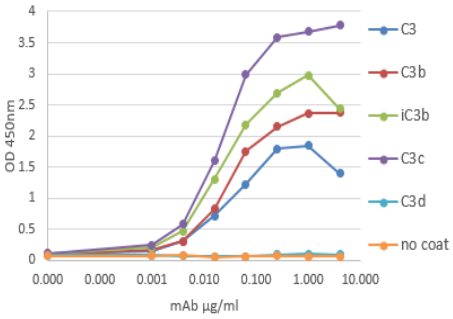
# CERTIFICATE OF ANALYSIS – TECHNICAL DATA SHEET

| | | | |
|---|---|---|---|
| **Product name** | C3, C3b, iC3b, C3c, Human, clone C3-16.4 | | |
| **Catalog number** | HM2401-100UG | | |
| **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 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 # | | | | | 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*




| coat 500 ng/ml | protein C3 | C3b | iC3b | C3c | C3d | no coat |
|---|---|---|---|---|---|---|
| µg/ml detection ab | *1* | *2* | *3* | *3* | *3* | *4* |
| 4.000 | 1.398 | 2.373 | 2.435 | 3.778 | 0.097 | 0.066 |
| 1.000 | 1.848 | 2.363 | 2.972 | 3.683 | 0.104 | 0.068 |
| 0.250 | 1.796 | 2.147 | 2.695 | 3.582 | 0.091 | 0.079 |
| 0.063 | 1.230 | 1.750 | 2.181 | 2.988 | 0.070 | 0.063 |
| 0.016 | 0.713 | 0.826 | 1.304 | 1.600 | 0.070 | 0.056 |
| 0.004 | 0.313 | 0.308 | 0.476 | 0.581 | 0.079 | 0.081 |
| 0.001 | 0.153 | 0.178 | 0.210 | 0.247 | 0.083 | 0.072 |
| blank | 0.109 | 0.096 | 0.110 | 0.121 | 0.081 | 0.071 |

IA: direct ELISA with antibodyC3-16.4 to determine specificity. Purified proteins were used as coating in a concentration of 500 ng/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.

- IA: antibody C3-16.4 was used in a direct ELISA.
- FS: the antibody blocks the formation of C3 convertase by alternative pathway (Ref.1)
- C3-16.4 recognizes a region around MG2 and MG6, and projects outward the C3b molecule (Ref.1)

## General Information

**Description**

The antibody clone C3-16.4 recognize complement C3, C3b, iC3b and C3c. The recognition domain is determined to MG2-MG6. The antibody blocks alternative pathway C3-covertase formation. 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 before an adaptive response can occur. It consists of a complex family of proteins and receptors which are found in the circulation, in tissues and other body-fluids. There are three pathways of complement activation. The classical pathway is initiated by Immune complexes; the lectin pathway by surface bound lectins; and the AP by all the surfaces that are not specifically protected against it. Each generates a C3 convertase, a serine protease that cleaves the central complement protein C3, and generates the major cleavage fragment C3b. The C3 and C5 convertases are enzymatic complexes that initiate and amplify the activity of the complement pathways and ultimately generate the cytolytic MAC. The synthesis of C3 is tissue-specific and is modulated in response to a variety of stimulatory agents. After cleavage by C3 convertase the anaphylotoxin C3a and activating C3b are formed. n the presence of complement regulatory molecules C3b may be further degraded sequentially to iC3b, C3c, C3dg and C3d. The disadvantage of most complement biomarkers is their short half-life, making reliable sample collection and measurements difficult. Unlike other C3 fragments, C3c does not bind to other structures like pathogens, cell surface (receptors) and other plasma proteins. Therefore, C3c is a stable complement biomarker which will appear in the fluid

| | |
|---|---|
| | phase only, without interference of other C3 based products. C3 is primarily produced by the liver but is also generated in macrophages, neutrophils, endothelial and epithelial cells. Due to the high levels in circulation with low biological reactivity, C3 is able to act in a fast and potent way when danger by e.g. pathogens is encountered. Defects in C3 can be unfavorable to the host leading to recurrent infections or auto-immune diseases. Although rare, C3 deficiency has been reported. These patients suffer from recurrent infections of eg S.pneumoniae or N.meningitidis due to lack of opsonization, but also impaired DC and Treg development. Polymorphism in C3 has been associated with AMD and aHUS. Besides clearance of pathogens, C3 is also important in removal of circulating immune-complexes by assisting the phagocytic capacity of macrophages. Malfunction of this system can lead to development of auto-immune disease and complement deposition in tissues. |
| **Immunogen** | Mixture of the human activated C3 fragments, C3b, iC3b, and C3dg, emulsified in complete Freund's adjuvant. Subsequently, mice were boosted three times at 2-week intervals with the same amount of C3 fragments in incomplete adjuvant. |
| **Aliases** | Complement component C3, C3b, iC3b, C3c |
| **References** | 1. Hildago, M et al; Functional and structural characterization of four mouse monoclonal antibodies to complement C3 with potential therapeutic and diagnostic applications. Eur J Immunol 2017, *47*:504 |
| **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

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