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Product Information

Complement C6 deficient serum human

Catalog Number **C1288**
Storage Temperature –70 °C

Product Description

Complement C6 is associated with a complex of complements C5b, C7, and C8, which mediates the polymerization of C9 molecules into a tube-like membrane attack complex that is inserted into the plasma membrane of unwanted organisms such as Gram-negative bacteria and viral infected cells. Complement C6 has also been implicated in facilitating axonal regeneration.

This product is prepared by C6 depletion of pooled, human serum by immunoadsorption as judged by a highly sensitive hemolytic assay. It is suitable for the determination of complement C6 activity. It is supplied as a solution containing 50 mM sodium phosphate, pH 7.4, and 150 mM sodium chloride.

The C6H50 unit is used to express the complement C6 hemolytic activity using C6 deficient serum. One C6H50 unit is defined as the amount of complement standard serum or sample containing complement C6 to yield 50% lysis of 3×10^7 antibody sensitized sheep erythrocytes when incubated in the presence of the recommended volume of C6 deficient serum for 30 minutes at 37 °C in a final volume of 500 µl.

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

The product ships on dry ice and storage at –70 °C is recommended. Repeated freezing and thawing is **not** recommended.

Procedure

The following procedure is used for the determination of C6 activity. The assay should be performed in an ice bath, except where otherwise indicated.

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

2. Thaw the C6 deficient serum in a 37 °C water bath. Do not thaw at 4 °C or at room temperature.

3. Place the thawed C6 deficient serum into an ice bath immediately and pipette the recommended volume (v, see lot-specific CofA) into the pre-cooled assay tubes.

4. Dilute the complement C6 to a concentration in the range of 10–50 ng/ml with ice cold gelatin veronal buffer (GVB^{2+}, Catalog Number G6514). If human whole serum is used, dilute 300 to 600-fold with ice cold GVB^{2+}.
   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 in GVB^{2+}. For an antibody sensitized sheep erythrocyte preparation protocol please visit sigma-aldrich.com/complement.

6. Pipette the diluted complement C6 or human whole serum, antibody sensitized sheep erythrocytes, GVB^{2+}, 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 GVB^{2+} to each tube immediately after incubation.

9. Centrifuge the tubes at 2,000 rpm at 2–8 °C for 10 minutes.

10. Read the absorbance of the supernatant of each tube at 412 nm.

11. Calculate the hemolytic activity for C6 as follows:
    a. Subtract the $OD_{412\ nm}$ of the "Spontaneous lysis" solution from the $OD_{412\ nm}$ of each assay solution (A, B,. . ., H) and from the $OD_{412\ nm}$ of the "100% lysis" solution. These values are represented as OD'_{412}. The $OD'_{412\ nm}$ of assay tube "A" represents the background activity. Note: Background activity should be determined every time for an assay with complement C6 deficient serum.

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

$$y = \frac{OD'_{412} \text{ of assay solution (A,B. . .H)}}{OD'_{412} \text{ of "100\% Lysis" solution}}$$

    c. Calculate the value of y/(1–y) for each assay solution (A, B,. . ., H).

    d. Plot the value of y/(1–y) against the corresponding volume of human whole serum or complement C6 used in each assay solution on a sheet of 2 × 3 cycle log-log graph paper.

    e. Determine the amount of human whole serum or complement C6, which gives a 50% lysis (i.e., y/(1–y) = 1). This value corresponds to one C6H50 unit. The hemolytic titer is calculated as the reciprocal of the dilution, which gives 50% lysis (i.e., the amount of C6H50 units/ml standard serum or sample.)

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Table 1.
The volumes indicated are an example only. Adjust the volumes of the C6-containing sample and GVB^{2+} as needed, keeping the total volume of the reaction mixture at 500 µl.

Assay Tubes	C6 deficient Serum (µl)	Diluted human whole serum or purified C6* (µl)	Antibody sensitized sheep erythrocytes (1.5×10^8 cells/ml) (µl)	GVB^{2+} (µl)	Distilled water (µl)
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	–

* Either dilute human whole serum or purified complement C6 can be added into the reaction mixture to restore C6 activity.

** The OD'_{412nm} of assay tube "A" represents the background activity.