Product Information

Complement C8 Deficient Serum

C1538

Storage Temperature −70 °C

Product Description

This product is prepared by C8 depletion of pooled, human serum by immunoadsorption as judged by a highly sensitive hemolytic assay. It is suitable for the determination of complement C8 activity.

The product is supplied as a solution in phosphate buffered saline (PBS), pH 7.3.

The C8H50 unit is used to express the complement C8 hemolytic activity using C8 deficient serum. One C8H50 unit is defined as the amount of complement standard serum or sample containing complement C8 to yield 50% lysis of 3×10^7 antibody sensitized sheep erythrocytes when incubated in the presence of the recommended volume of C8 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 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 C8 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 C8 deficient serum in a 37 °C water bath. Do not thaw at 4 °C or at room temperature.
3. Place the thawed C8 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 C8 to a concentration in the range of 50–100 ng/mL with ice cold gelatin veronal buffer (GVB^{2+}, Cat. No. G6514). If human whole serum is used, dilute 400 to 800-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+}.
6. Pipette the diluted complement C8 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 C8 as follows:

11.1. 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 C8 deficient serum.

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

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

11.3. Calculate the value of y/(1-y) for each assay solution (A, B, …, H).

11.4. Plot the value of y/(1-y) against the corresponding volume of human whole serum or complement C8 used in each assay solution on a sheet of 2 × 3 cycle log-log graph paper.

11.5. Determine the amount of human whole serum or complement C8 which gives 50% lysis (for example, y/(1-y) = 1). This value corresponds to one C8H50 unit. The hemolytic titer is calculated as the reciprocal of the dilution, which gives 50% lysis (the amount of C8H50 units/mL standard serum or sample, for example).

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Table 1.

The volumes indicated are an example only. Adjust the volumes of the C8-containing sample and GVB^{2+} as needed, keeping the total volume of the reaction mixture at 500 µL.

Assay Tubes	C8 deficient serum (µL)	Diluted human whole serum or purified C8* (µL)	Antibody Sensitized Sheep Erythrocytes (1.5 x 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 C8 can be to the reaction mixture to restore C8 activity.
** The $OD'_{412\ nm}$ of assay tube "A" represents the background activity.

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