



Product Information

ANTIBODY SENSITIZED SHEEP ERYTHROCYTES Sigma Prod. No. E7509

ACRONYM: EA7S

APPEARANCE: Red suspension

FORMULATION:

Sigma provides antibody sensitized sheep erythrocytes (EA7S) at a concentration of 1×10^9 cells/ml in a volume of two milliliters per vial. The erythrocytes have been sensitized with antibody against sheep erythrocytes developed in rabbit (S8014). The cells are suspended in gelatin veronal buffer containing 2.5 % glucose and 0.1 % sodium azide as a preservative.

STORAGE AND SHELF-LIFE:

The shelf life of E7509 is approximately four weeks when stored properly at 2 - 8°C. Protect against freezing at all times. Sigma produces and ships this product every two weeks.

USAGE:

Sigma has routinely used E7509 in several complement assay systems. Most assays are based on the classical complement pathway hemolytic system. The reported hemolytic activity of a given complement product is affected by the EA7S concentration of the assay suspension. Assay systems using low EA7S concentrations will yield higher hemolytic activities.

Washing Cells

Before the EA7S cell suspension is used for a hemolytic assay, the cells must be washed at 0 - 4°C using the following procedure:

1. Tap the vial gently and suspend the cells in solution.
2. Transfer the cells completely into a centrifuge tube by using 5 ml or more ice cold gelatin veronal buffer (GVB²⁺, Sigma Prod. No. G6514).
3. Centrifuge the suspension at 2,000 rpm at 0-4°C for 10 minutes.
4. Aspirate the supernatant fluid from the tube.
5. Tap the side of the centrifuge tube gently to evenly resuspend the cells before adding 10 ml of ice cold GVB²⁺ buffer.
6. Centrifuge the suspension again at 2,000 rpm at 0-4°C for 10 minutes.

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USAGE: (continued)

7. Repeat Steps 5 and 6 at least twice and then resuspend the cells in 10 ml of GVB²⁺ buffer.
8. Lyse 0.2 ml of cell suspension in 2.8 ml of dH₂O
9. Read OD at 415 nm in a spectrophotometer.
10. Calculate the final volume required to adjust the cell concentration to 1 x 10⁸ cells/ml

$$\frac{\text{OD}_{415} \text{ of sample}}{\text{OD}_{415} \text{ value given on label}} \times \text{initial volume of cell suspension (A)}$$

= Final volume of cell suspension (B)

11. If the initial volume (A) is smaller than the final volume (B), add the GVB²⁺ buffer with a volume difference between (A) and (B) to the cell suspension (A) to obtain a concentration of 1 x 10⁸ cells/ml.
12. If (B) is smaller than (A), centrifuge the cell suspension (A), remove the supernatant and resuspend the cells into sufficient GVB²⁺ buffer to obtain the final volume (B).
13. If another cell concentration is desired, adjust it based on the formula shown in step 10. The whole complement assay shown below requires a cell concentration of 5 X 10⁸ cells/ml.

The supernatant of the last wash step should be colorless to only faint red. If an intense red color persists in the wash-supernatants, fresh EA7S should be obtained.

Care should be taken to maintain the temperature at 0-4°C, and use gentle inversion for resuspending cells.

After each centrifugation and aspiration step, prior to the addition of buffer to the cell pellet, it is important to tap the side of the centrifuge tube gently to evenly suspend the cell suspension. Failure to do so will result in a cell pellet that will not resuspend properly.

Excessive agitation or temperature during these steps will result in spontaneous lysis of the cells.

Whole Complement Hemolytic Assay Procedure

Unit Definition:

A CH50 unit is defined as the amount of serum that will cause a 50% hemolysis of antibody sensitized sheep erythrocytes (EA7S, Sigma Prod. No. E7509) in the reaction mixture. This is an arbitrary unit, the magnitude of which will be dependent upon the reaction mixture used. Factors which 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.

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Whole Complement Hemolytic Assay Procedure (continued)

Assay Procedure:

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

1. Dilute serum with cold GVB²⁺ buffer (Sigma Prod. No. E7509):

Human serum	1:100
Guinea pig serum	1:500
Rat serum	1:150
Mouse serum	1:20
2. Wash the EA7S and adjust the concentration spectrophotometrically to 5×10^8 cells/ml with GVB²⁺ buffer.
3. 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 the indicated amount of GVB²⁺ buffer or deionized water to each tube (see Table 1).
4. Pipet 1.0 ml of the EA7S solution into each assay and control tube.
5. 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).
6. Incubate all tubes at 37°C for 60 minutes with shaking to prevent the cells from settling.
7. Centrifuge the tubes immediately at 2000 rpm for 10 minutes at 0-4°C.
8. Read OD₅₄₁ of supernatant fluid in a spectrophotometer.
9. Calculate the hemolytic titer as follows:
 - a. Subtract the OD₅₄₁ of "Spontaneous lysis" solution from the OD₅₄₁ of sample solution "A" through "F" and from the OD₅₄₁ of the 100% lysis solution. These values represent OD'₅₄₁.
 - b. Calculate the percent lysis (y) for each sample reaction mixture:
$$y = \frac{\text{OD}'_{541} \text{ of sample solution}}{\text{OD}'_{541} \text{ of 100\% lysis solution}}$$
 - c. Calculate the value of y/1-y for each sample level.
 - d. Plot the value of y/1-y vs. the corresponding volume of undiluted complement serum on 2 x 3 cycle log-log graph paper.
 - e. Determine the volume (ml) of 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 the CH50 unit.

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Whole Complement Hemolytic Assay Procedure (continued)

Table 1:

Assay Tubes	GVB ²⁺ buffer (ml)	EA7S (ml)	Complement serum (ml)	dH ₂ O (ml)
A	5.5	1.0	1.0	----
B	5.0	1.0	1.5	----
C	4.5	1.0	2.0	----
D	4.0	1.0	2.5	----
E	3.5	1.0	3.0	----
F	3.0	1.0	3.5	----
CONTROL TUBES				
100% LYSIS	----	1.0	----	6.5
SPONTANEOUS ** LYSIS	6.5	1.0	----	----

** The OD₅₄₁ of the spontaneous lysis tube should be below 0.1 for best results.

References:

Kabat, E.A. and Mayer, M.M., *Experimental Immunochemistry*, 2nd ed, pp. 149-153 (1961).