Name:	C4/FB-Dpl
Catalog Number:	A385
Sizes Available:	0.5 ml/vial
Concentration:	>50 mg/ml (see Certificate of Analysis for exact conc.)
Form:	Frozen liquid
Activity:	>70% versus normal human serum standard
Purity:	No C4 and factor B detectable by immunodiffusion
Buffer:	10 mM Sodium phosphate, 145 mM NaCl, pH 7.3
Presevarive:	None, 0.22 µm filtered.
Storage:	-70ºC 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/FB-Dpl is normal human serum in which C4 and factor B (fB) have been removed by immunoaffinity chromatography. The product is tested for the absence of C4 and factor B activity by testing classical and alternative pathway function and for the absence of C4 and factor B proteins by double immunodiffusion. The C4/FB-Dpl is certified to exhibit less than 5% classical pathway and alternative pathway activities. After reconstitution with purified factor B (0.2 mg/mL), C4/FB-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 (0.4 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.

Physical Characteristics & Structure

C4/FB-Dpl is supplied as a clear, straw-colored liquid containing all proteins of normal human serum except C4 and factor B.

Function

C4/FB-Dpl 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 0.4 mg/mL C4 (CompTech #A105) and 0.2 mg/mL factor B (CompTech #A135) and retested to verify that functional classical and alternative pathways are restored. The Certificate of Analysis provided with each lot gives a description of the assays for the depleted and reconstituted sera compared to normal human serum.

Assays

The unit of classical pathway activity is the CH50 and for the alternative pathway it is the AP50. A CH50 unit is the amount of complement needed to lyse 50% of 1 x 108 EA cells (antibody-sensitized sheep erythrocytes (CompTech #B200)) when that amount

of serum is incubated with the EA in GVB++ (CompTech #B100) in a total volume of 1.5 mL for 60 min at 37o C. See the Certificate of Analysis for lot specific values. An AP50 is defined as the amount of complement yielding 50% lysis of 1.5 x 107 rabbit erythrocytes (Er, CompTech #B300) when incubated for 30 min at 37o C in a total reaction volume of 100 µL of GVBo containing a final MgEGTA concentration of 5 mM.

Lectin pathway activity of C4/FB-Dpl is not tested but it would be expected to be inactive due to the absence of C4.

Applications

C4/FB-Dpl is made to supply a serum unable to activate complement via the classical, lectin and alternative pathways. Note that C1 and C2 may still be activated in the absence of C4. Although there is a C2 bypass system there does not appear to be an efficient C4 bypass 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

MSDS 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

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.

FOR RESEARCH USE ONLY.
NOT FOR HUMAN OR DRUG USE.

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