

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

- |                     |   |              |                           |
|---------------------|---|--------------|---------------------------|
| <b>#0010</b>        | Human Leptin                                |              |                           |
| <b>#200-120-AGH</b> | Human globular Adiponectin (gAcrp30)        |              |                           |
| <b>#0700</b>        | Human Sex Hormone Binding Glob (SHBG)       |              |                           |
| <b>#0900</b>        | Human IGF-Binding Protein 1 (IGFBP1)        |              |                           |
| <b>#1000</b>        | Human C-Reactive Protein (CRP)              |              |                           |
| <b>#100-110-RSH</b> | Human Resistin /FIZZ3                       |              |                           |
| <b>#100-140-ADH</b> | Human Adiponectin (Acrp30)                  |              |                           |
| <b>#100-160-ANH</b> | Human Angiogenin                            |              |                           |
| <b>#100-180-APH</b> | Human Angiopoietin-2 (Ang-2)                |              |                           |
| <b>#100-190-B7H</b> | Human Bone Morphogenic Protein 7 (BMP-7)    |              |                           |
| <b>#1190</b>        | Human Serum Albumin                         | <b>#1200</b> | Human Albumin (Urinary)   |
| <b>#1750</b>        | Human IgG (total)                           | <b>#1760</b> | Human IgM                 |
| <b>#1800</b>        | Human IgE                                   | <b>#1810</b> | Human Ferritin            |
| <b>#1210</b>        | Human Transferrin (Tf)                      | <b>#0020</b> | Beta-2 microglobulin      |
| <b>#1600</b>        | Human Growth Hormone (GH)                   |              |                           |
| <b>#0060</b>        | Human Pancreatic Colorectal cancer (CA-242) |              |                           |
| <b>#1820</b>        | Human Ovarian Cancer (CA125)                | <b>#1830</b> | Human CA153               |
| <b>#1840</b>        | Human Pancreatic & GI Cancer (CA199)        |              |                           |
| <b>#1310</b>        | Human Pancreatic Lipase                     |              |                           |
| <b>#1400</b>        | Human Prostatic Acid Phosphatase (PAP)      |              |                           |
| <b>#1500</b>        | Human Prostate Specific Antigen (PSA)       | <b>#1510</b> | free PSA (fPSA)           |
| <b>#0500</b>        | Human Alpha Fetoprotein (AFP)               |              |                           |
| <b>#0050</b>        | Human Neuron Specific Enolase (NSE)         |              |                           |
| <b>#0030</b>        | Human Insulin                               | <b>#0040</b> | Human C-peptide           |
| <b>#0100</b>        | Human Luteinizing Hormone (LH)              |              |                           |
| <b>#0200</b>        | Human Follicle Stimulating Hormone (FSH)    |              |                           |
| <b>#0300</b>        | Human Prolactin (PRL)                       |              |                           |
| <b>#0400</b>        | Human Chorionic Gonadotropin (HCG)          | <b>#0410</b> | HCG-free beta             |
| <b>#0600</b>        | Human Thyroid Stimulating Hormone (TSH)     |              |                           |
| <b>#1100</b>        | Human Total Thyroxine (T4)                  | <b>#1110</b> | Human Free T4 (fT4)       |
| <b>#1650</b>        | Human free triiodothyronine (fT3)           | <b>#1700</b> | Human T3 (total)          |
| <b>#1850</b>        | Human Cortisol                              | <b>#1860</b> | Human Progesterone        |
| <b>#1865</b>        | Human Pregnenolone                          | <b>#1875</b> | Human Aldosterone         |
| <b>#1880</b>        | Human Testosterone                          | <b>#1885</b> | Human free Testosterone   |
| <b>#1910</b>        | Human Androstenedione                       | <b>#1920</b> | Human Estradiol           |
| <b>#1925</b>        | Human Estrone                               | <b>#1940</b> | Dihydrotestosterone (DHT) |
| <b>#1950</b>        | Human DHEA-sulphate (DHEA-S)                |              |                           |
| <b>#3400</b>        | Human serum Neopterin                       |              |                           |
| <b>#3000</b>        | Human Rheumatoid Factors IgM (RF)           |              |                           |
| <b>#3100</b>        | Human anti-dsDNA                            |              |                           |
| <b>#3200</b>        | Anti-Nuclear Antibodies (ANA)               |              |                           |

Instruction Manual No. M-2960

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

**ELISA KIT Cat. No. 2960**

For Quantitative Determination of  
CIC C1q In Human Serum or plasma



*For In Vitro Research Use Only*

  
**ALPHA DIAGNOSTIC**  
**INTERNATIONAL**

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## Kit Contents: (Human CIC ELISA KIT # 2960)

<b>C o m p o n e n t s</b>	<b>q t y</b>
Anti-hC1q Coated Microwells (96 wells) #2961	1 p l a t e
Human CIC standards, 0, 16, and 64 ug Equivalent/ml (3 vials x1.5 ml each) ready to use #2962A-C	3 v i a l s
Human Anti-CIC negative control, 1.5 ml, #2963NC (lot specific values on the vial)	1 v i a l
Human Anti-CIC positive control, 1.5 ml, #2964PC (lot specific values on the vial)	1 v i a l
Incubation buffer (50 ml) # 2965	1 b o t t l e
Anti-human IgG-HRP Conj. (100x, 0.5 ml) #2966	1 v i a l
Conjugate Dilution buffer (20 ml) #2967	1 b o t t l e s
Wash Solution (10X), 50 ml x2 #2960WB	2 b o t t l e s
T MB Substrate solution (brown bottle) , 15 ml #2960SS	1 b o t t l e
Stop Solution, 15 ml (clear bottle) 2960ST	1 b o t t l e
Complete Instruction Manual	M - 2 9 6 0

### Intended Use

ADI's CIC C1q kit is an indirect ELISA for the quantitative determination of Immune complexes C1q in human serum or plasma. It is based upon capture of anti-C1q antibody coated on the plate. Bound CICs are detected by anti-human 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).

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 %

### Diagnostic Specificity and Sensitivity

Specimen obtained from 209 patients with systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) or other disorders were tested using the ADI CIC C1q kit and QUIDEL EIA kit. The obtained results are shown in the table below:

ADI	-	+	-	+
Quidel	-	+	-	+
RA Patients	20	12	4	4
RA Patients	38	25	12	6
Others	0	85	2	1

From the 209 tested samples the following diagnostic sensitivity and specificity are obtained:

ADI	RA	SLE	Others	RA+SLE	RA+SLE +Others
Sensitivity	75%	67%	97%	70%	87%
Specificity	83%	87%	-	85%	85%
Agreement	-	-	-	79%	86%

### 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.

### Comparative Data

Circulating Immunocomplexes (CIC) collected from 160 patients with systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), or other disorders subjects and 95 form normal, asymptomatic subject were measured. The overall agreement between the two test methods was 87 %. The average CIC concentration was 2.1 ug Equiv./mL (S.D. ug 1.6)

### 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.

## 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 %.

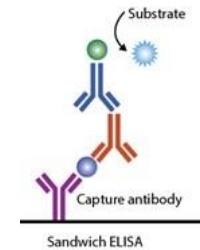
#### Inter Assay Variation

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

### Clinical Specificity and Sensitivity

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 %.

## PRINCIPLE OF THE TEST



Highly purified anti-human 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 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), H<sub>2</sub>SO<sub>4</sub> (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](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°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°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 already pre-diluted. Dispense sample 100 ul buffer into first 2 wells as blanks. **Pipet 100 ul 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 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.

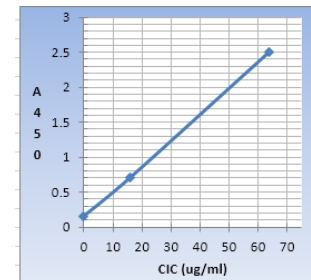
### WORKSHEET OF A TYPICAL ASSAY

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.



2960-Human-CIC-ELISA-Graphs/Arif-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.