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)

#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)

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Human Anti-C1q Antibody (Anti-C1q)

ELISA KIT Cat. No. 2950

For Quantitative Determination of Autoantibodies against C1q in Human Serum

For In Vitro Research Use Only

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**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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### 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 unbound 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 and 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 µl) 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 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°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:

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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 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 room temp (25-28°C).

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 15 minutes at room temp**.

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 for 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 TYPICAL ASSAY**

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

**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.

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