Name:	**C6 Protein**
Catalog Number:	**A123C**
Sizes Available:	250 µg/vial
Concentration:	1.0 mg/mL (see Certificate of Analysis for actual concentration)
Form:	Liquid
Activity:	>70% versus normal human serum standard
Purity:	>90% by SDS-PAGE
Buffer:	10 mM sodium phosphate, 145 mM NaCl, pH 7.3
Extinction Coeff.	$A_{280\ nm}$ = 1.08 at 1.0 mg/mL
Molecular Weight:	105,000 Da (single chain)
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

Native human C6 is a naturally glycosylated (11%) protein composed of a single polypeptide chain of 105,000 Da. C6 is essential for formation of the membrane attack complex (MAC) and is activated non-proteolytically by binding to recently-formed C5b at the cell membrane. Each pathway of complement activation generates proteolytic enzyme complexes (C3/C5 convertases) which are bound to the target surface (Law, S.K.A. and Reid, K.B.M. (1995); Ross, G.D. (1986)). These enzymes cleave a peptide bond in the larger alpha chain of C5 releasing the anaphylatoxin C5a and activating C5b. This is the only proteolytic step in the assembly of the C5b-9 complex. C5b is unstable, but it remains bound to the activating complex for a brief time (~2 min) during which it either binds a single C6 from the surrounding fluid or it decays and is no longer capable of forming MAC. The C5b,6 complex may also remain bound to the C3/C5 convertase where the binding of a single C7 exposes a membrane-binding region and C5b,6,7 can partially insert into the bilipid layer of the target cell. Up to this point the complex may diffuse away from the target cell and enter the membrane of a nearby cell. This is called bystander lysis or "reactive lysis" and can be a significant source of pathology. Each C5b-7 complex can bind one C8 protein molecule which results in the complex inserting more firmly into the membrane. This complex binds C9 and each bound C9 can bind another C9 initiating formation of a ring structure containing up to 18 C9 molecules (Podack, E.R. (1984)). C5b-9 complexes with one or more C9 are referred to as the Membrane Attack Complex (MAC) of complement. Not all C5b-8 complexes have complete rings of C9 with the average being only three C9 per C5b-8 complex. Completed protein rings of C9 form the pores seen on electron micrographs and they result in leakage of metabolites and small proteins out of the cell as well as movement of water into the cell. If sufficient numbers are inserted into a cell membrane then water flowing into the cell, due to osmotic pressure, will rupture the cell membrane allowing the entire contents of the target cell (or a bystander cell) to be released. Either process may result in cell death. Originally it was thought that this required only one C5b-9 complex per cell (referred to as the "one hit theory" of lysis (Rommel F.A. and Mayer, M.M. (1973)), but this is probably not correct. For example, an erythrocyte requires

~850 C5b-9 complexes, as measured by the number of C7 molecules, for lysis to occur (Bauer, J. et al. (1979)). Host cells protected from MAC by CD59 require sufficient numbers of C5b-9 to tie up all the CD59 and then ~850 C5b-9 in addition. Lysis of nucleated cells requires many more C5b-9 complexes due to their size and due to the presence of multiple defense mechanisms in such cells.

Physical Characteristics & Structure

Molecular weight: 105,000 Da composed of a single polypeptide chain. The pI of C6 is heterogeneous from 6.0 to 6.5.

CAS Number: 80295-56-3

Function

See General Description above.

Assays

The simplest assay for C6 is to use C6-depleted human serum and measure the lysis of EA (classical pathway) or Er (alternative pathway) as a function of the concentration of added test sample or standard purified C6. Each unique application might require appropriate conditions to be determined. However, a typical assay would involve mixing on wet ice 25 µL C6-Dpl, C6-containing sample diluted with GVB++ to contain from 0.1 to 1 ng C6 , and sufficient GVB++ to bring the volume to 300 µL. EA (3×10^7 cells in 200 µL) diluted in GVB++ should be added last. Purified C6 or normal human serum (NHS) may be used as a source of C6. The reaction mixture is incubated for 30 min at 37ºC and 1 mL of cold GVBE added, mixed and centrifuged to spin out unlysed cells. The released hemoglobin in the supernatant is then analyzed at 415 nm and compared to blanks without C6 (background lysis control) and cells incubated with 275 µL water instead of GVB++ and 25 µL C6-Dpl (100% lysis control).

Many other assays have been described using EA preloaded with C1 (EAC1 cells) or preloaded with the classical pathway C5 convertase (EAC1423 cells), however, all these assays require the use of multiple purified complement components or more difficult-to-prepare reagents (Dodds, A.W. and Sim, R.B. (1997); Morgan, B.P. (2000); Tack, B.F., et al. (1981)).

Applications

See General Description above.

In vivo

The normal serum concentration of C6 is 64 µg/mL (normal range 54 to 72 µg/mL). The primary site of synthesis is the liver. C6 is an acute phase protein and its synthesis is stimulated by the cytokines that stimulate increased biosynthesis of many other complement proteins.

Regulation

Many proteins and other components of plasma have an inhibitory effect on the lytic activity of C5b-9 complexes but there are no specific C6 inactivators. Most of the C5b-9 inhibitors interact with the complex after the C5b-7 stage. If any of the C5b-containing complexes fail to insert into a membrane they may self-aggregate or bind to

regulatory proteins the most prevalent of which is S Protein. S protein (also called vitronectin) is an 80,000 Da plasma protein that binds to C5b-9 complexes that fail to insert in the target cell membrane. This reduces damage to nearby host cells. Many other serum components inhibit or partially inhibit lysis by C5b-9 and these include SP40,40 (also known as clusterin and apolipoprotein J) and many plasma lipoprotein complexes (LDL, HDL, etc.).

Host cells protect themselves from C5b-9 by a variety of mechanisms. Membrane proteins DAF, MCP, and CR1 inhibit formation of C3/C5 convertases preventing MAC formation. CD59, also called "homologous restriction factor" and "protectin", is an 18,000 to 20,000 Da ubiquitous component of cell membranes that is very effective at binding to and inhibiting the lytic potential of C5b-8 and C5b-9 complexes. The species-specificity of CD59 is not absolute and many mammalian CD59 proteins do inhibit or partially inhibit MAC from other species. The specificity that is observed appears to be due to incompatibilities between C8 of one animal and the CD59 of another. Like DAF, CD59 contains a GPI anchor (a post-translationally added lipid tail that inserts into the bilipid layer of the cell). The disease PNH is caused by the loss of enzymes that attach the GPI tail, thus depriving cells of the ability to inactivate C3/C5 convertases and the ability to inactivate C5b-9. This results in complement-mediated damage to and eventual lysis of long-lived blood cells such as erythrocytes and platelets.

Genetics

Human chromosome location 5q 12-14. Accession number HSC6A. Mouse chromosome 15. Human genomic structure: the gene spans 80 kb with 18 exons.

Deficiencies

Human deficiencies have been found and exhibit autosomal recessive transmission. Patients generally exhibit abnormally high susceptibility to recurrent meningococcal meningitis and systemic neisserial infections. Partial deficiencies do not seem to show adverse clinical effects.

Diseases

See Deficiencies above.

Precautions/Toxicity/Hazards

This protein is purified from human plasma, 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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