Name:	**Cobra Venom Factor (CVF)**
Catalog Number:	**A150**
Sizes Available:	1 mg/vial
Concentration:	1.0 mg/mL (see Certificate of Analysis for actual concentration)
Form:	Frozen liquid
Extinction Coeff.:	$A_{280\ nm}$ = 1.0 at 1.0 mg/mL
Molecular weight:	146,000 Da (multiple chains and isoforms)
Activity:	>300 units/mg (see Certificate of Analysis).
Purity:	>95% by SDS-PAGE (see Certificate of Analysis).
Buffer:	10 mM sodium phosphate, 145 mM NaCl, pH 7.2
Preservative:	None, 0.22 µm filtered.
Storage:	-70°C or below. Avoid repeated freeze/thaw.
Source:	Venom from the Indian cobra *naja naja kaouthia* purchased from certified serpentariums located in the USA.
Restrictions:	Not available for sale outside the USA due to international endangered species laws.
Origin:	Manufactured in the USA.

General Description

Many types of venom contain components that activate complement. A major component of cobra venom is CVF which binds to the victim's factor B and this is then activated by factor D forming CVF,Bb. CVF, Bb is a serine protease specific for the victim's C3 and often C5 as well. The activity of this C3/C5 convertase is not affected by the complement regulatory proteins in blood and the complex is extremely stable (half-life approximately 7 hours) (Morgan, B.P. ed. (2000); Law, S.K.A. and Reid, K.B.M. (1995); Nolan, K.F. and Reid, K.B.M. (1993)). It is thought that the release of C3a is important for dilation of the blood vessels near the bite and this aids dispersion of the other venom components. C5a release provides a systemic activation of numerous immune cells and inflammatory reactions. CVF from *naja naja kaouthia* produces an enzyme that cleaves both human C3 and C5. The affinity of the *naja naja kaouthia* CVF for C5 is so high that it primarily consumes C5 first, then C3.

Physical Characteristics & Structure

CVF is a 146,000 dalton protein homologous to snake C3, but it is highly processed by proteases to a form that functions like C3b. Due to this extensive proteolysis the protein exists in many isoforms which vary in chain structure, but the most prominent species possesses three disulfide-linked chains (68,000, 48,000 and 30,000 daltons).

Function

CVF is an effective activator of complement in a wide array of species probably owing to the fact that the cobra hunts many different small animals for food and must defend itself against many large animals as well. CVF binds factor B in a metal-dependent interaction that is reversible. Once the complex is activated by factor D and CVF,Bb is generated the complex is extremely stable (half-life approximately 7 hours in blood). Cleavage of C5 to C5b and C5a proceeds slowly with a turnover number of only

a single C5 per 2 min at 37°C and a K_m = 36 nM (Rawal, N. and Pangburn, M.K. (2000)). C3 is cleaved to C3b and C3a with a turnover number of approximately 28 C3/min and a K_m = 12 µM.

Assays

The ability of CVF to activate complement and consume C3 and C5 is measured (Cochrane, C.G., et al. (1970)) by incubating 20 µL normal human serum (CompTech Cat# NHS) with various concentrations of CVF from 0.5 µg to 14 µg in GVB++ in a final volume of 500 µL for 30 min at 37°C. EA (2×10^7) are subsequently incubated with 50 µL of this CVF-treated NHS in a total volume of 90 µL for 30 min at 37°C. Cold GVBE (1 mL) is added and the percent lysis is plotted versus µg CVF to determine the amount of CVF giving 50% inhibition which equals one unit of activity. Purified CVF expresses a functional activity of approximately 250 to 450 Units/mg in this assay.

Applications

CVF has been widely used in animal studies to examine the role of the complement system in disease. Purified CVF has been injected i.p. or i.v. into many animal models (and even humans) to systemically inactivate the complement system for 3-6 days (Morgan B.P. (1990); Vogel, C-W. and Muller-Eberhard, H.J. (1984); von Zabern, I. (1993)). The treatment with CVF from the cobra species *naja naja kaouthia* depletes both C5 and C3. The typical level required for effective complement suppression is 0.5- 1.0 µg CVF/g body weight (Vogel, C-W. and Muller-Eberhard, H.J. (1984); von Zabern, I. (1993)). Effective doses that have been used are 10 µg CVF/20 g mouse, 80 µg/150 g rat, 150 µg/225 g guinea pig, and 1200 to 3000 µg/3 kg rabbit to reduce complement titers to less than 5% of normal. Subsequent injections are less effective due to immune responses to CVF so maintaining complement suppression for longer periods is impossible. De-complemented animals have been shown to have numerous impaired immune functions, but after a week complement titers begin to return to normal and are fully restored after 10 days (von Zabern, I. (1993)).

A cautionary note: many reports of CVF use in animals cite the amount used as Units/kg. Also, it is often sold as Units/mL. Unfortunately, there are many different types of "Units" and they vary more than 10-fold. Unless the source describes the assay used, the Units/kg must be considered as uncertain and irreproducible. CVF is extremely stable and in our experience the complement-consuming activity/mg does not vary significantly from year-to-year or lot-to-lot. Thus, the above cited amounts for in vivo use are the best representation of what has been used successfully by the majority of published studies. Each lot comes with a Certificate of Analysis giving the Units/mg and this should be used along with the mg administered when publishing results.

References

Cochrane, C.G., et al. (1970). J. Immunol. 105:55-69.

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Rawal, N. and Pangburn, M.K. (2000) Functional role of the noncatalytic subunit of complement C5 convertase. J. Immunol. 164:1379-1385.

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