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 Pregnolone **#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-2970

Monkey Circulating Immune Complexes (CIC) C1q ELISA Kit, 96 tests

ELISA KIT Cat. No. 2970

For Quantitative Determination of
CIC C1q In Monkey Serum or plasma

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

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Phone (210) 561-9515 • Fax (210) 561-9544;Toll Free (800) 786-5777

Email: service@4adi.com ;Web Site: www.4adi.com

DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.

Kit Contents: (Monkey CIC ELISA KIT # 2970

Components	qty
Anti-hC1q **Coated Microplate** (96 wells) #2971	1 plate
CIC **Standards**, **0, 16, and 64 ug** Equivalent/ml (3 vials x1.5 ml each) **ready to use** #2972A-C	3 vials
CIC **Negative control**, 1.5 ml, #2973NC (lot specific values on the vial)	1 vial
CIC **Positive control**, 1.5 ml, #2974PC (lot specific values on the vial)	1 vial
Incubation buffer (50 ml) # 2975	1 bottle
Anti-Monkey IgG-HRP Conj. (100x, 0.5 ml) #2976	1 vial
Conjugate Dilution buffer (20 ml) #2977	1 bottles
Wash Solution (10X), 50 mlx2 #2970WB	2 bottles
TMB Substrate solution (brown bottle) , 15 ml #2970SS	1 bottle
Stop Solution, 15 ml (clear bottle) 2970ST	1 bottle
Complete Instruction Manual	M-2970

Intended Use

ADI's CIC C1q kit is an indirect ELISA for the quantitative determination of Immune complex C1q in Monkey serum or plasma. It is based upon capture of CIC by anti-C1q antibody coated on the plate. Bound CICs are detected by anti-Monkey IgG-HRP Conjugate. This kit is for research use only, not for diagnostic or therapeutic use.

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.

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, is present in 25–50% of the cases and is the major cause of morbidity and mortality. Anti-C1q autoantibodies have been suggested to be closely associated with LN. This association is concluded from the correlation between anti-C1q autoantibody positivity and renal involvement, the predictive value of anti-C1q autoantibody titers for flares of nephritis, and the accumulation of anti-C1q autoantibodies in LN kidneys. 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. 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.

There are many tests for the determination of CIC, included the test of precipitation with PEG, radial immunodiffusion, and cellular tests like the test of Ray cell. There is no one procedure to determinate all types of immunocomplexes (e.g, C1q and C3d).

Clinical Specificity and Sensitivity (human studies)

92 serum and plasma specimens collected from normal and asymptomatic subjects were tested with CIC C1q ELISA. The overall specificity of the assay was 96 %.

125-serum and plasma specimen collected from patients with systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) or other disorders was tested with CIC C1q. The overall clinical sensitivity was 92 %

Analytical Sensitivity

The lowest detectable concentration of CIC C1q that can be distinguished from standard 0 is 1.0 µg Equiv./ml at the 99 % confidence limit.

Recovery

The recovery of 12.5 – 25 – 50 – 100 µg Equiv./ml IgG aggregates added to a sample gave values between 94.3% and 105.7%with reference to the original concentrations.

12. LIMITATIONS OF THE PROCEDURE

Sample(s), which are contaminated microbiologically, should not be used in the assay. Highly lipemic or haemolysed specimen(s) should similarly not be used. It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than one plate is used, it is recommended to repeat the dose response curve. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time deviation during reaction. Plate readers measure vertically. Do not touch the bottom of the wells. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.

Reference values

CIC concentration < 16 µg Equiv./ml	negative.
CIC concentration 16 – 18 µg Equiv./ml	uncertain.
CIC concentration > 18 µg Equiv./ml	positive.

The data above is given for human samples as no systemic reference values are available for monkey samples. We suggest that users establish their own reference values from a given population on monkey.

QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of CIC C1q for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends.

The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

Intra Assay Variation

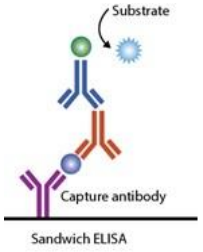
Within run variation was determined by replicate determination (16x) of two different control sera in one assay. The within assay variability is = 5.3 %.

Inter Assay Variation

Between run variation was determined by replicate measurements of two different control sera in 2 different lots. The between assay variability is = 6.0%.

The data above is given for human samples as no systemic reference values are available for monkey samples. We suggest that users establish their own reference values from a given population on monkey.

PRINCIPLE OF THE TEST



Highly purified anti-Monkey C1q IgG is bound to microwells. During first incubation C1q-fixing immune complexes (CIC) in samples and standards bind to the immobilized anti-C1q on the surface of the microtiter wells. Unbound CIC will be removed by a subsequent washing step. During second incubation specific anti-IgG antibodies conjugated with peroxidase bind to CIC. Unbound conjugate will be removed by a subsequent washing step. 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 and ELISA plate reader. The amount of color is directly proportional to the concentration of CIC present in the sample.

MATERIALS AND EQUIPMENT REQUIRED

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

PRECAUTIONS

The ADI's. CIC 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 Monkey 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 Prolcin-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 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^{O}C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum. Plasma (EDTA, Heparin, citrated etc) can also be used.

PREPARATION OF REAGENTS

Preparation of samples

Dilute samples 1:50 in incubation buffer (10 ul of samples in 500 ul incubation buffer and mix gently). Prepare sample dilutions before the test and keep at room temp until test is complete.

Preparation of 1X Wash Buffer

Dilute 1 bottle of 50 ml in 450 ml of distilled water. Store refrigerated. stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label. It should be brought to room temp for the test.

Preparation of 1X Antibody Conjugate

Dilute antibody conjugate (100x) by diluting with conjugate buffer (100 ul of 100x conjugate into 10 ml of conjugate buffer). Prepare 1 ml for each strip or 10 ml for the entire plate. Prepare 1X conjugate buffer as needed. Do not store 1X conjugate solution beyond the assay.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8^{o}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.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMP. BEFORE USE). Keeping the kit at room temp (25-28oC) for 1 hour and preparing required solutions is sufficient.*

1. Label, and secure the microtiter well strips to be used on the plate. **Dilute** serum samples (1:50). **Standards and Controls provided in the kit are ready-to-use**. Dispense sample 100 ul buffer into first 2 wells as blanks. Pipet 100 ul standards, negative, positive controls, , and *diluted* serum samples into appropriate wells in *duplicate*. Mix gently for 5-10 seconds, cover the plate and incubate for 30 minutes at 37oC.

2. **Aspirate and wash the wells 3 times** with 300 ul 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 ul of enzyme conjugate into each well**.** Mix gently for 5-10 seconds. Cover the plate and incubate for **30 minutes at 37oC**.

4. Aspirate and wash the wells 3 times as above.

5. Pipet 100 ul HRP substrate (TMB) solution into each well. Mix gently for 5 seconds. Cover the plate and incubate at room temp (25-28oC) 15 **minutes**. Blue color develops in positive wells.

6. Stop the reaction by adding 100 ul 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.

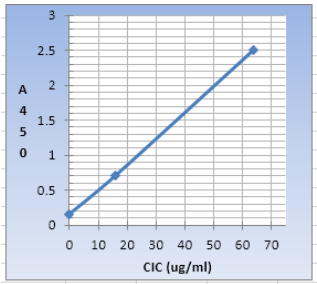
WORKSHEET OF A TYPICAL ASSAY

Wells	Calibrators /Samples	A450	Net A450	Results
A1/A2	Sample diluent	0.1000	-	
B1/B2	Std A (0 ug Eq/ml)	0.38	0.28	
C1/C2	Std B (16 ug Eq/ml)	0.54	0.44	
D1/D2	Std C (64 ug Eq/ml)	1.5	1.40	
D1/D2	Negative Control	0.54	0.44	
E1/E2	Positive control	1.15	1.05	+ve
F1/F2	Sample 1	0.410	0.310	-ve
G1/G2	Sample 2	0.910	0.810	+ve

Calculation of results

Calculate the mean absorbance for blanks, standards, controls, and samples. Subtract the average blank values from all values. Plot the values of the standards against concentration. Draw the best-fit curve through the plotted points. Interpolate the values of the samples on the standard curve to obtain the corresponding concentration of CIC expressed in µg Equiv./ml.

If Samples are diluted more than 1:50 (e.g. 1:200) then multiply the values by 4 to account for additional dilution.



2970-Monkey-CIC-ELISA-Graphs/AA-3

These data is for demonstration purpose only. Actual values may vary a little and the lot specific values must be used for the calculation of sample values.