



Product Information

HUMAN COMPLEMENT SERUM

Product No. **S 1764**

Product Description

Complement serum is prepared from pooled human plasma and lyophilized from the amount of serum indicated on the label.

Storage

Store desiccated below 0 °C.

Product Profile

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 which gives 50% cell lysis.

Procedure

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

1. Reconstitute the complement serum with the amount of cold deionized water indicated on the label.
2. Dilute the human complement 1:100 with ice cold gelatin veronal buffer (GVB²⁺, Sigma Product No. G 6514).
3. Wash the EA7S and adjust the concentration spectrophotometrically to 5×10^8 cells/ml with GVB²⁺ buffer.
4. Prepare six precooled assay tubes (13 x 100 mm) labeled "A" through "F" and two control tubes labeled "Spontaneous lysis" and "100% lysis" by adding the indicated amount of GVB²⁺ buffer or deionized water to each tube (see Table 1).
5. Pipet 1.0 ml of the EA7S solution into each assay and control tube.
6. Pipet the diluted complement serum, from 1.0 to 3.5 ml in 0.5 ml increments, into the six assay tubes (see Table 1).
7. Incubate all tubes in a 37 °C water bath for 60 minutes with shaking to prevent the cells from settling.
8. Centrifuge the tubes immediately at 2,000 rpm at 0-4 °C for 10 minutes.
9. Read the absorbance of the supernatant of each tube at 541 nm.
10. Calculate the hemolytic titer as follows:
 - a. Subtract the OD₅₄₁ of the "Spontaneous lysis" solution from the OD_{541 nm} of each assay solution (A, B, . . . F) and from the OD₅₄₁ of the "100% lysis" solution. These values are represented OD'₅₄₁.
 - b. Calculate the percent lysis (y) for each assay solution:
$$y = \frac{\text{OD}'_{541} \text{ of assay solution (A,B, . . . F)}}{\text{OD}'_{541} \text{ of "100\% lysis" solution}}$$
 - c. Calculate the value of $y/(1-y)$ for each assay solution.
 - 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) complement serum which gives a 50% lysis (i.e. $y/(1-y) = 1$). This value corresponds to one CH50 unit. The hemolytic titer is calculated as the reciprocal of the dilution which gives 50% lysis (i.e. the amount of CH50 units/ml complement serum.)

TABLE I

Assay Tubes	Complement serum (ml)	EA7S (5x10 ⁸ cells/ml) (ml)	GVB ²⁺ (ml)	dH ₂ O (ml)
A	1.0	1.0	5.5	--
B	1.5	1.0	5.0	--
C	2.0	1.0	4.5	--
D	2.5	1.0	4.0	--
E	3.0	1.0	3.5	--
F	3.5	1.0	3.0	--
Control Tubes				
100% lysis	--	1.0	--	6.5
Spontaneous lysis	--	1.0	6.5	--

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