Name:	**Factor I-Dpl**
Catalog Number:	**A338**
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 after reconstitution with factor I
Purity:	No factor I 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

Normal human serum was depleted of factor I by immunoaffinity chromatography. The product is tested for the absence of factor I by double immunodiffusion. Factor I is a regulator of complement activation (Pangburn, M.K., et al. (1977)). Factor I-Dpl is still capable of activating all three pathways of complement if metal ions are added. Activation of the alternative pathway is spontaneous and occurs in normal blood, in factor I deficient individuals and in Factor I-Dpl serum. Our depleted serum is stored with 0.1 mM EDTA to inhibit spontaneous activation. Upon the addition of magnesium ions spontaneous activation produces fluid phase C3b due to alternative pathway tick-over (Pangburn, M.K., Müller-Eberhard, H.J. (1980)). In normal human blood or serum this C3b would be rapidly inactivated to iC3b, by factors H and I (Pangburn, M.K., et al. (1977); (Laursen, S.B. et al. (1994)), however, in the absence of factor I, C3b remains as C3b. This C3b binds factor B and factor B is activated by factor D forming the C3b,Bb complex and the free Ba fragment. Because factor H is present in Factor I-Dpl Bb is rapidly decayed off the C3b,Bb complex. The free C3b then binds another factor B and the process repeats itself until little or no factor B remains. In factor I-deficient individuals their factor B levels are very low and they exhibit low or very low C3 levels (Davis, A.E.III, et al. (1977); Nilsson, S.C., et al. (2009)). Individuals with factor I deficiencies are susceptible to recurrent bacterial infections and exhibit little or no alternative pathway activity (Abramson, N. et al. (1971); Nilsson, S.C., et al. (2009)).

Factor I-Dpl is certified to possess a functional alternative pathway for complement activation only if factor I or a controlling factor with a function similar to that of factor I is added prior to the addition of metal ions (specifically Mg^{++}). Full reconstitution requires addition of 34 µg factor I/mL serum. It is also tested for and certified to contain functional classical pathway indicating that all other complement components necessary for classical and alternative pathway activation are present except for factor I (Morgan, B.P. (2000); Dodds, A.W. and Sim, R.B. (1997)).

Physical Characteristics & Structure

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

Function

Factor I-Dpl serum is not functionally deficient in either alternative or classical pathway activity, but without factor I the feedback loop of the alternative pathway will spontaneously activate within a few minutes if magnesium is added prior to addition of factor I or a factor I-like control protein. We verify that fully functional alternative and classical pathways are restored after addition of 34 µg/mL factor I (CompTech #A138). It is tested for classical pathway activity with assays using antibody-sensitized sheep erythrocytes (EA, CompTech #B200) and for alternative pathway function using rabbit erythrocytes (Er, CompTech #B300). 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. Lectin pathway activity is not routinely tested or certified, but it would be expected to be active in this depleted serum.

Assays

Because the complement system is still intact in Factor I-Dpl it is possible, to use this depleted serum to titer factor I or factor I-like proteins. If sufficient factor I or factor I-like activity is added back to stabilize the alternative pathway for the duration of the experiment then the alternative pathway can be assayed. An APH50 value is determined for Factor I-Dpl + factor I. The factor I should be titered to be equivalent to between 0 and 34 µg factor I/mL in the original undiluted serum plus 5 mM MgEGTA (CompTech #B106). After a preincubation period (10 to 30 min at 37ºC) a titer of the remaining alternative pathway activity or of factor B activity can be performed. In assays where insufficient factor I was present factor B will be consumed during the preincubation period and this will result in loss of alternative pathway activity. The remaining alternative pathway activity may be determined by measuring the amount needed to lyse 50% of 1.5 x 10^7 rabbit erythrocytes (CompTech #B300)) when incubated in GVBº (CompTech #B103) containing a final concentration of 5 mM MgEGTA (CompTech #B106) in a total volume of 100 µL for 30 min at 37ºC.

A more specific assay of factor I activity may also be performed. Radiolabeled C3b, biotinylated C3b or fluorescently tagged C3b may be added to the serum and SDS-PAGE used to detect cleavage of the labeled C3b alpha chain into two fragments characteristic of iC3b.

Applications

Factor I-Dpl can be used to assay the ability of factor I or of factor I-like regulatory proteins to stabilize the amplification system of the alternative pathway and yield a functional alternative pathway of complement.

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

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