occurs through the classical, lectin, or alternative pathway. Despite their different initiation mechanisms, all three pathways converge at the cleavage of complement component C3 and subsequently lead to the formation of C5 convertases and cleavage of C5, a 190 kDa circulating glycoprotein primarily synthesized in the liver. C5 consists of a disulfide-linked α-chain (~111 kDa) and β-chain (~75 kDa). Proteolytic cleavage of C5 produces the fragments C5a and C5b. Whereas C5b initiates formation of the membrane attack complex (MAC; TCC, C5b–9), which forms cytolytic pores in target membranes, C5a functions as a potent inflammatory mediator that orchestrates immune cell recruitment and activation.
C5a is a 74–amino acid polypeptide (9.8KDa) derived from the N-terminus of the C5 α-chain and represents one of the most potent anaphylatoxins generated during complement activation. In circulation, C5a is rapidly converted by serum carboxypeptidases into the more stable but still biologically active derivative C5a-desArg. In humans C5a exerts its biological effects primarily through two seven-transmembrane receptors: C5aR1 (CD88) and C5aR2 (C5L2/GPR77). These receptors are widely expressed, particularly on immune cells such as neutrophils, macrophages, monocytes, and T cells. Activation of C5aR1 initiates G-protein–coupled signaling pathways that stimulate the production of pro-inflammatory cytokines including TNF-α, IL-1β, IL-6, and IL-8. Through these signaling pathways, C5a induces smooth muscle contraction, increases vascular permeability, promotes mast cell and basophil degranulation, and stimulates the release of lysosomal enzymes. In addition, it acts as a powerful chemoattractant, directing these cells to migrate to sites of inflammation.
Although these responses are essential for host defense, excessive C5a generation contributes to the pathogenesis of numerous inflammatory and immune-mediated diseases, including sepsis, ischemia-reperfusion injury, autoimmune disorders, and cardiovascular disease. Consequently, the C5–C5a axis has emerged as an important therapeutic target. Inhibition of complement activation at the level of C5, using e.g. eculizumab, prevents the generation of C5a and the formation of the terminal complement complex. This makes complement inhibition a promising strategy for controlling complement-driven inflammation.
Antibody HM2445 is, at least but not limited to, applicable in the following applications: ELISA, BLI and western blotting. Please, contact Hycult Biotech for further information.

Immunogen

Purified human C5a

Aliases

C5a, Complement component 5a, human C5a.

Cross reactivity

Species:
Non-human primate: Yes;
Porcine: Yes

References

1. Oppermann, M., et Al; A sensitive enzyme immunoassay for the quantitation of human C5a/C5a(desArg) anaphylatoxin using a monoclonal antibody with specificity for a neotope, 1991. Complement Inflamm. 8(1):13-24
2. Oppermann, M., et Al; Assessment of complement activation in vivo, 1992. Immunopharm. 24, 119-134
3. Mollnes, T.E., et Al; Complement activation in septic baboons detected by neoepitope-specific assays for C3b/iC3b/C3c, C5a and the terminal C5b-9 complement complex (TCC), 1993. Clin Exp Immunol 91,295-300
4. Ammon, H. P. T., et Al; Improvement in the Long Term Stability of an Amperometric Glucose Sensor System by Introducing a Cellulose Membrane of Bacterial Origin, 1995. Anal. Chem. 67, 466-471
5. Nilsson, P., et Al; A novel human whole blood model preventing fibrin formation reveals that thrombin does not cleave C5 under physiological conditions, 2018. Abstract Mol Immunol *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.

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Approved by Manager of QC

Date

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