Name:	**C4-Dpl (Immuno C4-Dpl)**
Catalog Number:	**A308**
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 C4
Purity:	No C4 detectable by immunodiffusion
Buffer:	10 mM sodium phosphate, 145 mM NaCl, pH 7.4
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

C4-Dpl is normal human serum in which C4 has been removed by immunoaffinity chromatography. The product is tested for the absence of C4 by functional assays for classical and alternative pathway activity and for C4 protein by double immunodiffusion. C4-Dpl is certified to possess a functional alternative pathway for complement activation (Morgan, B.P. (2000); Dodds, A.W. and Sim, R.B. (1997)). Similarly, a functional classical pathway can be reconstituted by addition of purified C4 protein (0.40 mg/mL) indicating that all other complement components necessary for classical and alternative pathway activation are present. The absence of C4 also prevents complement activation by the lectin pathway, but the function of this pathway is not tested.

Note that this C4-Dpl serum does not contain C4-derived antigens because it has been depleted by immunoaffinity adsorption of C4.

Physical Characteristics

C4-Dpl is supplied as a clear, straw-colored liquid containing all proteins of normal human serum except complement component C4.

Function

The depleted serum is tested for remaining classical pathway activity by hemolytic assays using antibody-sensitized sheep erythrocytes (CompTech #B200). The depleted serum is reconstituted with human C4 (CompTech #A105) and retested to verify that a functional classical pathway is restored. C4-Dpl is also tested for normal alternative pathways function. 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 C4H50, is used to quantitate the activity of C4 and C4-Dpl serum. A C4H50 unit is the amount of functional C4 needed to lyse 50% of 3×10^7 EA cells (antibody-sensitized sheep erythrocytes (CompTech #B200)) when that amount of C4 (CompTech #A105) is

incubated with the recommended volume of C4-Dpl in GVB^{++} (CompTech #B100) in a total volume of 500 µL for 30 min at 37°C. This amount of C4 indicates the sensitivity of the assay for C4 which is typically less than 5 ng C4 with 30 µL C4-Dpl serum. See the Certificate of Analysis for lot-specific values.

Applications

C4-Dpl serum is used to assay C4 activity in samples and to supply a serum unable to activate complement via the classical or lectin pathways. Note that C1 and C2 may still be activated in the absence of C4. Although there is a C2 by-pass system there does not appear to be an efficient C4 by-pass mechanism. Low level lysis of EA in C4-Dpl serum has been shown to require activation of the early classical and the alternative pathways (Wagner, E. et al. (1999)).

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.

Wagner, E., Platt, J.L., Howell, D.N., Marsh, H.C. Jr and Frank, M.M. (1999) IgG and complement-mediated tissue damage in the absence of C2: evidence of a functionally active C2-bypass pathway in a guinea pig model. J. Immunol. 163:3549-3558.

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