ProductInformation

COBRA VENOM ANTI-COMPLEMENTARY PROTEIN
From *Naja naja kaouthia*
Cobra Venom Factor; CVF

Product Number **C8406**
Lot Number 010K4044
Storage Temperature: Below 0°C

Product Description
Lyophilized powder containing approx. 35% protein (Biuret); balance primarily sodium chloride and ammonium acetate.

Purity: Major band on polyacrylamide electrophoresis greater than 80%.

Activity: 55 units per mg protein. One unit is defined as the amount of cobra venom anti-complementary protein which causes 50% inhibition of lysis 2 x 10^8 antibody sensitized sheep erythrocytes (Sigma Product No. E7509) when incubated with human complement serum at 37°C for 15 minutes in a final volume of 1,000 µl.

Procedure
The following procedure is used for the determination of cobra venom anti-complementary protein (CVF) activity.

1. Prepare 5 assay tubes labeled "A" through "E" and 2 control tubes labeled "Spontaneous lysis" and "100% lysis.

2. Reconstitute the CVF to a concentration of 1 mg protein per ml with deionized water. Prepare four dilutions of 0.5, 0.25, 0.125 and 0.062 mg per ml (1:2, 1:4, 1:8 and 1:16) using ice cold gelatin veronal buffer (GVB^{2+}, Sigma Product No. G 6514). Label the solutions sequentially "B" through "E".

3. Reconstitute the human complement serum (Sigma Product No. S 1764) with cold deionized water and dilute 1:20 with ice cold GVB^{2+}.

4. Pipet 0.1 ml of each dilution of CVF into the corresponding assay tube. Pipet 0.1 ml of GVB^{2+} into assay tube "A". Add 0.5 ml of diluted human complement serum to each assay tube labeled "A" through "E". (Assay tube "A" corresponds to 100% complement activity.) Pipet 0.6 ml GVB^{2+} into the control tube labeled "Spontaneous lysis." Pipet 0.6 ml deionized water into the control tube labeled "100% lysis."

5. Pre-incubate all assay and control tubes for approximately 20 minutes in a 37°C water bath.

6. Prepare a suspension of 5 x 10^8 cells/ml of antibody sensitized sheep erythrocytes (Sigma Product No. E7509, EA7S) in ice cold GVB^{2+}.

7. Pipet 400 µl of the antibody sensitized sheep erythrocytes into each of the assay and control tubes. Incubate all tubes for 15 minutes at 37°C with shaking.

8. Add 2 ml of ice cold phosphate buffered saline, pH 7.0 (0.01 M phosphate 0.15 M NaCl) to each tube immediately after incubation.

9. Centrifuge the tubes at 2,000 rpm at 0-4°C for 10 minutes.

10. Read the absorbance of the supernatant of each tube at 415 nm.

11. Calculate the inhibition activity as follows:

a. Subtract the $OD_{415\ nm}$ of the "Spontaneous lysis" solution from the $OD_{415\ nm}$ of each assay solution (A, B, C, D, E) and from the $OD_{415\ nm}$ of the "100% lysis" solution. These values represent OD'_{415}.

b. Calculate the percent of lysis (y) for each assay tube:

$$y = \frac{OD'_{415} \text{ of assay solution (B,C,D,E)}}{OD'_{415} \text{ of assay solution "A"}}$$

where assay solution "A" represents 100% complement activity.

c. Calculate the value of y/1-y for each assay solution.

d. Plot the value of y/1-y against the corresponding amount of CVF used in each assay solution on a sheet of 2x3 cycle log-log graph papers.

e. Determine the amount of CVF which allows a 50% lysis (i.e. y/1-y = 1). This value corresponds to one unit.

TABLE I

The volumes shown are for example only. Adjust the volumes of the CVF and GVB^{2+} as needed, keeping the total volume of the reaction mixture at 1.0 ml.

Assay Tubes	CVF* (µl)	Diluted Complement Serum (µl)	EA7S (($5x10^8$ cells/ml) (µl)	GVB^{2+} (µl)	dH_2O (µl)
A**	--	500	400	100	--
B	100	500	400	--	--
C	100	500	400	--	--
D	100	500	400	--	--
E	100	500	400	--	--
Control Tubes					
100% lysis	--	--	400	--	600
Spontaneousl ysis	--	--	400	600	--

*Pipet 100 µl of each dilution into the corresponding tube.
**The OD'_{415nm} of assay tube "A" represents 100% complement activity.

Reference: Ballow, M. and Cochrane, C.G., J. Immunol., **103,** 944 (1969).