Name:	**C1s-Dpl**
Catalog Number:	**A304**
Sizes Available:	1.0 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 C1s 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

C1s depleted serum (C1s-Dpl) is normal human serum depleted of C1s by immunoaffinity chromatography. The product is tested for the absence of C1s by functional assays for classical pathway activity and for C1s protein by double immunodiffusion. C1s-Dpl is certified to possess a functional alternative pathway for complement activation (Morgan, B.P. (2000); Dodds, A.W. and Sim, R.B. (1997)).

In serum C1s is present in its proenzyme form (Valet, G and Cooper N.R. (1974); Ziccardi, R.J. and Cooper N.R. (1976)). C1s enzyme is the activated form of C1s proenzyme. C1s is a subunit of the C1 complex which is the first component in the classical pathway of complement (See product description for C1 Cat# A098). C1s proenzyme is an inactive zymogen until C1 is activated. C1r is activated when C1 binds to and is activated by antibodies bound to antigens (immune complexes) yielding C1r enzyme, the first protease that initiates the cascade. C1r enzyme in the C1 complex activates C1s proenzyme generating C1s enzyme. A functional classical pathway can be reconstituted by addition of purified C1s proenzyme (31 µg/mL) to the C1s-Dpl indicating that all other complement components necessary for classical pathway activation are present and active.

Regulation

Activation of C1s proenzyme in the C1 complex is regulated indirectly by C1- INH. C1r self-activation, and subsequent C1s activation, is controlled by a weak association of C1r with C1 esterase inhibitor (C1-INH) when it is in the C1 complex (Ziccardi, R.J. (1982)). Once activated, C1s enzyme is rapidly inactivated by C1-INH.

Physical Characteristics & Structure

C1s-Dpl is supplied as a clear, straw-colored liquid containing all proteins of normal human serum except complement component C1s.

Function

The depleted serum is tested for remaining classical pathway activity (CH50 assay) by hemolytic assays using antibody-sensitized sheep erythrocytes (CompTech #B200) and for alternative pathway function (AP50 assay) using rabbit erythrocytes (CompTech #B300). The depleted serum is reconstituted with 31 µg/mL C1s proenzyme (CompTech #A103) and retested to verify that a functional classical pathway is 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. The classical pathway activity is reported as the standard CH50 value for C1s-Dpl + C1s proenzyme (CompTech #A103) added equivalent to 31 µg C1s/mL in the undiluted serum. The CH50 activity is determined as the amount of reconstituted serum

needed to lyse 50% of 1 x 10^8 EA cells (antibody-sensitized sheep erythrocytes (CompTech #B200)) when incubated for 60 min at 37 ^oC in a total volume of 1.5mL GVB^{++}. Normal human serum is used as standard. See the Certificate of Analysis for lot specific titer values.

Alternative pathway titers are performed to document that this pathway of complement activation is fully functional in C1s-Dpl. Lectin pathway activity is not tested.

Applications

C1s-Dpl is used to assay C1s hemolytic activity in samples and to supply an alternative pathway activating system that is incapable of activating the classical 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 available upon request.

References

Morgan, B.P. ed. (2000) Complement Methods and Protocols. (ISBN 0-89603-654-5) Humana Press, Inc., Totowa, New Jersey.

Dodds, A.W. and Sim, R.B. editors (1997) Complement. A Practical Approach (ISBN 019963539) Oxford University Press, Oxford.

Valet, G. and Cooper N.R. (1974) J. Immunol. **112**, 1667.

Ziccardi, R.J. and Cooper N.R. (1976)) J. Immunol. **116**, 496.

Ziccardi, R.J. (1982) Spontaneous activation of the first component of human complement (C1) by an intramolecular autocatalytic mechanism. J. Immunol. 128:2500- 2504.

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