

Product Information

Antibody Sensitized Sheep Erythrocytes

Catalog Number **E9383**
Storage Temperature 2–8 °C

Synonym: EA7S

Product Description

Antibody sensitized sheep erythrocytes (EA7S) are supplied at a concentration of 1×10^9 cells/ml in a volume of two milliliters per vial. The cells are sensitized with an antibody against sheep erythrocytes (Catalog Number S8014) and suspended in gelatin veronal buffer, which contains 0.1 M sucrose as preservative.

This preparation is suitable for the assay of complement component activity (H_{50} units) and whole complement activity (CH_{50} units).

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 with wet ice and storage at 2–8 °C is recommended.

Procedure

Before the EA7S is used for a hemolytic assay, the cells must be washed at 2–8 °C using the following procedure:

1. Tap the vial gently to resuspend the cells in solution.
2. Transfer the cells completely into a centrifuge tube by using 5 ml or more of ice cold gelatin veronal buffer (GVB²⁺, Catalog Number G6514).
3. Centrifuge the suspension at 2,000 rpm at 2–8 °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 2–8 °C for 10 minutes.
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 distilled H₂O.
9. Read A_{415} in a spectrophotometer.
10. Calculate the final volume required to adjust the cell concentration to 1×10^8 cells/ml:

A_{415} of sample

$$A_{415} \text{ value given} \times \text{Volume (A)} = \text{Volume (B)}$$

A_{415} of sample – value from step 9

A_{415} value given – value on Certificate of Analysis
Volume (A) – volume of prepared cell suspension
(step 7, ~10 ml)

Volume (B) – final volume of cell suspension to
adjust the cell concentration to 1×10^8 cells/ml

11. If volume (A) is smaller than the final volume (B), add GVB²⁺ buffer to the cell suspension prepared in step 7 to obtain volume (B).
12. If the final volume (B) is smaller than volume (A), centrifuge the cell suspension prepared in step 7, remove the supernatant, and resuspend the cells in 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.

References

1. Kabat, E.A., and Mayer, M.M., *Experimental Immunochemistry*, 2nd edition, Charles C. Thomas, (Springfield, IL: 1961) pp. 149-153.

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