Name:	**C5b,6 Complex**
Catalog Number:	**A122**
Sizes Available:	50 µg/vial
Concentration:	0.2 mg/mL (see Certificate of Analysis for actual concentration)
Form:	Frozen liquid
Activity:	>10,000 Units/mg for lysis of sheep erythrocytes
Purity:	>90% by SDS-PAGE
Buffer:	10 mM HEPES, 120 mM NaCl, pH 7.2
Extinction Coeff.	$A_{280\ nm}$ = 1.03 at 1.0 mg/mL
Molecular Weight:	285,000 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

C5b,6 is a product of complement activation of C5 and an intermediate in the formation of the C5b-9 membrane attack complex (MAC) of complement. Each pathway of complement 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 highly potent anaphylatoxin C5a (74 amino acids) 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 time it either binds a single C6 from the surrounding fluid to form C5b,6 or it decays and aggregates and is no longer capable of binding C6 or of forming C5b-9 complexes. Normally the C5b,6 complex remains bound to the C3/C5 convertase until it binds a single C7 molecule. However, in the absence of the C7 protein, C5b,6 is released into the fluid phase. C5b,6 is a very stable complex. During complement activation some C5b,6 diffuses away from the target cell and may, after combining with C7, enter the membrane of a nearby cell. This is called bystander lysis or "reactive lysis" and can be a significant source of pathology.

Purified C5b,6 can be used to lyse any bilipid membrane surrounding a cell, virus or particle by mixing C5b,6 with the cell in the presence of a compatible source of C7, C8 and C9. Every cell requires a different amount of C5b,6 and conditions necessary for lysis vary widely depending on experimental conditions. Due to the inefficient insertion of C5b,6,7 complexes into membranes from the fluid phase, as compared to those formed directly on the surface, much higher concentrations of fluid phase C5b,6 are required to lyse cells than if complement is activated directly on that cell's surface.

Purified C5b,6 is prepared by cleavage of human C5 by the natural alternative pathway C3/C5 convertase. Activation is done in the absence of C7 so the resulting C5b,6 complexes are stable. Following isolation, these complexes can be frozen and thawed with little loss of activity. Purified C5b,6 can be used to produce C5b-9 complexes by addition of any compatible source of C7, C8 and C9. If any bilipid-enclosed cells or particles are present a small proportion of the C5b,6,7 complexes will

insert into these bilipid membranes. 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 eighteen 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 approximately 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 approximately 850 more 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. Thus, lysis of different cells with C5b,6 from the fluid phase may require vastly different amounts of C5b,6 depending on the cell type.

Physical Characteristics & Structure

Molecular weight: 285,000 Da. C5b,6 is composed of two disulfide linked chains from C5b (105,000 and 75,000) and one unlinked chain from C6 (105,000). On some SDS PAGE gel systems the two heavy chains (105,000 Da each) can be separated. Although the C5b and C6 components are not held together by covalent bonds this complex is very stable and requires harsh or denaturing conditions

Assays

Many cells may be used to measure the activity of C5b,6. Widely different titers will result with different cells (Morgan, B.P. ed. (2000); Dodds, A.W. and Sim, R.B. editors (1997)). The most convenient cell type is sheep erythrocytes (Es, CompTech #B210) and the most sensitive cell type is chicken erythrocytes. With Es, one unit of lytic activity is defined as the amount of C5b,6 complex required to yield 50% lysis of $1.5x10^7$ sheep erythrocytes when incubated for 30 minutes at 37^oC in a total reaction volume of 75 µL GVBE (10 mM EDTA) containing 500 ng C7, 500 ng C8, and 1000 ng C9. Typically this would require less than 100 ng C5b,6. The presence of other serum proteins will reduce the activity per mg of C5b,6 due to the presence of inhibitors of C5b,6,7 and the subsequent complexes with C8 and C9 .

Applications

C5b,6 may be used to directly assess the sensitivity of cells to the lytic action of complement by bypassing the activation of the complement system on the surface of a cell. It may also be used to measure the function of inhibitors of complement C5b-9 assembly, of insertion into membranes and of the C5b-9 complex itself.

In vivo

In normal blood with C7 present C5b,6 has a very short half-life. In the absence of C7 the C5b,6 complex is very stable and may be isolated from plasma intact and active.

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 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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