

Name:	iC3b
Catalog Number:	A115C
Sizes Available:	250 µg/vial
Concentration:	1.0 mg/mL (see Certificate of Analysis for actual concentration)
Form:	Frozen liquid
Purity:	>90% by SDS-PAGE
Buffer:	10 mM sodium phosphate, 145 mM NaCl, pH 7.2
Extinction Coeff.	$A_{280\text{ nm}} = 1.03$ at 1.0 mg/mL
Molecular Weight:	176,000 Da (3 chains)
Preservative:	None, 0.22 µm filtered
Storage:	-70°C or below. Avoid freeze/thaw.
Source:	Normal human serum (shown by certified tests to be negative for HBsAg and for antibodies to HCV, HIV-1 and HIV-II).
Precautions:	Use normal precautions for handling human blood products.
Origin:	Manufactured in the USA.

General Description

iC3b (inactivated C3b) is derived from C3b. Conversion of C3b to iC3b destroys almost all of the functional binding sites present on C3b. C3b itself is produced by all three pathways of complement (Law, S.K.A. and Reid, K.B.M. (1995)) when native C3 is cleaved releasing C3a. iC3b is prepared by cleavage of C3b by factor I in the presence of factor H. Cleavage by factors H and I occurs rapidly when the C3b is free in solution and is slower when it is attached to a surface. Other cofactors for factor I also permit cleavage of C3b to iC3b and these include the two membrane proteins CR1 (CD35) and MCP (CD46). Factor I can cleave C3b in two places in the alpha chain and if both sites are cleaved a small fragment (C3f, 2,000 Da) is released. If the C3b precursor was attached to a surface, the iC3b remains on that surface. The iC3b sold by CompTech is made from fluid phase C3b and is not capable of attaching to a surface. Surface-bound C3b and iC3b are linked to the target through a covalent bond which may be either an ester bond or an amide bond. Ester bonds are unstable resulting in the gradual release from the particle. Most of the C3b generated during complement activation never attaches to a surface because its thioester reacts with water forming fluid phase C3b. Surface-bound iC3b and its breakdown product C3d are recognized by numerous receptors on lymphoid and phagocytic cells which use these ligands to stimulate phagocytosis and antigen presentation to cells of the adaptive immune system. Receptors for iC3b are CR2 (CD21) found on B-cells and CR3 (CD11b/CD18) found on phagocytes (Dodds, A.W. and Sim, R.B. editors (1997); Morley, B.J. and Walport, M.J. (2000)). One of the results of iC3b-receptor interaction is an expansion of target-specific B-cell and T-cell populations.

Physical Characteristics & Structure

Molecular weight: 176,000 daltons composed of three disulfide linked chains. Human iC3b is glycosylated (~2.8%). The alpha prime chain of C3b is cleaved by factor I yielding two fragments (63,000 and 39,000 Da) both of which are disulfide-linked to the beta chain (75,000 Da) which is unchanged. There is some heterogeneity possible depending on whether factor I has cleaved the protein once or twice (Morley, B.J. and Walport, M.J. (2000); Law, S.K.A. and Reid, K.B.M. (1995); Dodds, A.W. and Sim, R.B.

editors (1997); Morgan, B.P. ed. (2000)). If factor I has cleaved twice C3f (2,000 Da) is released and the chains are approximately 61,000, 39,000 and 75,000 Da. The pI of iC3b is approx. 5.7.

Function

As its name implies “inactivated C3b” has lost most of the functions once expressed by C3b. Whereas C3b has binding sites for factor B, factor P, factor H, factor I, C5, DAF (CD55), MCP (CD46) and the receptor CR1, iC3b has undergone a structural change that destroys many of these sites (Gros, P., et al. (2008); Dodds, A.W. and Sim, R.B. editors (1997); Lambris, J.D. (1988)). Most critical, iC3b cannot bind factor B and is thus unable to participate in complement activation. Several activities remain, however, iC3b can still participate in the C5 convertase activity by binding C5, it can still bind properdin and it has acquired the ability to interact with the CR3 receptor important for phagocytosis and antigen presentation for B- and T-cell responses (Ghannam A, et al. (2008)).

Assays

There are no functional assays for iC3b. SDS gels are used to determine the chain structure of the protein.

In vivo

During complement activation C3b arises from the proteolytic cleavage of C3. During aggressive complement activation (in sepsis and at sites of infection) high concentrations of C3b may be formed, but most of it is fluid phase C3b. In blood, factors H and I rapidly cleave C3b forming iC3b. Although iC3b is very sensitive to trypsin-like enzymes it is very long lived in plasma or serum (half-life many hours). Because of the excess of protease inhibitors in plasma there is very little free thrombin, plasmin or other active proteases and iC3b remains as iC3b. In blood, however, the CR1 receptor on human erythrocytes induces a third cleavage by factor I and this releases C3c from C3dg (Dodds, A.W. and Sim, R.B. editors (1997)).

Regulation

iC3b is degraded by two mechanisms. Interaction of the iC3b with the receptor CR1 (CD35) provides the cofactor activity necessary for factor I to cleave iC3b into C3c (139,000 Da) and C3dg (38,000 Da). If iC3b is bound to a surface C3c is released while C3dg remains bound. Also, iC3b is extremely sensitive to trypsin and trypsin-like proteases in serum (plasmin, thrombin, etc.) and in areas where these are active iC3b is cleaved forming C3c and C3dg. These proteases also cleave C3dg releasing C3g (4,000 Da), and sometimes other small fragments, and leaving C3d (32,000 to 34,000 Da).

Genetics

Human chromosome location of the C3 gene is 19p13.3. The mouse chromosome location is chromosome 17 and the rat chromosome 9. Accession numbers K02765 (human) and K02782 (mouse). Human C3 genomic structure: the gene spans 41 kb with 41 exons

Precautions/Toxicity/Hazards

The source of this protein is human serum, therefore precautions appropriate for handling any blood-derived product must be used even though the source was shown by certified tests to be negative for HBsAg, HTLV-I/II, STS, and for antibodies to HCV, HIV-1 and HIV-II. MSDS sheet is available upon request.

References

Dodds, A.W. and Sim, R.B. editors (1997) *Complement. A Practical Approach* (ISBN 019963539) Oxford University Press, Oxford.

Ghannam A, et al. (2008) Human C3 deficiency associated with impairments in dendritic cell differentiation, memory B cells, and regulatory T cells. *J Immunol.* 181:5158-5166.

Gros, P., Milder, F.J., Janssen, B.J. (2008) Complement driven by conformational changes. *Nat Rev Immunol.* 8:48-58.

Lambris, J.D. (1988) The multifunctional role of C3, the third component of complement. *Immunol Today.* 9:387-93.

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

Morgan, B.P. ed. (2000) *Complement Methods and Protocols.* (ISBN 0-89603-654-5) Humana Press, Inc., Totowa, New Jersey.

Morley, B.J. and Walport, M.J. (2000) *The Complement Facts Book* (ISBN 0127333606) Academic Press, London.