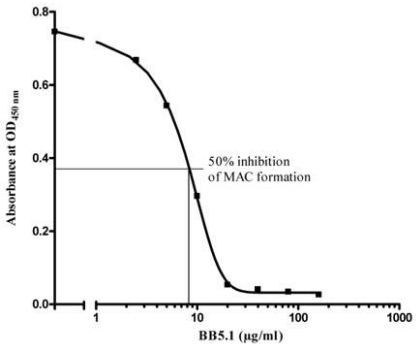
CERTIFICATE OF ANALYSIS – TECHNICAL DATA SHEET

Product name	C5, Mouse, clone BB5.1		
Catalog number	HM1073-10MG		
Lot number	-	**Expiry date**	-
Volume	-	**Amount**	10 mg
Formulation	0.2 µm filtered in PBS	**Concentration**	>0.5 mg/ml
Host Species	Mouse IgG1	**Conjugate**	None
Endotoxin	<24 EU/mg	**Purification**	Protein G
Storage	4°C		

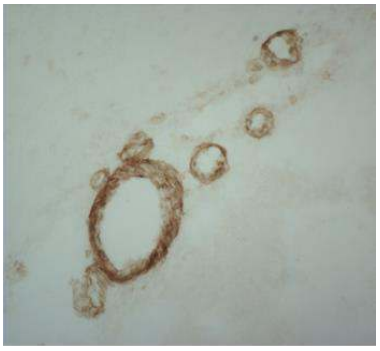
Application notes

	IHC-F	IHC-P	IF	FC	FS	IA	IP	W
Reference #	3	4,11	8,9		2,4,5,7,10,12	1,6	1	
Yes	●	●	●		●	●	●	
No								
N.D.				●				●

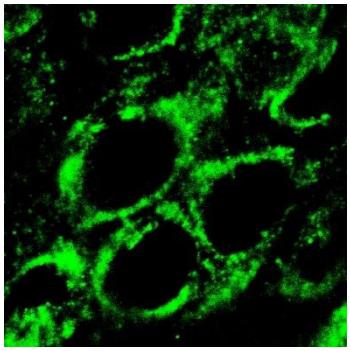
N.D.= Not Determined; IHC = Immuno histochemistry; F = Frozen sections; P = Paraffin sections; IF = Immuno Fluorescence; FC = Flow Cytometry; FS = Functional Studies; IA = Immuno Assays; IP = Immuno Precipitation; W = Western blot



FS: HM1073 inhibits membrane attack complex formation as determined using an anti-C9 polyclonal antibody.



IHC-F: Frozen section of rat brain, hippocampal area, 20x objective. Dilution of HM1073 used was 100x.



IHC-F: perfused frozen section of rat brain, hippocampus. C5 staining around neurons. Dilution of HM1073 used was 100x.

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: ex vivo: 50µg mAb / ml serum; in vivo:BALB/c mice 40mg mAb/kg/day, 0-2 days followed by twice a week
- IA: Wells were coated with 2-4µg purified BB5.1 in PBS as coat antibody for use in ELISA.
- IP: Mouse serum was pre-adsorbed with αmouse-Ig affinity gel and prot-A-sepharose for 30'at RT. Then C5 was precipitated with BB5.1 (10µg mAb/ml followed by prot-A sepharose for 1h. Precipitated samples were analysed on 7% SDS/PAGE.

General Information

Description

The monoclonal antibody BB5.1 binds the fifth component of mouse complement (C5). The complement system is a group of plasma and cell membrane proteins that play a key role in the immune system. All three pathways (classical, alternative & lectin) lead to the cleavage of C3 and eventually the formation of the cytolytic membrane attack complex C5b-9. If the activation cascade is allowed to proceed beyond the cleaving of C3 into C3a and C3b, an additional C3b molecule binds to the C3 convertases. This generates the C5 convertase (C3bBbC3b for the alternative pathway and C4bC2bC3b for the classical and lectin pathways). C5 convertase cleaves C5, releases the potent anaphylactic peptide C5a and generates C5b. C5b can initiate the terminal pathway , which recruits the components C6, C7, C8 and C9 to the surface of the target and inserts the C9 complex as a pore (termed the terminal complement complex) into the membrane. C5a is the most potent anaphylatoxin and a powerful chemotaxin for neutrophils and monocytes, with the ability to promote margination, extravasation, and activation of these cells. Thus, blocking C5 may be required for optimal inhibition of the inflammatory response. At the same time, inhibition of the complement cascade at C5 does not impair the generation of C3b through the classical and alternative pathways, preserving C3b-mediated opsonization of pathogenic microorganisms as well as opsonization and solubilization of immune complexes.

C5 is synthesised in the liver as a single polypeptide chain and is present in serum in a concentration of 50-80 µg/ml. 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). Monoclonal antibody BB5.1 has been shown to precipitate the two chains of C5 from normal mouse serum and inhibited C5-dependent hemolysis in a functional complement test. Furthermore, BB5.1 administration completely inhibits terminal complement activity in murine models for antibody-mediated rejection (AMR) during heart and kidney transplantation. In another mouse model, both pre-treatment as well as intervention with monoclonal antibody BB5.1 attenuates disease development during anti-MPO IgG-induced glomerulonephritis. Blockage of C5 activation by BB5.1 protects against renal ischemia-reperfusion injury by inhibition of late apoptosis and inflammation. In Lupus disease, combination therapy of anti-IL-10/anti-C5 (BB5.1) can both prevent and reduce the effect of the humoral immune response.

Immunogen Purified C5

Aliases Complement C5, Hemolytic complement

Gene Gene name: C5

Cross reactivity Rat C5: Yes.

References

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2. Thurman, J et al; C3a is required for the production of CXC chemokines by tubular epithelial cells after renal ischemia/reperfusion. J Immunol 2007, *178:* 1819
3. Mihai, S et al; The alternative pathway of complement activation is critical for blister induction in experimental epidermolysis bullosa acquisita. J Immunol 2007, *178*: 6514
4. Wang, H et al; Inhibition of terminal complement components in presensitized transplant recipients prevents antibody-mediated rejection leading to long-term graft survival and accommodation. J Immunol 2007, *179*: 4451
5. Huugen, D et al; Inhibition of complement factor C5 protects against anti-myeloperoxidase antibody-mediated glomerulonephritis in mice. Kidney Int 2007, *71*: 646
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7. Copland, D et al. Systemic and local anti-C5 therapy reduces the disease severity in experimental autoimmune uveoretinitis. Clin exp immunol 2010, *159*:303
8. Pavlovski, D et al; Generation of complement component C5a by ischemic neurons promotes neuronal apoptosis, Faseb 2012, *26*: 3680
9. Denny, K et al; C5a Receptor Signaling Prevents Folate Deficiency–Induced Neural Tube Defects in Mice. J Immunol 2013, *190*
10. Sommaggio, R et al; Inhibition of complement component C5 protects porcine chondrocytes from xenogeneic rejection. Osteoarth and Carti 2013, *21*:1958
11. Liu, Q et al; Stroke Damage Is Exacerbated by Nano-Size Particulate Matter in a Mouse Model. PLOS One 2016
12. Lagumersindez-Denis, N et al; Differential contribution of immune effector mechanisms to cortical demyelination in multiple sclerosis. Acta Neuopath 2017, *134*:15

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
Brenda Teunissen	07/10/2019

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