

# **CERTIFICATE OF ANALYSIS - TECHNICAL DATA SHEET**

Product name C3b/iC3b/C3c, Mouse, clone 2/11

Catalog number HM1065-20UG

Lot number - Expiry date -

**Volume** 200 μl **Amount** 20 μg

Formulation 0.2 µm filtered in PBS+0.1%BSA Concentration 100 µg/ml

Host Species Rat IgG1 Conjugate None

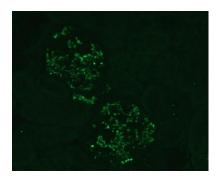
Endotoxin <24 EU/mg Purification Protein G

Storage 4°C

## **Application notes**

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



IHC-F: C3 fragments in frozen kidney tissue using clone 2/11 (Cat.# HM1065). C3 activation products are deposited on the glomeruli.

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.

- F: Fixation of sections in acetone for 5', pretreatment with 3% hydrogen peroxide in methanol at RT to quench endogenous peroxidases. As negative control, sections from C3-deficient mice were used. (Ref.1)
- FC: Mouse splenocytes were incubated with autologous, freshly drawn mouse serum to fix the C3 fragments before staining. As negative control splenocytes of C3 deficient mice was used. Antibody 2/11stains the neoantigenic site on the C3 activation fragments. (Ref.1)
- FS: Antibody 2/11 inhibits hemolysis in a dose-dependent manner. (Ref.1)
- W: A non-reduced sample treatment and SDS-PAGE was used. The band size is 150 kDa (Ref.1).
- IA: Supernatant of antibody 2/11 was incubated on anti-rat IgG Fc coated plates. After incubation with sample, HRP-conjugated polyclonal goat anti-mouse C3 was used for detection. (Ref.1)
- Positive control: Mouse splenocytes preincubated with serum from the same animal; Negative control: C3-deficient mouse cells

## **General Information**

### Description

The monoclonal antibody 2/11 recognizes activated fragments of the mouse complement protein C3. The complement system is an important part of the humoral response in innate immunity, consisting of three different pathways. The third complement component, C3, is central to the classical, alternative and lectin pathways of complement activation. Activation products of the complement cascade contain neo-epitopes that are not present in the individual native components.

The synthesis of C3 is tissue-specific and is modulated in response to a variety of stimulatory agents. C3 is the most abundant protein of the complement system with serum protein levels of about 1.3 mg/ml. An inherited deficiency of C3 predisposes the person to frequent assaults of bacterial infections. In ulcerative colitis, and idiopathic chronic inflammatory bowel disease, the deposition of C3 in the diseased mucosa has been reported.

Version: 05-2021

Proteolysis by certain enzymes results in the cleavage of C3 into C3a and C3b. C3b becomes attached to immune complexes and is further cleaved into iC3b, C3c, C3dg and C3f. The monoclonal antibody 2/11 is specific for cleaved C3 fragments C3b, iC3b, and C3c, and for activated C3. Therefore, positive reactivity in tissues is associated with activation of the complement cascade and C3 cleavage. In case of an acute inflammatory reaction lots of C3 are processed into the products recognized by 2/11 and is as such useful as marker for inflammatory reaction. In chronic inflammatory conditions minimal reactivity with 2/11 may observed. In such cases primarily the C3dg product resides at the place of inflammation (C3c being cleared) which is not recognized by antibody 2/11. The chronic processing/activation of C3 is taking place at a lower level, which would reduce detection of the C3 fragments C3b, iC3b, and C3c. Next to detection of C3 fragments C3b, iC3b, and C3c, the monoclonal antibody 2/11 inhibits the hemolytic activity of mouse complement in a dose-dependent manner.

#### **Immunogen**

Mouse C3

#### References

- 1. Mastellos, D et al; Novel monoclonal antibodies against mouse C3 interfering with complement activation: description of fine specificity and applications to various immunoassays. Mol Immunol 2004, 40: 1213
- Markiewski, M et al; C3a and C3b activation products of the third component of complement (C3) are critical for normal liver recovery after toxic injury. J Immunol 2004, 173: 747
- 3. Markiewski, M et al, Modulation of the antitumor immune response by complement. Nat Immunol 2008, 9: 1225

#### 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 Brenda Teunissen

Date 13/07/2021

Do you have any questions or comments regarding this product? Please contact us via <a href="mailto:support@hycultbiotech.com">support@hycultbiotech.com</a>.

Version: 05-2021