

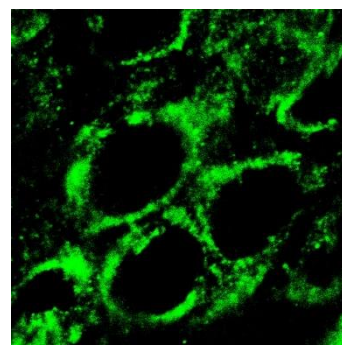
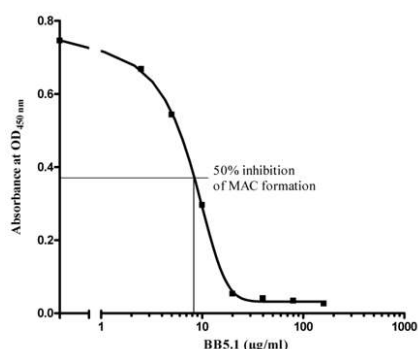
## CERTIFICATE OF ANALYSIS – TECHNICAL DATA SHEET

|                       |                        |                      |            |
|-----------------------|------------------------|----------------------|------------|
| <b>Product name</b>   | C5, Mouse, clone BB5.1 |                      |            |
| <b>Catalog number</b> | HM1073-100MG           |                      |            |
| <b>Lot number</b>     | -                      | <b>Expiry date</b>   | -          |
| <b>Volume</b>         | -                      | <b>Amount</b>        | 100 mg     |
| <b>Formulation</b>    | 0.2 µm filtered in PBS | <b>Concentration</b> | >0.5 mg/ml |
| <b>Host Species</b>   | Mouse IgG1             | <b>Conjugate</b>     | None       |
| <b>Endotoxin</b>      | <24 EU/mg              | <b>Purification</b>  | Protein G  |
| <b>Storage</b>        | 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 amouse-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.

|                              |   |
|------------------------------|---|
| <b>Immunogen</b>             | Purified C5   |
| <b>Aliases</b>               | Complement C5, Hemolytic complement   |
| <b>Gene</b>                  | Gene name: C5   |
| <b>Cross reactivity</b>      | Rat C5: Yes.  |
| <b>References</b>            | <ol style="list-style-type: none"><li>1. Frei, Y et al; Generation of a monoclonal antibody to mouse C5 application in an ELISA assay for detection of anti-C5 antibodies. <i>Mol Cell Probes</i> 1987, <i>1</i>: 141</li><li>2. Thurman, J et al; C3a is required for the production of CXC chemokines by tubular epithelial cells after renal ischemia/reperfusion. <i>J Immunol</i> 2007, <i>178</i>: 1819</li><li>3. Mihai, S et al; The alternative pathway of complement activation is critical for blister induction in experimental epidermolysis bullosa acquisita. <i>J Immunol</i> 2007, <i>178</i>: 6514</li><li>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. <i>J Immunol</i> 2007, <i>179</i>: 4451</li><li>5. Huugen, D et al; Inhibition of complement factor C5 protects against anti-myeloperoxidase antibody-mediated glomerulonephritis in mice. <i>Kidney Int</i> 2007, <i>71</i>: 646</li><li>6. Nilsson, K et al. Enhanced susceptibility to low-dose collagen-induced arthritis in CR1/2-deficient female mice – possible role of estrogen on CR1 expression. <i>Faseb</i> 2009, <i>23</i>: 2450</li><li>7. Copland, D et al. Systemic and local anti-C5 therapy reduces the disease severity in experimental autoimmune uveoretinitis. <i>Clin exp immunol</i> 2010, <i>159</i>:303</li><li>8. Pavlovski, D et al; Generation of complement component C5a by ischemic neurons promotes neuronal apoptosis, <i>Faseb</i> 2012, <i>26</i>: 3680</li><li>9. Denny, K et al; C5a Receptor Signaling Prevents Folate Deficiency–Induced Neural Tube Defects in Mice. <i>J Immunol</i> 2013, <i>190</i></li><li>10. Sommaggio, R et al; Inhibition of complement component C5 protects porcine chondrocytes from xenogeneic rejection. <i>Osteoarthritis and Cartilage</i> 2013, <i>21</i>:1958</li><li>11. Liu, Q et al; Stroke Damage Is Exacerbated by Nano-Size Particulate Matter in a Mouse Model. <i>PLOS One</i> 2016</li><li>12. Lagumersindez-Denis, N et al; Differential contribution of immune effector mechanisms to cortical demyelination in multiple sclerosis. <i>Acta Neuopath</i> 2017, <i>134</i>:15</li></ol> |
| <b>Storage&amp;stability</b> | Product should be stored at 4°C. Under recommended storage conditions, product is stable for at least one year.   |
| <b>Precautions</b>           | 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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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  
Brenda Teunissen

Date  
07/10/2019

Do you have any questions or comments regarding this product? Please contact us via [support@hycultbiotech.com](mailto:support@hycultbiotech.com).