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Product Information

Complement C2 Deficient Serum human

Catalog Number **C0913**
Storage Temperature –70 °C

Product Description

This product is prepared by C2 depletion of pooled, human serum by immunoadsorption as judged by a highly sensitive hemolytic assay and an Ouchterlony immunodiffusion method. It is suitable for the determination of complement C2 activity.

The product is supplied in a solution of phosphate buffered saline (PBS), pH 7.2.

The C2H50 unit is used to express the complement C2 hemolytic activity using C2 deficient serum. One C2H50 unit is defined as the amount of complement standard serum or sample containing complement C2 to yield 50% lysis of 3×10^7 antibody sensitized sheep erythrocytes when incubated in the presence of the recommended volume of C2 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 C2 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 C2 deficient serum in a 37 °C water bath. Do not thaw at 4 °C or at room temperature.
3. Place the thawed C2 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 C2 to a concentration in the range of 10–100 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 C2 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 C2 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 C2 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 C2 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 C2 which gives 50% lysis (i.e., y/(1-y) = 1). This value corresponds to one C2H50 unit. The hemolytic titer is calculated as the reciprocal of the dilution, which gives 50% lysis (i.e., the amount of C2H50 units/ml standard serum or sample.)

CS,RBG,MAM 05/09-1

Table 1.
The volumes indicated are an example only. Adjust the volumes of the C2-containing sample and GVB^{2+} as needed, keeping the total volume of the reaction mixture at 500 µl.

Assay Tubes	C2 deficient serum (µl)	Diluted human whole serum or purified C2* (µl)	EA7S (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 C2 can be added to the reaction mixture to restore C2 activity.

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