



COMPLEMENT C9 DEFICIENT SERUM, HUMAN

Product No. **C1663**
Lot 089H0844

Product Description

Solution containing 50 mM sodium phosphate, 150 mM sodium chloride, pH 7.4.

Storage

Store at -70°C or below. Repeated freezing and thawing is **not** recommended.

Precautions

Potential Biohazard. Handle as if capable of transmitting infectious agents. Refer to Material Safety Data Sheet.

Product Profile

The C9H50 unit is used to express the complement C9 hemolytic activity using C9 deficient serum. One C9H50 unit is defined as the amount of complement standard serum or sample containing complement C9 to yield 50% lysis of 3×10^7 antibody sensitized sheep erythrocytes (Sigma Product No. E 7509) when incubated in the presence of the recommended volume of C9 deficient serum for 30 minutes at 37°C in a final volume of 500 μ l.

Recommended volume of C9 deficient serum: 4 μ l(v)

Amount of purified C9 to yield one C9H50 unit: 0.38 ng

Background activity: $OD'_{415\text{ nm}} = 0.178$. Note: Background activity should be determined at the time of assay each time complement C9 deficient serum is used. Calculation described in Step 11a.

Procedure

The following procedure is used for the determination of C9 activity. The assay should be performed in an ice bath, except where otherwise indicated.

1. Prepare 8 precooled assay tubes labeled "A" through "H" and 2 precooled control tubes labeled "Spontaneous lysis" and "100% lysis."

Product Information

2. Thaw the C9 deficient serum in a 37°C water bath. Do not thaw at 4°C or at room temperature.

3. Place the thawed C9 deficient serum into an ice bath immediately and pipet the recommended volume into the precooled assay tubes.

4. Dilute the complement C9 to a concentration in the range of 10-100 ng/ml with ice cold Gelatin Veronal Buffer (GVB²⁺, Sigma Product No. G 6514). If human whole serum is used, dilute to a concentration in the range of 1:100 to 1:500 with ice cold GVB²⁺. **Note:** The above serum dilution range is a suggestion only. Due to variability in sera, the actual serum dilution required should be determined by the investigator.

5. Prepare a suspension of 1.5×10^8 cells/ml of antibody sensitized sheep erythrocytes (Sigma Product No. E 7509, EA7S) in GVB²⁺.

6. Pipet the diluted complement C9 or human whole serum, antibody sensitized sheep erythrocytes, GVB²⁺ and distilled water into the assay tubes according to Table I.

7. Incubate all tubes in a 37°C water bath with shaking for 30 minutes.

8. Add 1.0 ml of ice cold GVB²⁺ 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 hemolytic activity for C9 as follows:

a. Subtract the $OD_{415\text{ nm}}$ of the "Spontaneous lysis" solution from the $OD_{415\text{ nm}}$ of each assay solution (A, B, . . . H) and from the $OD_{415\text{ nm}}$ of the "100% lysis" solution. These values are represented as OD'_{415} .

b. Calculate the value of y for each assay solution:

$$y = \frac{\text{OD}'_{415} \text{ of assay solution (A,B. . .H)}}{\text{OD}'_{415} \text{ of "100% lysis" solution}}$$

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

d. Plot the value of $y/(1-y)$ against the corresponding volume of human whole serum or complement C9 used in each assay solution on a sheet of 2x3 cycle log-log graph paper.

e. Determine the amount of human whole serum or complement C9 which gives a 50% lysis (i.e. $y/(1-y) = 1$). This value corresponds to one C9H50 unit. The hemolytic titer is calculated as the reciprocal of the dilution which gives 50% lysis (i.e. the amount of C9H50 units/ml standard serum or sample.)

TABLE I

The volumes indicated are an example only. Adjust the volumes of the C9-containing sample and GVB²⁺ as needed, keeping the total volume of the reaction mixture at 500 μ l.

Assay Tubes	C9 deficient serum (μ l)	Diluted human whole serum or purified C9** (μ l)	EA7S (1.5×10^8 cells/ml) (μ l)	GVB ²⁺ (μ l)	dH ₂ O (μ l)
A***	v	--	200	300-v	--
B	v	5	200	295-v	--
C	v	10	200	290-v	--
D	v	20	200	280-v	--
E	v	30	200	270-v	--
F	v	40	200	260-v	--
G	v	50	200	250-v	--
H	v	60	200	240-v	--
Control Tubes					
100% lysis	--	--	200	--	300
Spontaneous lysis	--	--	200	300	--

*Value obtained when assayed using above conditions.

**Either dilute human whole serum or purified C9 can be added into the reaction mixture to restore C9 activity.

***The OD_{415nm} of assay tube "A" represents the background activity.

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