Name:	**Factor H-Dpl**
Catalog Number:	**A337**
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 H
Purity:	No factor H detectable by immunodiffusion
Buffer:	10 mM sodium phosphate, 145 mM NaCl, pH 7.3
Preservative:	None, 0.22 µm filtered
Storage:	-70^{o}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 H by immunoaffinity chromatography. The product is tested for the absence of factor H by double immunodiffusion. Factor H is a regulator of complement activation. As a result, factor H-Dpl is still capable of activating the alternative pathway and does so spontaneously and very rapidly in the absence of factor H. In fact, activation occurs in less than 2 min in the fluid phase without an activating particle resulting in the consumption of both C3 and factor B. For this reason this depleted serum is stored with 0.1 mM EDTA to inhibit spontaneous activation. Factor H-Dpl is certified to possess a functional alternative pathway for complement activation only if a controlling factor, such as factor H, is added prior to the addition of metal ions (specifically Mg^{++}). Full reconstitution requires addition of 500 µg factor H/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 H (Morgan, B.P. (2000); Dodds, A.W. and Sim, R.B. (1997)).

Physical Characteristics & Structure

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

Function

Factor H-Dpl serum is not functionally deficient in either alternative or classical pathway activity, but without factor H the feedback loop of the alternative pathway will spontaneously activate in less that 2 min if magnesium is added prior to addition of factor H or a factor H-like control protein (Pangburn, M.K. (2002)). The depleted serum is reconstituted with 500 µg/mL Factor H (CompTech #A137) and tested to verify that fully functional alternative and classical pathways are restored. 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.

Assays

The unit of classical pathway activity is the CH50 and the unit of alternative pathway activity is the APH50. Because the pathways are both still active in factor H-Dpl it is difficult, but not impossible, to use this depleted serum to titer factor H. If sufficient factor H or factor H-like activity is added back to stabilize the alternative pathway for the intended duration of the experiment then the alternative pathway can be assayed (Pangburn, M.K. (2002)). An APH50 value is determined for Factor H-Dpl + factor H (sufficient factor H is added to be equivalent to 500 ug factor H/mL in the undiluted serum) by measuring the amount needed to lyse 50% of 1.5 x 10^7 rabbit erythrocytes (CompTech #B300)) when incubated in GVB^o (CompTech #B103) containing a final concentration of 5 mM MgEGTA (CompTech #B106) in a total volume of 100 µL for 30 min at 37°C. Various MgEGTA concentrations, from 3 mM to 13 mM, have been reported to be effective. The classical pathway activity is reported as the standard CH50 value for Factor H-Dpl + factor H (added equivalent to 500 ug factor H/mL in the undiluted serum). The CH50 activity is determined as the amount of reconstituted serum needed to lyse 50% of 3 x 10^7 EA cells (antibody-sensitized sheep erythrocytes (CompTech #B200)) when incubated with the recommended volume of serum in GVB^{++} (CompTech #B100) in a total volume of 500 µL for 30 min at 37°C. See the Certificate of Analysis for lot specific titer values.

Lectin pathway activity is not routinely tested or certified, but it would be expected to be active.

Applications

Factor H-Dpl can be used to assay the ability of factor H or of factor H-like regulatory proteins to stabilize the amplification system of the alternative pathway and yield a functional alternative pathway of complement (Pangburn, M.K. (2002)).

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.

Pangburn, M.K. (2002) Cutting edge: localization of the host recognition functions of complement factor H at the carboxyl-terminal: implications for hemolytic uremic syndrome. J. Immunol. 169:4702-4706.

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