Name:	**C3-Dpl (Immuno C3-Dpl)**
Catalog Number:	**A314**
Sizes Available:	1.0 mL/vial
Concentration:	>50 mg protein/mL (see Certificate of Analysis for actual conc.)
Form:	Frozen liquid
Activity:	>80% versus NHS standard when reconstituted with C3
Purity:	No C3 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

C3-Dpl is normal human serum in which C3 has been removed by immunoaffinity chromatography. The product is tested for the absence of C3 activity by classical and alternative pathway functional assays and for C3 protein by double immunodiffusion. C3-Dpl is certified to possess a functional alternative pathway for complement activation after reconstitution with 1.3 mg C3/mL (Morgan, B.P. (2000); Dodds, A.W. and Sim, R.B. (1997)). Similarly, a functional classical pathway can be reconstituted by addition of purified C3 protein (1.3 mg/mL) indicating that all other complement components necessary for classical and alternative pathway activation are present. Although a functional lectin pathway should also be reconstituted by the addition of C3, the function of this pathway is not tested.

Note that there is a weak C3 by-pass mechanism whereby the convertase C4b,C2a is able to activate C5 (Rawal, N. & Pangburn, M.K. 2003) and form C5b-9 complexes capable of causing low grade lysis. This system of C5 activation is functional in both the classical and the lectin pathways and is approximately 1000-fold less active than in the presence of C3.

C3-Dpl serum does not contain C3-derived antigens because it has been depleted by immunoaffinity adsorption of C3.

Physical Characteristics & Structure

C3-Dpl is supplied as a clear, straw-colored liquid containing all proteins of normal human serum except complement component C3.

Function

The depleted serum is tested for classical pathway activity by hemolytic assays using antibody-sensitized sheep erythrocytes (CompTech #B200) and for alternative pathway function using rabbit erythrocytes (CompTech #B300). The depleted serum is reconstituted with 1.3 mg/mL C3 (CompTech #A113c) and retested to verify that a functional classical and alternative pathways are 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 C3H50, is used to quantitate the activity of C3 and C3-Dpl. A C3H50 unit is the amount of functional C3 needed to lyse 50% of 3 x 10^7 EA cells (antibody-sensitized sheep erythrocytes (CompTech #B200)) when that amount of C3 (CompTech #A113) is incubated with the recommended volume of C3-Dpl in GVB^{++} (CompTech #B100) in a total volume of 500 µL for 30 min at 37°C. This amount of C3 indicates the sensitivity of the assay for C3 which is typically less than 200 ng C3 with 5 µL C3-Dpl. See the Certificate of Analysis for lot specific values.

Alternative pathway titers are performed to document that this pathway of complement activation is fully functional in reconstituted C3-Dpl. Lectin pathway activity is not tested, but it would be expected to be inactive due to the absence of C3.

Applications

C3-Dpl is used to assay C3 activity in experimental samples and to supply a serum with minimal ability to activate any of the three pathways of complement past C1, C4 and C2 in the classical pathway and MBL or ficolins, and C4 and C2 in the lectin pathway.

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.

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

Rawal N, Pangburn MK. (2003) Formation of high affinity C5 convertase of the classical pathway of complement. J. Biol. Chem. 278: 38476-83.

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Complement Technology, Inc.
4801 Troup Hwy, Suite 701
Tyler, Texas 75703 USA
Phone: 903-581-8284
FAX: 903-581-0491
Email: contactCTI@complementtech.com
Web: www.ComplementTech.com