Name:	**iC3b (Mouse)**
Catalog Number:	**M115**
Sizes Available:	50 µg/vial
Concentration:	0.5 mg/mL (see Certificate of Analysis for actual concentration)
Form:	Frozen liquid
Purity:	$\geq$ 85% by SDS PAGE
Buffer:	10 mM sodium phosphate, 145 mM NaCl, pH 7.2
Extinction Coeff.	$A_{280\ nm}$ = 0.994 at 1.0 mg/ml for pure iC3b
Molecular weight:	~175,000 Da (3 chains)
Preservative:	None, 0.22 µm filtered.
Storage:	-70°C or below. Avoid freeze/thaw.
Source:	Normal mouse serum from healthy animals of mixed gender
Precautions:	Use normal precautions for handling animal blood products.
Origin:	Manufactured in the USA.

General Description

Mouse iC3b (inactivated C3b) is derived from purified mouse C3b (CompTech #M114). Mouse iC3b is prepared by cleavage of mouse C3b by factor I in the presence of factor H. 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. Mouse 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 if 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 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.

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

Mouse iC3b has a molecular weight of ~175,000 daltons and is composed of three disulfide linked chains. The alpha prime chain of C3b is cleaved by factor I, yielding two fragments (61,000 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.

Using the Novex NuPAGE gel electrophoresis system with MOPS buffer and a 4-12% Tris-Glycine gel, mouse iC3b shows a single peptide band with a mobility like that of mouse C3b (175,000 Da). Under reduced conditions three peptide chains (75,000, 61,000 and 39000 Da) are observed. The 75,000 Da beta chain of mouse iC3b migrates as a ~62,000 Da band and the larger cleavage peptide (61,000 Da) derived from the alpha prime chain of mouse C3b migrates just behind the beta chain of mouse iC3b (see gel image under CoA).

The concentration of purified mouse iC3b is determined by using the calculated extinction coefficient of mouse C3 ($E^{1\%}/_{280}$nm = 9.94) 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

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 importantly, 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.

Precautions/Toxicity/Hazards

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

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.

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