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

Catalog#	ProdDescription
3100	Human anti-dsDNA IgG ELISA Kit, 96 tests, Quantitative
3105	Human anti-dsDNA IgM ELISA Kit, 96 tests, Quantitative
3110	Human anti-dsDNA IgA ELISA Kit, 96 tests, Quantitative
3115	Human anti-ssDNA IgG ELISA Kit, 96 tests, Quantitative
3205	Human Anti-Nuclear Antibodies (ANA) ELISA Kit, 96 tests, Semi-Quantitative
3210-SSA	Human anti-SS-A (60 Kda/Ro IgG ELISA Kit, 96 tests, Quantitative
3215-SSA	Human anti-SS-A (52 Kda/Ro IgG ELISA Kit, 96 tests, Quantitative
3220-SSB	Human anti-SS-B/La IgG ELISA Kit, 96 tests, Quantitative
3250	Anti-thyroid peroxidase ELISA kit, Semi-Quantitative
3300	Anti-helicobacter pylori IgG ELISA kit, Semi-Quantitative
3300-100-SMG	Human Anti-Smith antigen (Sm) IgG ELISA kit, 96 tests, Quantitative
3300-110-SRG	Human Anti-Smith antigen/RNP (Sm/RNP) IgG ELISA kit, 96 tests,
3300-120-RNG	Human Anti-RNP (RNP-70) IgG ELISA kit, 96 tests, Quantitative
3300-130-HNG	Human Anti-histones IgG ELISA kit, 96 tests, Quantitative
3300-140-SCG	Human Anti-Scl-70 (Scleroderma 70 Kda/DNA-topoisomerase-1) IgG ELISA kit,
3300-150-JOG	Human Anti-Jo-1 (Scleroderma 70 Kda/DNA-topoisomerase-1) IgG ELISA kit, 96
3300-160-AFG	Human Anti-Alpha Fodrin IgG ELISA kit, 96 tests, Quantitative
3300-170-CLG	Human Anti-Cardiolipin IgG ELISA kit, 96 tests, Quantitative
3300-175-CLM	Human Anti-Cardiolipin IgM ELISA kit, 96 tests, Quantitative
3300-185-CLA	Human Anti-Cardiolipin IgA ELISA kit, 96 tests, Quantitative
3300-190-B2G	Human Anti-Beta2-Glycoprotein 1 IgG ELISA kit, 96 tests, Quantitative
3300-195-B2M	Human Anti-Beta2-Glycoprotein 1 IgM ELISA kit, 96 tests, Quantitative
3300-200-B2A	Human Anti-Beta2-Glycoprotein 1 IgA ELISA kit, 96 tests, Quantitative
3300-205-APS	Human Anti-Phospholipid Screen (anti-Phosphatidyl Serine, Phosphatidyl Inositol, Phosphatidic Acid and beta-2-Glycoprotein I) IgG/IgM ELISA kit, 96 tests, Quantitative
3300-210-PSS	Human Anti-Phosphotidyl serine IgG/IgM ELISA kit, 96 tests, Quantitative
3300-215-PIS	Human Anti-Phosphotidyl Inositol IgG/IgM ELISA kit, 96 tests, Quantitative
3300-220-PAS	Human Anti-Phosphotidic Acid IgG/IgM ELISA kit, 96 tests, Quantitative
3300-230-APG	Human Anti-Prothrombin IgG/IgM ELISA kit, 96 tests, Quantitative
3300-235-APA	Human Anti-Prothrombin IgA ELISA kit, 96 tests, Quantitative
3300-240-AVA	Human Anti-Annexin V IgG ELISA kit, 96 tests, Quantitative
3300-250-ANG	Human ANCA Screen (Anti-PR3 and Anti-MPO) IgG ELISA kit, 96 tests,
3300-255-PRG	Human ANCA (Anti-PR3) IgG ELISA kit, 96 tests, Quantitative
3300-260-LFG	Human Anti-Lactoferrin IgG ELISA kit, 96 tests, Quantitative
3300-265-MPG	Human ANCA (Anti-MPO) IgG ELISA kit, 96 tests, Quantitative
3300-270-GBG	Human Anti-glomerular basement membrane (GBM) IgG ELISA kit, 96 tests,
3300-280-BPG	Human Anti-bactericidal permeability increasing (BPI) protein IgG ELISA kit, 96
3300-290-ELG	Human Anti-Elastase IgG ELISA kit, 96 tests, Quantitative
3300-300-GLG	Human Anti-Gliadin IgG ELISA kit, 96 tests, Quantitative
3300-305-GLM	Human Anti-Gliadin IgM ELISA kit, 96 tests, Quantitative
3300-310-GLA	Human Anti-Gliadin IgA ELISA kit, 96 tests, Quantitative
3300-315-PRG	Human Anti-Parietal cell (alpha and beta subunits of the Parietal Cell (H//K/ATPase) IgG ELISA kit, 96 tests, Quantitative
3300-320-ASC	Human Anti-ASCA (mannan from Saccharomyces cerevisiae) IgA/IgG ELISA kit, 96 tests, Quantitative
3300-330-ASG	Human Anti-Sperm IgG ELISA kit, 96 tests, Quantitative
3300-340-CCG	Human Anti-Cyclic Citrullinated Peptide (CCP) IgG ELISA kit, 96 tests,
3300-350-TPG	Human Anti-thyroid peroxidase (TPO) IgG ELISA kit, 96 tests, Quantitative
3300-360-TGG	Human Anti-thyroglobulin (TG) IgG ELISA kit, 96 tests, Quantitative

Instruction Manual No. M-3300-170-CLG

Human Anti-Cardiolipin IgG

ELISA KIT Cat. No. 3300-170-CLG

for the detection of IgG autoantibodies specific for cardiolipin in human serum and plasma

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

4638 N Loop 1604 West • San Antonio• Texas 78249 • USA.

Phone (210) 561-9515 • Fax (210) 561-9544

Toll Free (800) 786-5777

Email: Techsupport@4adi.com

Web Site: www.4adi.com

Anti-Cardiolipin IgG ELISA KIT Cat. No. 3300-170-CLG

Kit Contents: (reagents for 96 tests)

Components	
Cardiolipin Antigen coated microwell strip plate (96 wells);#3300171	96 wells (1 plate)
Anti-Cardiolipin **Std. A**, 1.5 ml, 0 GPL U/mL, #3300172A	1 vial
Anti-Cardiolipin **Std. B**, 1.5 ml, 7.5 GPL U/mL, #3300172B	1 vial
Anti-Cardiolipin **Std. C**, 1.5 ml, 15 GPL U/mL, #3300172C	1 vial
Anti-Cardiolipin **Std. D**, 1.5 ml, 30 GPL U/mL, #3300172D	1 vial
Anti-Cardiolipin **Std. E**, 1.5 ml, 60 GPL U/mL, #3300172E	1 vial
Anti-Cardiolipin **Std. F**, 1.5 ml, 120 GPL U/mL, #3300172F	1 vial
Anti-Cardiolipin **Positive Control, 1.5 ml**#3300170P	1 vial
Anti-Cardiolipin **Negative Control, 1.5 ml**#3300170N	1 vial
lot sp. Conc. mentioned on each vial	
Anti-Cardiolipin **Sample Diluent (5X)** 20 ml, #3300173	1 bottle
Anti-Cardiolipin IgG **Conjugate**, 15 ml, #3300174	1 bottle
HRP **substrate Solution**, 15 ml # #3300170TM	1 bottle
Wash buffer (50X), 20 ml #3300170-WB	1 bottle
Stop solution (ready-to-use), 15 ml, #3300170-ST	1 bottle
Complete Instruction Manual, M-3300-170-CLG	1

Intended Use

Anti-Cardiolipin IgG is an indirect enzyme immunoassay (ELISA) for the determination of IgG class autoantibodies against Cardiolipin in human serum or plasma. ADI's Anti-Cardiolipin IgG ELISA KIT is intended for research use only, not for use in diagnostic procedures.

General Information

Anti-cardiolipin antibodies (aCL) can be considered as one of three groups of anti-phospholipid antibodies, the others being VDRL and lupus anticoagulant (LAC). aCL and LAC antibodies appear to be closely related, or to recognize similar antigenic determinants. Of the anti-phospholipid antibodies, aCL are observed most frequently and can be associated with a diverse set of clinical manifestations including neurological dysfunction, venous or arterial thrombosis, thrombocytopaenia and recurrent foetal loss5. Measuring aCL antibody levels may well be more useful than other anti-phospholipids because of these clinical associations6,7. The IgG isotype appears to provide the best correlation, IgM is also found but IgA is more rare.

PERFORMANCE CHARACTERISTICS

1. PRECISION

Intra-assay precision: *Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.*

Sample	Mean Value GPL U/mL	%CV
1	10.9	5.5
2	20.5	5.4
3	73.0	5.4

Inter-assay precision: *Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.*

Sample	Mean Value GPL U/mL	%CV
1	11.8	5.3
2	21.1	3.7
3	70.5	6.3

Detection Limit

The lower limit of detection was determined to be 1 GPL-U/ml

Measuring range

0 - 120 GPL-U/ml

Interferences:

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

References:

Reynaud Q (2014) Autoimmune Red. In press; Saadatnia M (2007) Neurosci. 12, 124-126; Coulam CB (1997) J. Assist. Reprod. Genet. 14, 603-608; Arnold LW (1993) Intl. Immunol. 5, 1365-1373; Colaco CB (1985) Clin. Exp. Immunol. 59, 449-456; Triplett DA (2002) Arch. Pathol. Lab. Med. 126, 1424-1429; Miyakis (2006) J. Throm. Res. 4, 295-306;

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples GPL U/mL	Mean A_{450nm}
A1, A2	**Std. A** (0)	0.15
B1, B2	**Std. B** (7.5)	0.35
C1, C2	**Std. C** (15)	0.75
D1, D2	**Std. D** (30)	1.15
E1, E2	**Std. E** (60)	1.5
F1, F2	**Std. F (120)**	1.95

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.

CALCULATION OF RESULTS:

Consider each assay separately when calculating and interpreting results For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve. The concentration of patient samples may then be estimated from the calibration curve by interpolation. Using data reduction software a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice

PRINCIPLE OF THE TEST

The wells of the microtiter strips are coated with highly purified cardiolipin antigen. During the first incubation, specific autoantibodies in diluted serum or plasma bind to the antigen-coated surface; the wells are then washed to remove unbound components. In the second incubation, the Conjugate, enzyme-labelled antibodies to human IgG, binds any surface-bound autoantibodies. After further washing, specific autoantibodies are traced by incubation with the Substrate. Addition of Stop Solution terminates the reaction, resulting in a colored end-product. The amount of Conjugate bound is measured in absorbance units. In the qualitative protocol, the amount of Conjugate bound by the sample is compared with that bound by the Reference Control. In the quantitative protocol, the concentration of anti-cardiolipin autoantibody can be estimated by interpolation from a dose-response curve based on Standards.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (25-100 µl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plate Reader.

LIMITATIONS

1.The Alpha Diagnostic International ELISA test is intended for *in vitro research* use only.
Although the presence of high titres of aCL antibodies is associated with clinical symptoms the information is an aid to diagnosis only, and must be considered in light of other clinical and laboratory findings.
2. If a current or prior syphilis infection is suspected this should be confirmed or ruled out by a specific test for anti-treponemal antibodies, as the patient may have a positive result without increased risk of thrombosis.
3. Low to moderate levels of aCL antibodies have been reported in acute infection (32%)10, asymptomatic elderly patients (2-52%)4-7, and healthy blood donors (2%)4. In the majority of cases, these conditions are not reported to be accompanied by thrombotic events, and clinical interpretation is unclear. If such patients test positive while there are clinical signs, e.g. infection, the test should be repeated after six months.

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).
All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).

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

REAGENTS PREPRATION:

Dilute the contents of one vial of the buffered **wash solution concentrate (50x)** with distilled or deionised water to a final volume of 1000 ml prior to use.. Store at 4ºC.

Dilute Sample Diluent (5X): 20 ml with 100 ml distilled water.

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 TEMPERATURE BEFORE USE).* **Dilute wash buffer & Sample Diluent as per detail on page 3.**

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag.

Quantitative protocol: run Standards (A-F), Positive and Negative Controls, and samples.

1. Label or mark the microtiter well strips to be used on the plate. Reference wells for identification.

2. Pipet **100 µl of standards**, Positive and Negative Controls, and pre-diluted patient samples into appropriate wells. Remember to change pipette tips between additions. Cover the plate and incubate for **30 minutes** at room temperature (20-28 °C).

3. Aspirate and wash the wells **3 times** with **300 µl wash buffer**. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

4. Pipet **100 µl** Conjugate to each well. Cover the plate and incubate for **15 minutes at room temperature.**

5. Aspirate and wash the wells **3 times** with 300 µl wash buffer as above.

6. Dispense **100 µl TMB substrate per well**. Mix gently for 5-10 seconds.

7. Cover the plate and incubate for **15 minutes at room temperature.**

8. Stop the reaction by adding **100 µl** of stop solution to all wells. Mix gently for 5-10 seconds. Blue color turns yellow. Read the plate at 450 nm within 30 min.

Expected Values:

110 serum samples comprising an almost equal ratio of male to female, aged between 18 and 61 years, were assayed for IgG anti-cardiolipin antibodies. The reference range was established based on the mean plus three standard deviations. No differences attributable to age or gender were observed in this population. It is recommended that users establish reference ranges for the populations served by their own laboratories; the following is only intended as a guide to the interpretation of results.

Quantitative Protocol:

Concentration GPL U/mL	Suggested Interpretation
<=10	Negative for antibodies to cardiolipin.
>10 to 15	Borderline for antibodies to cardiolipin. A repeat test should be carried out on a subsequent sample. Note that the clinical significance of borderline levels of anti-cardiolipin antibodies is the subject of debate, and these results should be considered in light of other diagnostic and clinical information.
>15 to 60	Positive for antibodies to cardiolipin.
>60	High levels of cardiolipin antibodies.

Quantitative Protocol:

Calculate the mean absorbance value of each Standard and plot against log10 Standard concentration (see following table) on suitable graph paper. The concentration of samples and Controls can then be read from the standard curve; a typical plot is shown below for reference purposes, it must not be used for interpreting results Smooth spline, weighted 4- or 5-parameter logistic, log/logit, or lin/linit are also satisfactory.

Samples with absorbances above Standard F (120 GPL U/mL) are outside the range of the assay, and should be reported as >120 GPL U/mL, diluted and re-assayed, correcting for the dilution factor.