Name:	**C3/FB-Dpl**
Catalog Number:	**A345C**
Sizes Available:	0.5 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 and factor B.
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/FB-Depleted Serum is normal human serum in which C3 and factor B (fB) have been removed by immunoaffinity chromatography. The product is tested for the absence of C3 and factor B activity by testing classical and alternative pathway function and for the absence of C3 and factor B proteins by double immunodiffusion. The C3/FB-depleted serum is certified to exhibit less than 5% classical pathway and alternative pathway activities. A functional classical pathway can be reconstituted by addition of purified C3 protein (1.3 mg/mL) whereas a functional alternative pathway can be reconstituted by addition of purified C3 protein (1.3 mg/mL) and factor B protein (0.2 mg/mL) indicating that all other complement components necessary for classical and alternative pathway activation are present. After reconstitution with C3 (1.3 mg C3/mL) the C3/FB-Depleted Serum is certified to possess a functional classical pathway for complement activation whereas after reconstitution with C3 (1.3 mg C3/mL) and factor B (0.2 mg/mL), the product is certified to possess a functional alternative pathway for complement activation (Morgan, B.P. (2000); Dodds, A.W. and Sim, R.B. (1997)). Although a functional lectin pathway should also be reconstituted by the addition of similar concentrations 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.

Physical Characteristics & Structure

C3/FB-Depleted Serum is supplied as a clear, straw-colored liquid containing all proteins of normal human serum except C3 and factor B.

Function

C3/FB-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 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 and specific titers 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 10^8 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 x 10^7 rabbit erythrocytes (Er, CompTech #B300) when incubated for 30 min at 37°C in a total reaction volume of 100 µL of GVB° containing a final MgEGTA concentration of 5 mM. Lectin pathway activity of C3/FB-Depleted Serum is not tested, but it would be expected to be inactive due to the absence of C3.

Applications

C3/FB-Depleted Serum is used as a source of serum with minimal ability to activate any of the three pathways of complement. However, C1 can be activated as well as the lectin pathway complexes of the lectins and MASPs. These complexes will then activate C2 that will attach to C4 generating the convertase C4b,C2a which is able to activate C5 (Rawal, N. & Pangburn, M.K. 2003) via a weak C3 by-pass mechanism. 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.

Precautions/Toxicity/Hazards

The source is human serum, therefore precautions appropriate for handling any bloodderived 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.