

Name:	C5/FB-Dpl
Catalog Number:	A355
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 C5 and factor B detectable by immunodiffusion
Buffer:	10 mM Sodium phosphate, 145 mM NaCl, pH 7.3
Preseervative:	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

C5/FB-Dpl is normal human serum in which C5 and factor B (fB) have been removed by immunoaffinity chromatography. The product is tested for the absence of C5 and factor B activity by testing classical and alternative pathway function and for the absence of C5 and factor B proteins by double immunodiffusion. The C5/FB-Dpl is certified to exhibit less than 5% classical pathway and alternative pathway activities. A functional alternative pathway is restored by addition of purified C5 (75 ug/mL) and factor B (200 ug/mL) (Morgan, B.P. (2000); Dodds, A.W. and Sim, R.B. (1997)). Similarly, a functional classical pathway is restored after reconstitution with purified C5 protein (75 ug/mL) indicating that all other complement components necessary for classical and alternative pathway activation are present. The function of the lectin pathway is not tested.

Physical Characteristics & Structure

C5/FB-Dpl is supplied as a clear, straw-colored liquid containing all proteins of normal human serum except C5 and factor B.

Function

C5/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 75 ug/mL C5 (CompTech #A120) and 200 ug/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 37°C. See the Certificate of Analysis for lot specific values. An

AP50 is defined as the amount of complement yielding 50% lysis of 1.5×10^7 rabbit erythrocytes (Er, CompTech #B300) when incubated for 30 min at 37°C in a total reaction volume of 100 μ L of GVBo containing a final MgEGTA concentration of 5 mM.

Applications

C5/FB-Dpl is made to supply a serum unable to activate the alternative pathway of complement but able to activate the classical pathway up to the step of C5 activation. C5/FB-Dpl can be used as an activating system that is capable of C3b deposition via the classical pathway but incapable of activating the formation of the membrane attack complex (C5b-9) of complement due to the absence of C5. Lectin pathway activity of C5/FB-Dpl has not been tested.

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

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