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ProductInformation

Guinea Pig Complement Serum

Product Number **S 1639**

Product Description
Complement serum is prepared from pooled guinea pig plasma and lyophilized from the amount of serum indicated on the label.

Storage/Stability
The product ships on dry ice and it is recommended to store desiccated below –20 °C.

Procedure
The hemolytic assay of whole complement should be carried out in an ice bath or at 0 °C.

1. Reconstitute the lyophilized complement serum with the amount of cold water indicated on the label.
2. Dilute the reconstituted guinea pig complement 1:500 with ice cold gelatin veronal buffer (GVB^{2+}, Product No. G 6514).
3. Wash the EA7S and adjust the cell concentration spectrophotometrically to 5×10^8 cells/ml with GVB^{2+} buffer.
4. Prepare six precooled assay tubes (13 x 100 mm) labeled "A" through "F" and two control tubes labeled "100% lysis" and "spontaneous lysis" by adding 1.0 ml of EA7S cell solution to each tube.
5. Pipette the diluted complement serum from 1 to 3.5 ml, in 0.5 ml increments, into the six assay tubes.
6. Adjust the volume of all assay tubes to 7.5 ml with GVB^{2+} buffer.
7. Add 6.5 ml of water into tube labeled "100% lysis" and 6.5 ml of GVB^{2+} buffer into tube labeled "Spontaneous lysis."
8. Incubate all tubes at 37 °C for 60 minutes with shaking to prevent cells from settling.
9. Centrifuge the tubes immediately at 2000 rpm for 10 minutes at 0-4 °C.
10. Read A_{541} of supernatant in a spectrophotometer against a GVB^{2+} buffer reference.
11. Calculate the hemolytic titer as follows:
    a. Substract the A_{541} of "spontaneous lysis" solution from the A_{541} of sample solutions "A" through "F" and from the A_{541} of the "100% lysis" solution. These values represent A'_{541}.
    b. Calculate the percent lysis (y) for each sample reaction mixture:

$$y = \frac{A'_{541} \text{ of assay solution (A,B. . .F)}}{A'_{541} \text{ of "100% lysis" solution}}$$

    c. Calculate the value of y/1-y for each sample level.
    d. Plot the value of y/1-y against the corresponding volume of undiluted complement serum used in each assay solution on a sheet of 2 x 3 cycle log-log graph paper.
    e. Determine the volume (ml) of complement serum which gives a 50% lysis (i.e., y/1-y = 1). This value corresponds to one CH50 unit.
    f. Calculate the hemolytic titer as the reciprocal of CH50 unit.

Note: A CH50 unit is defined as the amount of serum that will cause a 50% hemolysis of antibody sensitized sheep erythrocytes (EA7S, Product No. E 9383) in the reaction mixture. This is an arbitrary unit, the magnitude of which will be dependent upon the reaction mixture used. Factors that will affect this unit are cell number, ionic strength, the concentration of magnesium and calcium, and the nature of the antibody used for sensitization. The hemolytic titer is the number of CH50 units per ml of serum and is calculated as the reciprocal of the serum dilution that gives 50% cell lysis.

References
1. Kabat, E.A., and Mayer, M.M., in Experimental Immunochemistry, Charles C. Thomas, 2nd Edition (Springfield, Ill: 1961) pp. 149-153.

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