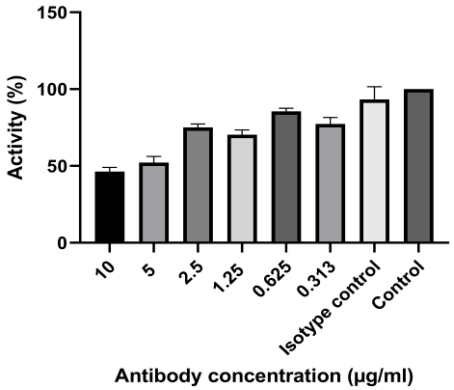
CERTIFICATE OF ANALYSIS – TECHNICAL DATA SHEET

Product name	C3b/iC3b, Human, clone 3E7		
Catalog number	HM2286-1MG		
Lot number	xxxxxXxxxx-X	**Expiry date**	MMM YYYY
Volume	xx ml	**Amount**	1 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 #			1-3,8	1-5	1-7			
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: Alternative pathway inhibition by anti C3/iC3b antibody (HM2286)

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: The inhibition of the alternative pathway by HM2286, targeting C3/iC3b, was evaluated at varying concentrations using pooled human serum within the HK3012 human Alternative Complement Pathway assay. An isotype control (MOPC-21, BioLegend) was also included to discern isotype-specific interactions. The pooled human serum served as a control.

General Information

Description

Monoclonal antibody 3E7 recognizes human complement C3b/iC3b and blocks activation of the alternative pathway (AP). 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. There are three pathways of complement activation. The classical pathway is initiated by Immune complexes; the lectin pathway by surface bound mannan binding lectin; 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. Upon activation of C3 two fragments are generated. The smaller anaphylatoxin C3a and the larger short lived C3b. The latter is highly reactive and can bind to complement-activating particles or immune-complexes. Unlike the classical pathway, the AP is in state of continuous activation. The AP plays an important role in tissues damage and inflammation associated with certain autoimmune diseases and with ischemia-reperfusion injury. Increasing evidence suggests blocking activation of AP can prevent or reduce certain disease pathologies and maintain host defense afforded by CP and LP. Deposition of C3b on cell surfaces can opsonize cells for destruction. Cell bound C3b can be degraded to inactive forms, iC3b and then C3dg. Antibody 3E7 shows enhanced specificity for C3b(i) attached to a cell surface and it can bind to C3b(i)-

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opsonized cells in whole blood. The antibody blocks AP based on its capacity to prevent C3b deposition on the surface of a variety AP activators and also inhibits AP-promoted lysis of rabbit erythrocytes, as used in the standard AP50 test. Clone 3E7 competes with factor B and H for binding to C3b-opsonized substrates. The use of 3E7 has been shown to enhance the immunotherapeutic action of Rituximab. The CP is not affected or enhanced by this antibody.

Immunogen C3b(i)-Sepharose.

References

1. Kennedy, A et al; An anti-C3b(i) mAb enhances complement activation, C3b(i) deposition and killing of CD20 cells by rituximab. Blood 2003, *101*:3
2. Kennedy, A et al; Rituximab infusion promotes rapid complement depletion and acute CD20 loss in Chronic Lymphocytic Leukemia. Journal of Immunology 2004, *172*:5
3. Pawluczkowycz, A et al; Hematin promotes complement alternative pathway-mediated deposition of C3 activation fragments on human erythrocytes: Potential implications for the pathogenesis of anemia in malaria. Journal of Immunology 2007, *178*:8
4. Risitano, AM; Paroxysmal nocturnal hemoglobinuria and other complement-mediated hematological disorders. Immunobiology 2012, *217*:11
5. Lindorfer, M et al; A novel approach to preventing the hemolysis of paroxysmal nocturnal hemoglobinuria: both complement-mediated cytolysis and C3 deposition are blocked by a monoclonal antibody specific for the alternative pathway of complement. Blood 2010, *115*:11
6. DiLillo, D et al; Selective and efficient inhibition of the alternative pathway of complement by a mAb that recognizes C3b/iC3b. Molecular Immunology 2006, *43*:7
7. Paixão-Cavalvante, D et al; A humanized antibody that regulates the alternative pathway convertase: Potential for therapy of renal disease associated with nephritic factors. Journal of Immunology 2014, *192*:10
8. Beum, P et al; Complement activation on B lymphocytes opsonized with rituximab or ofatumumab produces substantial changes in membrane structure preceding cell lysis. Journal of immunology 2008, *181*:1

Storage&stability Product should be stored at 4°C. Under recommended storage conditions, product is stable for at least one year.

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