

Name:	C4-Deficient Guinea Pig Serum
Catalog Number:	A305C
Sizes Available:	1.0 mL/vial
Concentration:	>30 mg protein/mL (see Certificate of Analysis for actual conc.)
Form:	Frozen liquid
Activity:	>1,000,000 C4H50 units/mg C4 added to assay with C4-D GPS
Purity:	No C4 detectable by hemolytic assay
Buffer:	None added
Preservative:	None, 0.22 µm filtered
Storage:	-70°C or below. Minimize freeze/thaw cycles
Source:	Guinea pigs genetically deficient in C4
Precautions:	Use normal precautions for handling animal blood products.
Origin:	Manufactured in the USA.

General Description

C4-deficient guinea pigs were characterized in 1971 as being completely genetically and functionally deficient in C4 (Ellman, L. et al. (1970); Ellman, L. et al. (1971); Frank, M.M., et al. (1971)). This line of guinea pigs has been maintained and used extensively for the characterization of complement. In fact, these animals were a key element in the rediscovery and detailed characterization of the alternative pathway of complement (Ellman, L. et al. (1971); Frank, M.M., et al. (1971)). The product is tested for the absence of C4 by functional assays for classical pathway activity. C4-D guinea pig serum reconstituted with human C4 is certified to possess a functional classical pathway for complement activation after reconstitution (Morgan, B.P. (2000); Dodds, A.W. and Sim, R.B. (1997)). The absence of C4 would also be predicted to prevent complement activation by the lectin pathway, but the function of this pathway is not tested.

Physical Characteristics

C4-D guinea pig serum is supplied as a clear, straw-colored liquid containing all proteins of normal guinea pig serum except complement component C4.

Function

The depleted serum is tested for remaining classical pathway activity by hemolytic assays using antibody-sensitized sheep erythrocytes (CompTech #B200). The depleted serum is reconstituted with human C4 (CompTech #A105) and retested to verify that a 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. A similar unit, the C4H50, is used to quantitate the activity of C4 and C4-D guinea pig serum. A C4H50 unit is the amount of functional C4 needed to lyse 50% of 3×10^7 EA cells (antibody-sensitized sheep erythrocytes (CompTech #B200)) when that amount of C4 (CompTech #A105) is incubated with the recommended volume of C4-D guinea pig serum in GVB⁺⁺ (CompTech #B100) in a total volume of 500 µL for 30 min at 37°C. This amount of C4

indicates the sensitivity of the assay for C4 which is typically less than 2 ng C4 with 10 µL C4-D guinea pig serum. See the Certificate of Analysis for lot specific values.

Applications

C4-D guinea pig serum is used to assay C4 activity in samples and to supply a serum unable to activate complement via the classical pathway. Note that C1 and C2 may still be activated in the absence of C4, but whereas there is a C2 by-pass system there does not appear to be an efficient C4 by-pass mechanism. Low level lysis of EA in C4-D guinea pig serum has been shown to require activation of the early classical and the alternative pathways (Wagner, E. et al. (1999)).

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.

Ellman, L., Green, I. and Frank, M.M. (1970) Genetically controlled total deficiency of the fourth component of complement in the guinea pig. *Science* 170:74-75.

Ellman, L., Green, I., Judge, F. and Frank, M.M. (1971) *In vivo* studies in C4-deficient guinea pigs. *J. Exp. Med.* 134:162-175.

Frank, M.M., May, J., Gaither, T. and Ellman, L. (1971) *In vitro* studies of complement function in sera of C4-deficient guinea pigs. *J. Exp. Med.* 134:176-187.

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

Wagner, E., Platt, J.L., Howell, D.N., Marsh, H.C. Jr and Frank, M.M. (1999) IgG and complement-mediated tissue damage in the absence of C2: evidence of a functionally active C2-bypass pathway in a guinea pig model. *J. Immunol.* 163:3549-3558.

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www.tecomedical.com

e-mail: info@tecomedical.com

