

Product Information

Cobra Venom Anti-Complementary Protein From *Naja naja kaouthia*

Product Number **C8406**
Storage Temperature $-20\text{ }^{\circ}\text{C}$

Synonyms: Cobra Venom Factor; CVF

Product Description

This product is a lyophilized powder containing 25-35% protein (Biuret) with the balance primarily sodium chloride and ammonium acetate.

Purity: minimum 80% (PAGE)

Unit Definition: One unit is defined as the amount of cobra venom anti-complementary protein, which causes 50% inhibition of lysis of 2×10^8 antibody sensitized sheep erythrocytes (Product No. E9383) when incubated with human complement serum at $37\text{ }^{\circ}\text{C}$ for 15 minutes in a final volume of $1,000\text{ }\mu\text{l}$.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store the product at $-20\text{ }^{\circ}\text{C}$.

Procedure

This 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 water. Prepare four dilutions of 0.5, 0.25, 0.125, and 0.062 mg per ml (2, 4, 8, and 16-fold dilutions) using ice cold Gelatin Veronal Buffer (GVB²⁺, Product No. G 6514). Label the solutions sequentially "B" through "E".
3. Reconstitute the human complement serum (Product No. S1764) with cold water and dilute 20-fold with ice cold GVB²⁺.
4. Pipette 0.1 ml of each dilution of CVF into the corresponding assay tube. Pipette 0.1 ml of GVB²⁺ into assay tube "A". Add 0.5 ml of the diluted human complement serum to each assay tube labeled "A" through "E". Assay tube "A" corresponds to 100% complement activity. Pipette 0.6 ml of GVB²⁺ into the control tube labeled "Spontaneous lysis." Pipette 0.6 ml of water into the control tube labeled "100% lysis". (See Table 1)
5. Pre-incubate all assay and control tubes for approximately 20 minutes in a $37\text{ }^{\circ}\text{C}$ water bath.
6. Prepare a suspension of 5×10^8 cells/ml of antibody sensitized sheep erythrocytes, EA7S, (Product No. E9383) in ice cold GVB²⁺.
7. Pipette $400\text{ }\mu\text{l}$ of the antibody sensitized sheep erythrocytes into each of the assay and control tubes. Incubate all tubes for 15 minutes at $37\text{ }^{\circ}\text{C}$ with shaking.
8. Add 2 ml of ice cold phosphate buffered saline, pH 7.0, (0.01 M phosphate buffer with 0.15 M NaCl) to each tube immediately after incubation.
9. Centrifuge the tubes at 2,000 rpm at $0-8\text{ }^{\circ}\text{C}$ for 10 minutes.
10. Read the absorbance of the supernatant of each tube at 415 nm.
11. Calculate the inhibition activity.

Calculations

- a. Subtract the A_{415} of the "Spontaneous lysis" solution from the A_{415} of each assay solution (Tubes A, B, C, D, and E) and from the A_{415} of the "100% lysis" solution. These values represent A'_{415} .
- b. Calculate the percent of lysis (y) for each assay tube:

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

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 2 x 3 cycle log-log graph paper.
- e. Determine the amount of CVF which allows a 50% lysis (i.e. $y/1-y = 1$). This value corresponds to one unit.

Table 1.

The volumes shown are for example only. Adjust the volumes of the CVF and GVB²⁺ as needed, keeping the total volume of the reaction mixture at 1.0 ml.

Assay Tubes	CVF* (μl)	Diluted Complement Serum (μl)	EA7S (5 x 10 ⁸ cells/ml) (μl)	GVB ²⁺ (μl)	Water (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
Spontaneous lysis	--	--	400	600	--

*Pipette 100 μl of each dilution into the corresponding tube.

**The A'_{415nm} of assay tube "A" represents 100% complement activity.

References

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

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