

ELISA kits available from ADI (see details at the web site)

#0010	Human Leptin		
#200-120-AGH	Human globular Adiponectin (gAcrp30)		
#0700	Human Sex Hormone Binding Glob (SHBG)		
#0900	Human IGF-Binding Protein 1 (IGFBP1)		
#1000	Human C-Reactive Protein (CRP)		
#100-110-RSH	Human Resistin /FIZZ3		
#100-140-ADH	Human Adiponectin (Acrp30)		
#100-160-ANH	Human Angiogenin		
#100-180-APH	Human Angiopoietin-2 (Ang-2)		
#100-190-B7H	Human Bone Morphogenic Protein 7 (BMP-7)		
#1190	Human Serum Albumin	#1200	Human Albumin (Urinary)
#1750	Human IgG (total)	#1760	Human IgM
#1800	Human IgE	#1810	Human Ferritin
#1210	Human Transferrin (Tf)	#0020	Beta-2 microglobulin
#1600	Human Growth Hormone (GH)		
#0060	Human Pancreatic Colorectal cancer (CA-242)		
#1820	Human Ovarian Cancer (CA125)	#1830	Human CA153
#1840	Human Pancreatic & GI Cancer (CA199)		
#1310	Human Pancreatic Lipase		
#1400	Human Prostatic Acid Phosphatase (PAP)		
#1500	Human Prostate Specific Antigen (PSA)	#1510	free PSA (fPSA)
#0500	Human Alpha Fetoprotein (AFP)		
#0050	Human Neuron Specific Enolase (NSE)		
#0030	Human Insulin	#0040	Human C-peptide
#0100	Human Luteinizing Hormone (LH)		
#0200	Human Follicle Stimulating Hormone (FSH)		
#0300	Human Prolactin (PRL)		
#0400	Human Chorionic Gonadotropin (HCG)	#0410	HCG-free beta
#0600	Human Thyroid Stimulating Hormone (TSH)		
#1100	Human Total Thyroxine (T4)	#1110	Human Free T4 (ft4)
#1650	Human free triiodothyronine (ft3)	#1700	Human T3 (total)
#1850	Human Cortisol	#1860	Human Progesterone
#1865	Human Pregnenolone	#1875	Human Aldosterone
#1880	Human Testosterone	#1885	Human free Testosterone
#1910	Human Androstenedione	#1920	Human Estradiol
#1925	Human Estrone	#1940	Dihydrotestosterone (DHT)
#1950	Human DHEA-sulphate (DHEA-S)		
#3400	Human serum Neopterin		
#3000	Human Rheumatoid Factors IgM (RF)		
#3100	Human anti-dsDNA		
#3200	Anti-Nuclear Antibodies (ANA)		

Instruction Manual No. M-2950

Human Anti-C1q Antibody (Anti-C1q)

ELISA KIT Cat. No. 2950, 96 Tests

For Quantitative Determination of
Autoantibodies against C1q In Human Serum



For In Vitro Research Use Only



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Kit Contents: (Human Anti-C1q ELISA KIT # 2950)

Components	#
Purified C1q Coated Microwells (8x12 wells) , 2 9 5 1	
Human Anti-C1q IgG stds , 0, 6.3, 12.5, 25, 50 and 100 U/ml (1.5 ml each in serum/buffer base) #2952A-F	6 vials
Human Anti-C1q negative control , 1.5 ml, in serum/buffer base) (lot sp. values on the vial) #2953	1 vial
Human Anti-C1q positive control , 1.5 ml, in serum/buffer base) (lot sp. values on the vial) #2954	1 vial
Sample buffer conc 5X, (20 ml), yellow color #2955	1 bottle
Anti-human IgG-HRP Conj. (15 ml, red color) #2956	1 bottle
Wash Solution (50X), 20 ml, #2957	1 bottle
T MB Substrate solution , 15 ml, #2958	1 bottle
Stop Solution (diluted acid), 15 ml #2959	1 bottle
Complete Instruction Manual, M-2950	1

Intended Use

Anti-C1q is an indirect solid phase enzyme immunoassay (ELISA) for the determination of IgG class autoantibodies against C1q in human serum or plasma. The assay is intended for research use only, not for use in diagnostic procedures.

INTRODUCTION

The complement system, a central component of innate immunity, exhibits three pathways of activation classical, alternative, and lectin-mediated. C1, a key component of the classical pathway, is actually a complex of three proteins C1q, C1r, and C1s. C1q is serum glycoprotein of 18-polypeptides chains consisting of three non-identical subunits, A (29 kDa), B (26 kDa), and C (246 aa, 19 kDa) in molar ratio of 1:2:2. C1Q in the plasma is complexed with two proenzymes C1r and two C1s molecule to form the first component of complement (C1). Activation of complement via classical pathway is triggered by binding of globular head of C1q to immune complexes containing IgG (Fc-region) or IgM or to a variety of other activating substances, including C-reactive protein, retrovirus, and mitochondria. Subsequent to C1q binding, c1r and C1s are converted to proteolytic enzymes that are responsible to continuation of activation via the classical pathway.

Alternatively, high-affinity autoantibodies directly recognize the collagenous "tail" portion of C1q through the antibody F(ab) antigen- combining sites rather than via the Fc domain. **Anti-C1q autoantibodies** have been commonly identified in patients with autoimmune diseases such as systemic lupus erythematosus (SLE) and hypocomplementemic urticarial vasculitis. Anti-C1q antibodies preferentially localized in the glomeruli of patients with SLE. Lupus nephritis (LN), the renal disease that accompanies SLE. Anti-C1q autoantibodies have been suggested to be closely associated with LN. Conversely, in the absence of anti-C1q autoantibodies, no LN develops. Serial measurement of Anti-C1q titers will be an effective tool for the guidance of immunosuppressive therapy in SLE patients. Anti-C1q autoantibodies may be especially relevant for monitoring of lupus nephritis activity.

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Specificity

The microplate is coated with C1q. The antigen preparation is highly purified by affinity chromatography. The Anti-C1q test is specific only for autoantibodies directed against Anti-C1q.

The conjugate (anti-human IgG-HRP) used in the kit is designed to detect the IgG or its subtypes without any substantial reactivity with other subtypes (IgM, IgA etc). However, it is possible to use subtype specific anti-human IgG (IgG1-4) or IgM to determine the anti-C1q subtype specific in samples.

Species crossreactivity

This kit is designed for human samples only and it has not been tested in other species (such as monkey or animals). ADI also provide anti-C1q ELISA kit for mouse samples (Cat # 5020).

Calibrators values Vs International reference

Since no international reference preparations for Anti-C1q autoantibodies is available, the assay system is calibrated in arbitrary units.

LIMITATIONS OF PROCEDURE

The Anti-C1q ELISA is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated.

INTERFERING SUBSTANCES

No interference has been observed with haemolytic (up to 1000 mg/dL), lipemic (up to 3 g/dL triglycerides) or bilirubin (up to 40 mg/dL) containing sera. Nor have any interfering effects been observed with the use of anticoagulants. However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

General references and Information on C1q

Blom, A.M. 2004. *Mol. Immunol.* **40**, 1333; Wen, L., 2004. *J. Allergy Clin. Immunol.* **113**, 585; Swierzko, A.S. 2003. *Eur. J. Immunol.* **33**, 2842; Niculescu, F., 1994. *JBC* **269**, 4417; Rivas, G., 1994. *Biochem.* **33**, 2341; Muller-Eberhard, H.J. 1988. *Annu. Rev. Biochem.* **57**, 321; Ziccardi, R.J. 1982. *J. Immunol.* **128**, 2500; Hu, V.W., 1981. *J. Immunol.* **127**, 380; Pangburn, M.K., 1981. *J. Exp. Med.* **154**, 856; Polley, M.J., 1971. *J. Exp. Med.* **133**, 53; Cooper, N.R., and Muller-Eberhard, H.J. 1970. *J. Exp. Med.* **132**, 775

Calculation of results

For Anti-C1q IgG a 4-Parameter-Fit with linear-log coordinates for optical density and concentration is the data reduction method of choice.

First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

Interpretation of results

In a normal range study with serum samples from healthy blood donors the following ranges have been established for the Anti-C1q test: Anti-C1q IgG [U/ml]

Normal: < 10

Elevated: > 10

Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually. It is recommended that each laboratory establishes its own normal and pathological ranges of serum Anti-C1q antibodies. The above reference ranges should be regarded as guidelines only.

Quality Control

This test is only valid if the optical density at 450 nm for Positive Control (1) and Negative Control (2) as well as for the Calibrator A and F complies within acceptable range. If any of these criteria is not fulfilled, the results are invalid and the test should be repeated.

PERFORMANCE CHARACTERISTICS

Parallelism

In dilution experiments sera with high antibody concentrations were diluted with sample buffer and assayed in the Anti-C1q kit. The assay showed linearity over the full measuring range.

Precision (Reproducibility)

Statistics for coefficients of variation (CV) were calculated for each of three samples from the results of 24 determinations in a single run for Intra-Assay precision. Run-to-run precision was calculated for each of 3 samples from the results of 16 determinations in two runs.

Intra-Assay

Mean 25.2, 58.6, and 75.4 with 3.7%, 2.9% and 2.8% CV respectively.

Intra-Assay

Mean 22.2, 33.6, and 53.4 with 4.7%, 2.5% and 1.9% CV respectively.

Sensitivity

The lower detection limit for the Anti-C1q test was determined at 0.5 U/ml.

The highest Anti-C1q titers were found in patients with active lupus nephritis. It was also demonstrated that rises in Anti-C1q titers have predictive value for ensuing relapses of lupus nephritis. It is described that in some cases patients with clinical active lupus were found as Anti-ds DNA negative, so Anti-C1q antibodies may serve as an additional tool for rheumatologist to document lupus activity.

PRINCIPLE OF THE TEST

Highly purified human C1q protein is bound to microwells. Antibodies against this antigen, if present in diluted serum, bind to the respective antigen. Washing of the microwells removes unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human IgG immunologically detects the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured at 450 nm using an ELISA plate reader. The amount of color is directly proportional to the concn of IgG antibodies present in the sample.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (20-100 μ l) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The ADI's Anti-C1q ELISA test is intended for *in vitro research* use only. The reagents contain proclin 300 as preservative; necessary care should be taken when disposing solutions. The standards and controls have been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), H₂SO₄ (stop solution), and Proclin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

SAMPLE COLLECTION AND HANDLING

Blood should be collected by venipuncture, allowed to clot, and serum separated by centrifugation at room temperature. Do not heat inactivate the serum. If sera can not be immediately assayed, these could be stored at -20°C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

PREPARATION OF REAGENTS

Preparation of sample buffer

Dilute the contents of each vial of the sample buffer concentrate (5x) with distilled or deionized water to a final volume of 100 ml prior to use. Store refrigerated:

stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

Preparation of wash solution

Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled or deionized water to a final volume of 1000 ml prior to use. Store refrigerated: stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

Sample preparation

Dilute all patient samples 1:100 with sample buffer before assay. Therefore combine 10 µl of sample with 990 µl of sample buffer in a polystyrene tube. Mix well. Controls are ready to use and need not be diluted.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 12 months from the date of shipping under appropriate storage conditions. Do not contaminate the bottles. Withdraw solutions in a separate clean tube or dispensing trays. Any unused solution should be discarded and not returned to the bottle. Do not use HRP substrate solution if this solution is blue. Do not expose these solutions to strong light.

TEST PROCEDURE (ALLOW ALL REAGENTS TO REACH ROOM TEMP. BEFORE USE).

1. Label, and secure the microtiter well strips to be used on the plate. **Dilute** serum samples (1:100). Controls provided in the kit are already pre-diluted. **Pipet 100 µl pre-diluted negative, positive controls, standards, and diluted serum samples into appropriate wells in duplicate.** Mix gently for 5-10 seconds, cover the plate and incubate for **30 minutes** at room temp (25-28oC).
2. **Aspirate and wash the wells 3 times** with 300 µl of diluted wash buffer. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.
3. **Add 100 µl of enzyme conjugate** into each well. Mix gently for 5-10 seconds. Cover the plate and **incubate for 15 minutes** at room temp.
4. **Aspirate and wash the wells 3 times** as above.
5. **Pipet 100 µl HRP substrate (TMB) solution** into each well. Mix gently for 5 seconds. Cover the plate and **incubate at room temp for 15 minutes.** Blue color develops in positive wells.

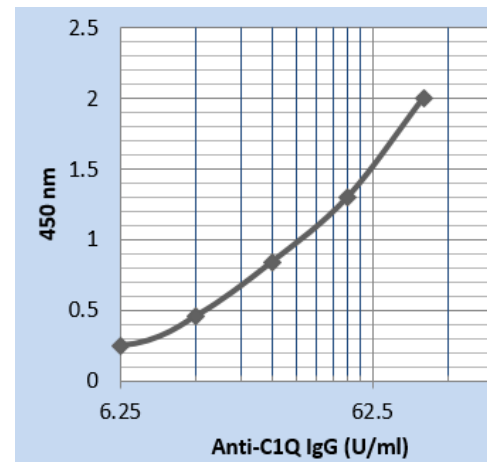
6. **Stop the reaction by adding 100 µl of stopping solution** to all wells at the same timed intervals. Mix gently for 5-10 seconds. Blue color turns yellow. Measure the absorbance at 450 nm using an ELISA reader.

NOTES:

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed five minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a set of negative & positive standards and calibrator on each plate. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A _{450nm}
A1, A2	Std. A (0 U/ml)	0.17
B1, B2	Std. B (6.25 U/ml)	0.25
C1, C2	Std. C (12.5 U/ml)	0.46
D1, D2	Std. D (25 U/ml)	0.84
E1, E2	Std. E (50 U/ml)	1.3
F1, F2	Std. F (100 U/ml)	2.003
G1, G2	-ve control	0.18
H1, H2	+ve control	1.45



NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.