



Complement Reagents: Cobra Venom Factor

**For Research Use Only.
Not For Use in Diagnostic Procedures.**

Specifications

Catalog Number:	A600
Protein concentration:	1.0-1.2 mg/ml
Purity:	≥ 95% by SDS PAGE
Volume/Vial:	1.0 ml
Activity/Vial:	≥350 units
Storage:	≤ -70°C
Buffer:	Phosphate Buffered Saline (pH 7.2 ± 0.2)

Background

Cobra Venom Factor (CVF), sometimes referred to as C3b_(Cobra), is the non-toxic, complement activating component of cobra venom.⁽¹⁻³⁾ Like naturally occurring C3b, CVF forms a complex, with complement components Factor B and Factor D. This CVFBbD convertase is capable of activating C3 in a wide variety of species via the alternative complement pathway. Unlike the naturally occurring convertase (C3bBbD), C3b_(Cobra)BbD convertase is Factor H resistant and is therefore not inactivated by Factor I or CR1. Given appropriate incubation time, CVF will convert nearly 100% of the C3 to C3 end products. Unlike CVF purified from the *Naja naja haje* species, CVF from *Naja naja kaouthia* activates the terminal pathway directly by forming a C5 convertase.^(4,5) This depletes C5 in a manner analogous to that described above for C3. Levels of iC3b, C3a, SC5b-9, C5a and the Factor B cleavage product Bb are all extremely high in CVF treated sera.

Preparation

Cobra Venom Factor is purified from lyophilized, whole cobra venom (*Naja naja kaouthia*) using standard chromatographic techniques. The purified CVF is tested for phospholipase activity using a commercially available PLA-2 EIA Kit.

Analysis and Testing

Quidel's CVF is tested for purity by SDS PAGE and is greater than 95% pure.

Activity units are assigned to this material following a modification of the procedure described by Muller-Eberhard and Fjellstrom. Briefly, 20 µl of serial dilutions of purified CVF are incubated with 1:40 normal human serum complement (Quidel Item **A113**) at 37°C for 30 minutes. Assay tubes are immediately placed on ice and 780 µl of cold GVB⁺⁺ and 100 µl of EA (5x10⁸ cells/ml) are added. The tubes are again incubated for 30 minutes at 37°C, after which the cells are pelleted and the absorbance (A₅₄₀) for each is determined. By definition, one unit of CVF gives 50% inhibition of lysis in this assay. **In general, one unit of CVF is equal to 2-6 µg of CVF.**

Storage and Handling

Purified CVF may be stored at -70°C until the expiration date listed on the vial and the accompanying Certificate of Analysis. CVF should be thawed rapidly at 37°C and immediately placed on ice until use.

Applications

Note: When using any CVF *in vivo* or *in vitro*, it is important to monitor units of activity rather than µg/ml as activity/µg can vary slightly between preparations and suppliers.

Quidel's CVF has been used in a variety of *in vitro* and *in vivo* models to deplete complement. For *in vitro* experiments, 8-20 units/ml of serum is adequate to convert nearly all the available C3 to C3 fragments when incubated with neat human serum for 60-90 minutes at 37° (data on file at Quidel). This will also convert nearly all the available C5 to C5a and SC5b-9.

Quidel's CVF has also been used successfully in a variety of animal models,⁽⁶⁻⁸⁾ including mice, rats, guinea pigs, various primates, dogs, pigs and sheep to deplete complement *in vivo*. **This application has not been tested or verified at Quidel.** For a list of studies, please refer to Quidel's expanded bibliographic references for this product, available upon request from Quidel Technical Service.

Related Products

A113:	Normal Human Serum Complement
A114:	Classical Pathway Activator

Ordering and Further Technical Information

To place an order or for technical assistance in the United States, please call (800) 524-6318, Monday through Friday, between 8:00 am and 5:00 pm, Pacific Time. Orders may also be placed by fax at (408) 616-4311 anytime. For orders placed outside the U.S. and Canada, please contact your local Quidel distributor.

Information about Quidel, our affiliated distributors and our products is available online at www.quidel.com.

Selected References

1. Fritzing, D.C., Bredehorst, R. Vogel, C-W. "Molecular cloning and derived primary structure of cobra venom factor" *PNAS* **91**:26, 12775-12779 (1994).
2. O'Keefe, M.C.; Caporale, L.H.; Vogel, C-W. "Comparison of the Alpha Chain Fragments of C30 and C3c and CVF implications for C3 convertase formation" *Complement* **4**:3-4 (1987).
3. Gowda D.C., et al "Immunoreactivity and function of oligosaccharides in cobra venom factor" *J Immunol* **152**:5, 2977-86 (1994).
4. Van Den Berg, C.W., et al "In vivo anti complementary activities of cobra venom factors from *Naja naja* and *Naja haje*" *J Immunol Meth* **12**:6, 287-294 (1991).
5. Bauman, N. "Lack of complement C5 convertase generating activity in *Naja haje* cobra venom factor" *J Immunol* **120**:5, 1763-1764 (1978).
6. Till, G.O. et al "Activation of C5 by CVF is required in neutrophil-mediated lung injury in the rat" *Am J Pathol* **129**:144-53 (1987).
7. Rajasinghe, H. et al "Key role of the alternative pathway in hyperacute rejection of rat hearts transplanted into fetal sheep" *Transplantation* **62**:3, 407-426, 1996.
8. Koymada, N. Bach, F. "Transient complement inhibition plus T-Cell immunosuppression induces long term graft survival of mouse to rat cardiac xenografts" *Transplantation* **66**:9, 1210-1215 (1998).

