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Product Information

Complement C7 Deficient Serum human

Catalog Number **C1413**
Storage Temperature –70 °C

Product Description

This product is prepared by C7 depletion of pooled, human serum by immunoadsorption as judged by a highly sensitive hemolytic assay. It is suitable for the determination of complement C7 activity.

The product is supplied as a solution in phosphate buffered saline (PBS), pH 7.4.

Human complement C7 is a β_2-globulin, which is composed of a single polypeptide chain.[1] The initial binding of C7 and C6 to C5b is thought to form a stable complex for subsequent binding of C8 and C9. The resulting activated complex is responsible for membrane attack and lysis.[2]

The C7H50 unit is used to express the complement C7 hemolytic activity using C7 deficient serum. One C7H50 unit is defined as the amount of complement standard serum or sample containing complement C7 to yield 50% lysis of 3×10^7 antibody sensitized sheep erythrocytes when incubated in the presence of the recommended volume of C7 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 C7 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".
2. Thaw the C7 deficient serum in a 37 °C water bath. Do not thaw at 4 °C or at room temperature.
3. Place the thawed C7 deficient serum into an ice bath immediately and pipette the recommended volume (v, see lot-specific CofA) into the precooled assay tubes.
4. Dilute the complement C7 to a concentration in the range of 50–100 ng/ml with ice cold gelatin veronal buffer (GVB^{2+}, Catalog Number G6514). If human whole serum is used, dilute 200 to 400-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 a procedure to prepare antibody sensitized sheep erythrocytes please visit sigma-aldrich.com/complement.
6. Pipette the diluted complement C7 or human whole serum, antibody sensitized sheep erythrocytes, GVB^{2+}, and distilled water into the assay tubes according to Table 1.
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 C7 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 C7 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 C7 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 C7 which gives 50% lysis (i.e., y/(1-y) = 1). This value corresponds to one C7H50 unit. The hemolytic titer is calculated as the reciprocal of the dilution, which gives 50% lysis (i.e., the amount of C7H50 units/ml standard serum or sample).

References

1. Podack, E.R., *et al.*, J. Immunol., **121,** 484 (1978).
2. Götze, O., and Müller-Eberhard, H.J., N. Engl. J. Med., **286,** 180 (1972).

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Table 1.

The volumes indicated are an example only. Adjust the volumes of the C7-containing sample and GVB^{2+} as needed, keeping the total volume of the reaction mixture at 500 µl.

Assay Tubes	C7 deficient serum (µl)	Diluted human whole serum or purified C7* (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 C7 can be added to the reaction mixture to restore C7 activity.

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