ProductInformation

COMPLEMENT C1q DEFICIENT SERUM, HUMAN

Product No. **C8567**
Lot 49H9276
Store at 0◦C

Product Description
Protein: Determined by Biuret method.
Form: Lyophilized from amount of serum indicated on the label.
Source: Prepared from freshly clotted whole blood. C1q is depleted by the method of Kolb, *et al.*[1]
Background hemolytic activity (OD_{415}) (Tube A): Less than 5% of normal serum complement activity. The background activity is determined in a 15 minute assay using diluted, reconstituted C1q deficient serum. Hemolytic activity is restored after adding back C1q to the assay.
Recommended dilution of this lot of the reconstituted C1q deficient serum with GVB^{2+} for the hemolytic assay of complement C1q: 1:3
Note: Failure to dilute the serum prior to the assay will result in an increase in background activity.

Precautions and Disclaimer
For laboratory use only. Not for drug, household or other uses. POTENTIAL BIOHAZARD. Handle as if capable of transmitting infectious agents. Refer to Material Safety Data Sheet.

Storage
Store below 0◦C. After reconstitution, divide into smaller aliquots and store at -70◦C. Repeated freezing and thawing is **not** recommended.

Procedure
The following procedure is used for the hemolytic assay of complement C1q activity.

1. Reconstitute the C1q deficient serum with 1 ml ice cold deionized water.

2. Add 20 µl of a solution containing 0.3 M $CaCl_2$ and 1 M $MgCl_2$ to each ml of C1q deficient serum.

3. Dilute an aliquot of the C1q deficient serum with gelatin veronal buffer (GVB^{2+}, Product No. G6514) as indicated above.

4. Prepare nine precooled assay tubes labeled "A" through "I" and two control tubes labeled "100% lysis" and "Spontaneous lysis".

5. Dilute purified complement C1q (Product No. C0660) or other complement C1q containing sample to a concentration of approximately 5 μg/ml complement C1q with GVB^{2+}.

6. Prepare a suspension of 1.5 x 10^8 cells/ml of antibody sensitized sheep erythrocytes (Product No. E9383, EA7S) in GVB^{2+}.

7. Prepare reaction tubes on ice according to Table 1.

8. Incubate all tubes in a 37◦C water bath with shaking for 3-15 minutes.

9. Add 1.0 ml of ice cold GVB^{2+} buffer immediately at the end of the incubation time.

10. Centrifuge all tubes at 2,000 rpm at 0-4◦C for 10 minutes.

11. Read the absorbance of each supernatant at 412 nm.

12. Subtract the OD_{412} of "Spontaneous lysis" tube from the OD_{412} of each assay tube (A,B,...,I). These values represent OD_{412}. Tube B containing normal serum (Product No. S1764) is considered to be 100% of complement activity.

13. The percent of normal complement activity for each tube is calculated as the OD_{412} of each tube divided by the OD_{412} of tube B.

TABLE I

Assay Tubes	GVB^{2+} (μl)	dH_2O (μl)	C1q deficient serum (μl)	Normal Serum (μl)	Purified C1q (μl)	EA7S (1.5×10^8 cells/ml) (μl)
(A)	290	--	10	--	--	200
(B)	290	--	--	10	--	200
(C)	289	--	10	--	1	200
(D)	285	--	10	--	5	200
(E)	280	--	10	--	10	200
(F)	278	--	10	--	12	200
(G)	276	--	10	--	14	200
(H)	274	--	10	--	16	200
(I)	272	--	10	--	18	200
Control Tubes: (J) 100% lysis	--	300	--	--	--	200
(K) Spontaneous Lysis	300	--	--	--	--	200

References 4/99

1. Kolb, *et al.,* J. Immunol., **122,** 2103 (1979)
2. Kabat, E.A. and Mayer, M.M., Experimental Immunochemistry, Springfield, IL, Charles C. Thomas, 2nd edition, 149 (1961)