Name:	**C1 Complex (PURE)**
Catalog Number:	**A098**
Sizes Available:	200 µg C1 in 1.0 mL
Concentration:	>0.20 mg C1/mL (see Certificate of Analysis for actual concentration)
Form:	Frozen liquid
Activity:	>250,000 C1H50 units/mg total protein
Purity:	>95% by SDS Page
Buffer:	50 mM sodium acetate, 50 mM EACA, 10 mM benzamidine, 10 mM, EDTA, 300 mM NaCl, 40% glycerol, pH 5.5
Extinction Coeff.	$A_{280\ nm}$ = 0.89 at 1.0 mg/mL for pure C1
Molecular Weight:	766,000 Da (22 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, 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

C1 is the first complement component in the cascade referred to as the classical pathway of complement. C1 binds to and is activated by antibodies bound to antigens (immune complexes) yielding a protease that initiates the cascade. C1 is actually a non-covalent complex of three different proteins (C1q, C1r and C1s) bound together in a calcium-dependent complex. C1q binds through two or more of its six arms to the Fc domains of IgG or IgM. The binding of multiple arms to immune complexes is thought to introduce stress which causes the two C1r proteins in the complex (protease zymogens) to auto-activate themselves producing two active C1r serine proteases (Morikis, D. and Lambris, J.D. (2005)). These activated C1r subunits cleave and activate the two C1s protease zymogens in the complex. Activated C1s cleaves complement component C4 releasing C4a and initiating covalent attachment of C4b to the activating surface. Activated C1s also cleaves C2 and the larger fragment of C2 binds to the surface-attached C4b forming C4b,C2a which is the C3/C5 convertase of the classical pathway.

Physical Characteristics & Structure

C1 is a high molecular weight complex (766,000 daltons) composed of one C1q molecule (410,000 daltons), two C1r molecules (92,000 daltons) and two C1s molecules (86,000 daltons). The complex of C1q, C1r and C1s is stable in the presence of calcium, but easily dissociates if calcium is removed. C1q itself is composed of 18 polypeptide chains with six each of three different types of chains. When C1 is activated the C1r and C1s subunits are each cleaved into two chain molecules due to proteolytic activation. Thus, the SDS gel pattern of C1 is very complex.

C1 is difficult to separate from other high molecular weight complexes in serum and extensive handling results in spontaneous activation (Dodds, A.W. and Sim, R.B. (1997)). Thus, the C1 sold by CompTech was not a pure protein. However, we have recently been successful at making a pure preparation of C1 (beginning with Lot # 17).

Function

C1 can be used to prepare cells bearing active C1 on their surface by incubating C1 with EA (Dodds, A.W. and Sim, R.B. editors (1997); Morgan, B.P. ed. (2000)). EA are sheep erythrocytes with rabbit IgM anti-sheep erythrocytes antibodies bound to their surface (CompTech #B200). These cells (EAC1 cells) can be washed with $SGVB^{++}$ buffer (see Assays below) and they maintain bound active C1 if not washed too much or for too long. EAC14 cells may be prepared from EAC1 by addition of C4 which may subsequently be used to make EAC142 cells by addition of C2. Similarly, EAC1 cells can be used to assay C4 or C2 (Morgan, B.P. ed. (2000)).

Assays

The unit of classical pathway activity is the CH50 and the unit of alternative pathway activity is the APH50. Similarly, the unit used to quantitate the activity of C1 is the C1H50. A C1H50 unit is the amount of functional C1 needed to lyse 50% of 3×10^7 EA (CompTech #B200)) when that amount of C1 is incubated with 1 µg C4 and 0.2 µg C2 for 12 min at 30°C in a total volume of 500 µL $SGVB^{++}$ followed by addition of 1 mL of guinea pig serum (GPS) diluted 1:50 in GVB^{o} containing 40 mM EDTA pH 7.2, for 30 min at 37°C. $SGVB^{++}$ buffer is a low ionic strength, slightly hypertonic buffer containing 170 mM sucrose, 0.1% gelatin, 5 mM sodium barbital, 57 mM NaCl, 0.15 mM $CaCl_2$, 0.5 mM $MgCl_2$, and 0.02% sodium azide.

Applications

See section titled Function above.

Regulation

Activated C1 is rapidly inactivated by C1-INH (C1 esterase inhibitor, CompTech #A140) In serum or plasma the spontaneous activation of C1 is minimized by the presence of C1-INH which rapidly inactivates spontaneously activated C1r and C1s. The mechanism is thought to involve the existence of a weak complex between C1-INH and native C1 in plasma. This association apparently stabilizes C1 thus preventing spontaneous activation (Ziccardi, R.J. (1982)). Separation of C1-INH from C1 during purification is one of the reasons that isolated C1 is unstable and prone to spontaneous activation.

Genetics

The EMBL/Genbank cDNA accession numbers are: C1q A chain (P02745), C1q B chain (P02746), C1q C chain (P02747), C1r (M14058, and C1s (J04080). The genes for C1q chains A, B and C are all located on chromosome 1p in the order A-C-B. C1r and C1s genes are closely linked and located on chromosome 12p.

Deficiencies

Deficiencies of each of the three components of C1 have been found (Ross, G.D. (1986)). Patients lacking C1q generally have immune-complex-mediated renal disease and skin lesions. Like all patients lacking early classical pathway components C1q deficient individuals are prone to systemic lupus erythematosus (SLE). They lack classical pathway function and may or may

not exhibit C1q antigen in blood. C1r and C1s deficient patients are similarly likely to have SLE and recurrent pyogenic infections (Rother, K., et al. (1998)).

Diseases

See section titled Deficiencies above.

Precautions/Toxicity/Hazards

This protein is purified from human serum and 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.

References

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

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

Morikis, D. and Lambris, J.D. editors. (2005) Structural Biology of the Complement System. (ISBN 0-8247-2540-9) Taylor & Francis Group, Florida.

Ross, G.D. (1986) Immunobiology of the Complement System. (ISBN 0-12-5976402) Academic Press, Orlando.

Rother, K., Till, G.O., and Hänsch, G.M. (1998) The Complement System. (ISBN 3-540-61894-5) Springer-Verlag, Heidelberg.

Ziccardi, R.J. (1982) Spontaneous activation of the first component of human complement (C1) by an intramolecular autocatalytic mechanism. J. Immunol. 128:2500-2504.

Ziccardi, RJ. (1982) A new role for C-1-inhibitor in homeostasis: control of activation of the first component of human complement. J. Immunol. 128:2505-2508.

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