Name:	**C6-Dpl**
Catalog Number:	**A323**
Sizes Available:	1.0 mL/vial
Concentration:	>50 mg protein/mL (see Certificate of Analysis for actual conc.)
Form:	Frozen liquid
Activity:	>80% versus normal human serum standard
Purity:	No C6 detectable by immunodiffusion
Buffer:	10 mM sodium phosphate, 145 mM NaCl, pH 7.3
Preservative:	None, 0.22 µm filtered
Storage:	-70°C or below. Minimize freeze/thaw cycles.
Source:	Normal human serum (shown by certified tests to be negative for HBsAg and for antibodies to HCV, HIV-1 and HIV-II).
Precautions:	Use normal precautions for handling human blood products.
Origin:	Manufactured in the USA.

General Description

Normal human serum depleted of complement C6 protein by immunoaffinity chromatography. The product is tested for the absence of C6 by functional assays for classical pathway activity and alternative pathway activity and for C6 protein by double immunodiffusion. C6-Dpl is certified to possess functional classical and alternative pathways for complement activation up to the step of C6 binding to C5b (Law, S.K.A. and Reid, K.B.M. (1995); Morgan, B.P. ed. (2000); Dodds, A.W. and Sim, R.B. editors (1997)). Functional complement systems can be reconstituted by addition of purified C6 protein (64 µg/mL) indicating that all other complement components necessary for complement activation are present.

Physical Characteristics

Clear, straw-colored liquid containing all proteins of normal human serum except complement component C6.

Function

The depleted serum is tested for remaining classical pathway hemolytic activity using antibody-sensitized sheep erythrocytes (CompTech #B200) and for alternative pathway function using rabbit erythrocytes (CompTech #B300). The depleted serum is reconstituted with 64 µg/mL C6 (CompTech #A123) and retested to verify that a hemolytic function is restored. The Certificate of Analysis provided with each lot gives a description of the assays and specific titers for the depleted and reconstituted sera compared to normal human serum.

Assays

The unit of classical pathway activity is the CH50. A similar unit, the C6H50, is used to quantitate the activity of C6 and C6-Dpl. A C6H50 unit is the amount of C6 needed to lyse 50% of 3×10^7 EA cells (antibody- sensitized sheep erythrocytes (CompTech #B200)) when that amount of C6 (CompTech #A123) is incubated with the recommended volume of C6-Dpl in GVB^{++} in a total volume of 500 µL for 30 min at 37°C. This amount of C6 indicates the sensitivity of the assay for C6 which is typically about <5 ng C6 with 25 µL C6-Dpl. See the Certificate of Analysis for lot specific

values. Controls without C6 exhibit typically <5 % lysis. After full reconstitution (64 µg C6/mL C6-Dpl) lysis should be 100% in this assay.

Applications

C6-Dpl is used to assay C6 hemolytic activity in samples and to supply an activating system that is incapable of activating the membrane attack complex (C5b-9) of complement.

Precautions/Toxicity/Hazards

The source is human serum, therefore precautions appropriate for handling any blood-derived product must be used even though the source was shown by certified tests to be negative for HBsAg and for antibodies to HCV, HIV-1 and HIV-II.

Hazard Code: B WGK Germany 3

MSDS is available upon request.

References

Dodds, A.W. and Sim, R.B. editors (1997) Complement. A Practical Approach (ISBN 019963539) Oxford University Press, Oxford.

Law, S.K.A. and Reid, K.B.M. (1995) Complement 2nd Edition (ISBN 0199633568) Oxford University Press, Oxford.

Morgan, B.P. ed. (2000) Complement Methods and Protocols. (ISBN 0-89603-654-5) Humana Press, Inc., Totowa, New Jersey.

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