CERTIFICATE OF ANALYSIS

Complement Technology, Inc.
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Tyler, Texas 75703, USA

Product: **Cyno C1q-Dpl**
Catalog # **CY300** Lot #
Exp. Date:

Description: Cyno C1q-Depleted Serum is depleted of C1q as determined by immunochemical analysis and CH50 functional activity.*

Specifications	Limits	Results
PROTEIN CONCENTRATION	≥ 40 mg/ml using an extinction coefficient of $E^{1\%}/_{280\ nm}$ = 10	69 mg/mL
FILL VOLUME	0.50 – 0.55 mL	0.505 mL
PHYSICAL APPEARANCE	Clear, straw colored	Clear, straw colored
BUFFER	Phosphate Buffered Saline, pH 7.4	Conforms
PRESERVATIVE	None, filtered through a 0.22 µm pore size filter	Conforms
STARTING MATERIAL	Pooled cynomolgus monkey serum from healthy animals	Conforms
IMMUNOCHEMICAL ANALYSIS		
Ouchterlony	No cyno C1q antigen detected using goat anti-human C1q antiserum	Conforms

CH50 FUNCTIONAL ACTIVITY **

NCYS Standard	≥ 75 CH50/mL	156 CH50/mL
Cyno C1q-Dpl	≤ 5 CH50/mL	< 1 CH50/mL
Ratio: CH50/mL Cyno C1q-Dpl / CH50/mL NCYS Std	≤ 0.1	< 0.1
Cyno C1q-Dpl reconstituted with 70µg cyno C1q/mL	≥ 40 CH50/mL	67 CH50/mL
Ratio: CH50/mL Cyno C1q-Dpl + cyno C1q / CH50/mL NCYS Std	≥ 0.30	0.43

ALTERNATIVE PATHWAY (AP) ACTIVITY ***

NCYS Standard	≥ 50 APH50/mL	211 APH50/mL
Input NCYS Std to yield 1 APH50	≤ 20 µL	4.75 µL
Cyno C1q-Dpl	≥ 50 APH50/mL	229 APH50/mL
Input Cyno C1q-Dpl to yield 1 APH50	≤ 20 µL	4.4 µL

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Product: **Cyno C1q-Dpl # 2 (continued)**

AP Ratio $\frac{\text{APH50/mL Cyno C1q-Dpl}}{\text{APH50/mL NCYS Std}}$	$\geq$ 0.50	1.1

* The Cynomolgus monkey serum is not available for sale or usage outside the USA due to international endangered species laws.

** Cyno C1q-Dpl reconstituted with 70ug cyno C1q retained partial classical pathway activity (43% CH50 activity) when compared to cyno normal serum (NCYS) as complement standard. One CH50 unit is defined as the input of cyno C1q-Dpl reconstituted with cyno C1q (70ug/ml), or NCYS complement standard yielding 50% lysis of $1x10^8$ EA when incubated for 60 minutes at 37 ^oC in a total reaction volume of 1.5 mL GVB^{++}.

*** One unit of whole alternative pathway activity (APH50) is defined as the input of cyno C1q-Dpl or NCYS complement standard yielding 50% lysis of $1.5x10^7$ rabbit erythrocytes (Er) when incubated for 30 minutes at 37 ^oC in a total reaction volume of 100 µL GVB^o containing a final Mg-EGTA concentration of 10 mM.

STORE AT -70^{o}C or BELOW.
Thaw quickly at 37°C, mix, and put in an ice+water bath to cool.
Avoid Repeated Freeze/Thaw

FOR RESEARCH USE ONLY
NOT FOR HUMAN OR DRUG USE

Signature of Analyst

Date of Analysis

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Product: **Cyno C1q-Dpl # 2 (continued)**

CLASSICAL PATHWAY ACTIVITY*

Recommended volume of cyno C1q-Dpl serum per assay	40 µL or less	20 µL
C1qH50 Units/mg purified cyno C1q at the recommended input of C1q-Dpl	≥12,500 Units/mg	1,230,000 Units/mg
Input of purified cyno C1q to yield 1 Unit	≤ 80 ng/Unit	0.81 ng/Unit
C1qH50 Units/ml NCYS Complement Std (at 70 µg cyno C1q/ml) at the recommended input of cyno C1q-Dpl	≥ 3,500 Units/ml	73,000 Units/ml
Input of cyno C1q in NCYS Std to yield 1 Unit	≤ 20 ng/Unit	0.96 ng/Unit
Ratio: $\frac{\text{C1qH50/mg C1q}}{\text{C1qH50/mg C1q in NCYS Std}}$	≥ 0.2	1.2
Background A_{412} EA blank reading at the recommended input of C1q-Dpl	≤ 0.250	0.059

* One C1qH50 unit measured by classical pathway activation is defined as the amount of C1q required to yield 50% lysis of $3x10^7$ EA when incubated in the presence of the recommended volume of cyno C1q-Dpl serum for 30 minutes at 37 °C in a total reaction volume of 500 µL GVB^{++}.

General Description Normal human serum depleted of complement C1q protein by immunoaffinity chromatography. The product is tested for the absence of C1q by functional assays for classical pathway activity and for C1q protein by double immunodiffusion. C1q-Dpl is certified to possess a functional alternative pathway for complement activation (Morgan, B.P. (2000); Dodds, A.W. and Sim, R.B. (1997)). A functional classical pathway can be reconstituted by addition of purified C1q protein (70 µg/mL) indicating that all other complement components necessary for classical pathway activation are present and active. The absence of C1q does not prevent complement activation by the lectin and alternative pathways on appropriate surfaces.

The material would be used to validate custom and commercial cyno C1q ELISA assays for our non-human primate studies. The sera does not need to retain complement activity but rather be depleted of C1q. We would use it as a matrix along with purified cyno C1q to evaluate the ELISA assays. We would need very little material.

It would be helpful to know what the cyno C1q-Dol will be used for? Our preliminary analysis of making Cyno C1q-Dpl indicates that we can successfully deplete cyno C1q from cyno serum as determined by double immunodiffusion assays (ouchterlony) and by hemolytic assays. The cyno C1q-dpl was active for the alternative pathway of complement in the absence of C1q and the activity was comparable to that of normal cyno serum.

Preliminary analysis of the cyno C1q-Dpl after reconstituting it with purified cyno C1q restored the classical pathway activity. But the activity was less when compared to normal cyno and human serum. Addition of purified human C2 and C4 to the cyno C1q-Dpl serum resulted in robust classical pathway activity comparable to that of normal human and cyno serum.

Before we proceed to make cyno C1q-Dpl, we would like to know if the cyno C1q-Dpl reconstituted with purified human C2 and C4 will work for you.