

Name:	C3b (Cynomolgus monkey)
Catalog Number:	CY114
Sizes Available:	100 µg/vial
Concentration:	1.0 mg/ml (see Certificate of Analysis for exact conc.)
Form:	Frozen liquid
Purity:	≥ 85% by SDS PAGE
Buffer:	10 mM sodium phosphate, 145 mM NaCl, pH 7.2
Extinction Coeff.	$A_{280\text{ nm}} = 0.974$ at 1.0 mg/ml for pure C3b
Molecular weight:	176,000 Da (2 chains)
Preservative:	None, 0.22 µm filtered.
Storage:	-70°C or below. Avoid freeze/thaw.
Source:	Normal cynomolgus serum from healthy animals of mixed gender
Precautions:	Use normal precautions for handling animal blood products.
Origin:	Manufactured in the USA.

General Description

Cynomolgus monkey C3b (cyno C3b) is derived from native purified cynomolgus monkey C3 (cyno C3, CompTech #CY113) upon cleavage and release of C3a with the alternative pathway C3 convertase. C3b is central to the function of all three pathways of complement (Law, S.K.A. and Reid, K.B.M. (1995)). Initiation of each pathway generates proteolytic enzyme complexes (C3 convertases) which are bound to the target surface. These enzymes cleave a peptide bond in C3 releasing the anaphylatoxin C3a and activating C3b. For a brief time (~60 µs) this nascent C3b is capable of reacting with and covalently coupling to hydroxyl groups on the target surface. Carbohydrates are the favored target, but protein hydroxyls and amino groups also react. This process of tagging the target surface with C3b is called opsonization. The reactive site in nascent C3b is a thioester (Tack B.J., et al. (1980); Pangburn M.K. and MüllerEberhard H.J. (1980)) and C3b is linked to the target through a covalent ester bond (an amide bond is formed if C3b is attached to amino groups). Most of the C3 activated during complement activation never attaches to the surface because its thioester reacts with water forming fluid phase C3b which is rapidly inactivated by factors H and I forming iC3b. Surface-bound C3b is necessary in all three pathways for efficient activation of C5 and formation of C5b-9 complexes that lyse the target cell membrane. Surface-bound C3b and its breakdown products iC3b and C3d are recognized by numerous receptors on lymphoid and phagocytic cells which use the C3b ligand to stimulate antigen presentation to cells of the adaptive immune system. The end result is an expansion of target-specific B-cell and T-cell populations.

Physical Characteristics & Structure

Cynomolgus monkey C3b is composed of two disulfide-linked chains. Analysis of purified cyno C3b by SDS/polyacrylamide gel electrophoresis under non-reduced conditions shows the mobility of cyno C3b to be similar to that of human C3b (176,000 daltons). Under reduced conditions, the migration of the alpha prime chain (α' -chain) of cyno C3b is similar to that of human C3b alpha prime chain (101,000 daltons) while the beta chain migrates slightly ahead of the human C3b beta chain (75,000 daltons).

The concentration of purified cyno C3b is determined by using the calculated extinction coefficient of cyno C3 based on its amino acid sequence using ProtParam and

assumes all pairs of Cys residues form cystines (i.e. a pair of cystine molecules are joined by a disulfide bond).

Function

The biological functions of C3b are described under human C3b.

Assays

Functional activity is determined by cleavage of cyno C3b by the human protease factor I in the presence of the cofactor human factor H. This assay is very convenient as it only requires factor H and I and SDS gels to analyze the cleavage of the alpha prime chain of C3b in 67,000 and 43,000 Da fragments.

Precautions/Toxicity/Hazards

This protein is purified from animal serum and therefore precautions appropriate for handling any animal blood-derived product must be used.

References

Law, S.K.A. and Reid, K.B.M. (1995) Complement 2nd Edition (ISBN 0199633568) Oxford University Press, Oxford.

Tack BF, Harrison RA, Janatova J, Thomas ML, Prahl JW. (1980) Evidence for presence of an internal thioester bond in third component of human complement. Proc Natl Acad Sci U S A. 77:5764-8.

Pangburn M.K. and Müller-Eberhard H.J. (1980) Relation of putative thioester bond in C3 to activation of the alternative pathway and the binding of C3b to biological targets of complement. J Exp Med. 152:1102-14.

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