Name:	**C9-Dpl**
Catalog Number:	**A326C**
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 C9 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 C9 protein by immunoaffinity chromatography. The product is tested for the absence of C9 by functional assays for classical pathway activity and alternative pathway activity and for C9 protein by double immunodiffusion. C9-Dpl is certified to possess functional classical and alternative pathways for complement activation up to the step of C9 binding to C5b678 (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 C9 protein (60 µg/mL) indicating that all other complement components necessary for complement activation are present. Note that the C5b678 complex has considerable hemolytic potential without C9 and therefore C9-Dpl is capable of lysing EA and most other cells directly without added C9 if activation is strong enough and if the concentration of depleted serum used is high enough.

Physical Characteristics

C9-Dpl is a clear, straw-colored liquid containing all proteins of normal human serum except complement component C9.

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 60 µg/mL C9 (CompTech #A126) and retested to verify that full 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 C9H50, is used to quantitate the activity of C9 and C9-Dpl. A C9H50 unit is the amount of C9 needed to lyse 50% of 3×10^7 EA cells (antibody- sensitized sheep erythrocytes (CompTech #B200)) when that amount of C9 (CompTech #A126) is incubated with the

recommended volume of C9-Dpl in GVB^{++} in a total volume of 500 µL for 30 min at 37°C. This amount of C9 indicates the sensitivity of the assay for C9 which is typically about <5 ng C9 with 5 µL C9-Dpl. See the Certificate of Analysis for lot specific values of recommended amounts of C9-Dpl to use. Controls without C9 typically exhibit ~10 % lysis. After full reconstitution (60 µg C9/mL C9-Dpl) lysis should be 100% in this assay. Note as mentioned above, inputs of C9-Dpl higher than ~5 µL cause increasing lysis of EA and other target cells even in the absence of C9 depending on the susceptibility of the cells to C5b-8.

Applications

C9-Dpl is used to assay C9 hemolytic activity in samples and to supply an activating system that is incapable of activating the membrane attack complex of complement up to the C5b678 stage.

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.