Name:	**C3 Protein Concentrated**
Catalog Number:	**A113c**
Sizes Available:	1000 µg/vial
Concentration:	5.0 mg/mL (see Certificate of Analysis for actual concentration)
Form:	Frozen liquid
Activity:	>70% versus normal human serum standard (see Cert of Analysis).
Purity:	>95% by SDS-PAGE
Buffer:	10 mM sodium phosphate, 145 mM NaCl, pH 7.2
Extinction Coeff.	$A_{280\ nm}$ = 1.03 at 1.0 mg/mL
Molecular Weight:	185,000 Da (2 chains)
Preservative:	None, 0.22 µm filtered
Storage:	-70°C or below. Avoid freeze/thaw (>10 % activity loss per thaw).
Source:	Normal human serum (shown by certified tests to be negative for HBsAg, HTLV-I/II, STS, 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

Concentrated C3 is available primarily because the normal concentration of C3 in serum is high (~1.2 mg/ml) and reconstituting depleted serum thus requires high concentrations of this complement protein. Native human C3 is a naturally glycosylated (~2.7%) polypeptide containing two disulfide-linked chains. C3 is central to the activation of all three pathways of complement activation (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 Müller-Eberhard 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

Molecular weight: 185,000 daltons composed of two disulfide linked chains. The alpha chain is 110,000 daltons (contains C3a and C3d domains) and the beta chain is 75,000 daltons. Alpha and beta chains are linked through a single disulfide bond. The pI of C3 is approx. 5.9

Upon cleavage of C3 by C3 convertases, C3a (77 amino acid fragment, 9083 Da) is released from the N-terminal of the alpha chain and C3b (176,000 Da) becomes attached covalently to the surface of the activator. The crystal-derived structures of both C3 and C3b have been described (Gros, P. (2008)) and these show that large conformational changes occur in the C3b portion of C3 following cleavage of the C3a-C3b peptide bond.

Native C3 and C4 circulate in plasma with intramolecular thioester bonds linking a glycine and a glutamine residue in their C3d or C4d domains. These thioester bonds are susceptible to nuleophilic attack by amines such as ammonia, methylamine, hydroxylamine and hydrazine, all of which have been used to inactivate complement in serum.

CAS Number: 80295-41-6
MDL Number: MFCD00130836

Function

C3 is essential for effective complement activation and subsequent presentation of antigens to the cells of the adaptive immune system (Lambris, J.D. (1988)). Following recognition of a target, complement is activated by one of the three complement pathways and enzymes (C3 convertases) are formed on the target's surface. These enzymes (C4b,C2a or C3b,Bb) cleave C3 after Arg 77 of the alpha chain releasing the anaphylatoxin C3a and depositing C3b on the target surface. Although there is a very weak C3 bypass system that operates through the classical and lectin pathways (C4b,C2a can activate C5 without C3b at about 1/2000 the rate of C3b,C4b,C2a), C3b is necessary for effective C5 activation (Rawal N. and Pangburn M.K. (2003)).

Purified C4 is extremely sensitive to freeze/thaw losing 5-10% of its activity with each freeze/thaw cycle. It is also sensitive to intermediate temperatures such as -20^{o}C. The longer it remains at intermediate temperatures the more activity is lost. A few hours at -20^{o}C can completely inactivate it, even though it remains completely frozen.

Assays

Complement activation requires C3. Typical assays for C3 function therefore use cell lysis endpoints in systems that lack C3 except from the source being assayed. There are three basic assays. 1) Antibody-sensitized sheep erythrocytes (EA) can be used in a CH50-type assay using C3-depleted human serum. The sensitivity of this assay is approximately 50 ng C3. 2) EA and purified components C1, C4 and C2 can be used to make EAC142 cells which utilize C3 for effective C5 activation and lysis (Dodds, A.W. and Sim, R.B. (1997)). The sensitivity of this assay is approximately 5 ng C3. 3) An alternative pathway assay may be used that employs rabbit erythrocytes and C3-depleted human serum in the presence of 5 mM MgEGTA. The sensitivity of this assay is about 200 ng C3.

In vivo

Serum concentration is 1.0 to 1.5 mg/mL with the average of 1.2 mg/mL which makes C3 the most abundant complement protein in blood. It represents approx. 2.5% of the total protein in blood and excluding albumin and immunoglobulins it is ~8% of the protein present in plasma. The primary site of synthesis is the liver, but C3 is also made

in macrophages, neutrophils, astrocytes, and in endothelial and epithelial cells in many tissues of the body.

Regulation

Biosynthesis of C3 is upregulated in most cells that synthesize C3 by IL-1, IL-6, TNF-alpha, and LPS. Neutrophils are downregulated by IL-1 and IFN-gamma

Genetics

Human chromosome location 19p13.3-p13.2. Mouse chromosome location chromosome 17 and rat chromosome 9. Accession numbers K02765 (human) and K02782 (mouse). Human genomic structure: the gene spans 41 kb with 41 exons

Deficiencies

Complete human C3 deficiencies are rare but a number of cases have been found. Importantly, adults with this condition have been found so although there is a high risk due to impaired immunity, it is not necessarily fatal. A well characterized case of a deficient two year old child demonstrated that the deficiency is associated with recurrent pyogenic infections, impaired dendritic cell differentiation, impaired ability to acquire B cell memory and deficient regulatory T cell development. Vaccination produced only a small, short term antibody response (Ghannam A, et al. (2008)). Other human cases and numerous animal experimental models support these conclusions (Singer, L, et al., (1994)). The association of these immune system defects with C3 deficiency strongly supports a major role for C3 in innate and adaptive immune responses.

The absence of C3 also results in failure to opsonize bacteria resulting in reduced phagocytosis, failure to release C3a and severely reduced ability to generate C5a and C5b which impairs generation the terminal complement complex C5b-9.

Diseases

The deposition of C3, that is, the attachment of C3b to microorganisms or host tissues is the hallmark of complement activation at inflammatory sites. Many diseases exhibit histochemically identifiable C3b deposits as part of their pathology, or at least as markers of pathology (Law, S.K.A. and Reid, K.B.M. (1995); Ross, G.D. (1986)). These diseases include ischemia/reperfusion events such as heart attacks and strokes and bacterial, viral, parasitic and fungal infections. Antibody-mediated autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, and autoimmune hemolytic anemia are characterized by C3b deposition on tissues. Even when the antibody response is not directed at the host complement can be depositied. A major function of complement is to aid the macrophage phagocytic system in the removal of circulating immune complexes. High levels of complexes can overwhelm this system leading to the deposition of complement and the immune complexes in tissues and the kidney leading to glomerulonephritis, dense deposit disease, and arthritis. Variants of C3 that increase or decrease complement activity are associated with diseases such as age-related macular degeneration. C3 deposits also signal complement activation in diseases such as paroxysmal nocturnal hemoglobinuria, inherited hemolytic uremic syndrome, transplant rejection and inflammatory skin diseases such as angioedema.

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.

Hazard Code: B WGK Germany 3

MSDS available upon request.

References

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