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

3300-200-B2A	Human Anti-Beta2-Glycoprotein 1 IgA ELISA kit, 96 tests
3300-210-PSS	Human Anti-Phosphatidyl serine IgG/IgM ELISA kit, 96 tests
3300-215-PIS	Human Anti-Phosphotidyl Inositol IgG/IgM ELISA kit, 96 tests
3300-220-PAS	Human Anti-Phosphotidic Acid IgG/IgM ELISA kit, 96 tests
3300-230-APG	Human Anti-Prothrombin IgG/IgM ELISA kit, 96 tests
3300-235-APA	Human Anti-Prothrombin IgA ELISA kit, 96 tests
3300-240-AVA	Human Anti-Annexin V IgG ELISA kit, 96 tests
3300-250-ANG	Human ANCA Screen (Anti-PR3 and Anti-MPO) IgG ELISA kit
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
3300-290-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
3300-350-TPG	Human Anti-thyroid peroxidase (TPO) IgG ELISA kit
3300-360-TGG	Human Anti-thyroglobulin (TG) IgG ELISA kit, 96 tests
3300-410-HTA	Human Anti-Toxoplasma IgA (Toxo-IgA) ELISA kit, 96 tests
3300-415-HTG	Human Anti-Toxoplasma IgG (Toxo-IgG) ELISA kit, 96 tests
3300-420-HTM	Human Anti-Toxoplasma IgM (Toxo-IgM) ELISA kit
3300-500-HCA	Human Anti-Chlamydia Trachomatis IgA ELISA kit, 96 tests
3300-510-HCG	Human Anti-Chlamydia Trachomatis IgG ELISA kit, 96 tests
3300-520-HCM	Human Anti-Chlamydia Trachomatis IgM ELISA kit, 96 tests

Instruction Manual No. M-3300-240-AVA

Human Anti-Annexin V IgG Elisa Kit

Cat. No. 3300-240-AVA, 96 Tests

For Quantitative Determination of IgG to Anti-Annexin V

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

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Email: Techsupport@4adi.com Web Site: www.4adi.com

Human Anti-Annexin V IgG # 3300-240-AVA

For Quantitative Determination of anti-Annexin V Antibodies

Kit Contents: (reagents for 96 tests)

Components	
Purified Annexin V coated microwell strips (12 X 8 wells), Ready-to-use, 3300-241-P	1 Plate
Anti-Annexin V IgG **Standard A** (0 U/ml) 1.5 ml, #3300-242A	1 vial
Anti-Annexin V IgG **Standard B** (6.3 U/ml) 1.5 ml, #3300-242B	1 vial
Anti- Annexin V IgG **Standard C** (12.5 U/ml) 1.5 ml, #3300-242C	1 vial
Anti- Annexin V IgG **Standard D** (25 U/ml) 1.5 ml, #3300-242D	1 vial
Anti- Annexin V IgG **Standard E** (50 U/ml) 1.5 ml, #3300-242E	1 vial
Anti- Annexin V IgG **Standard F** (100 U/ml) 1.5 ml, #3300-242F	1 vial
Anti- Annexin V IgG **Postive control** IgG, 1.5 ml, #3300-242PC	1 vial
Anti- Annexin V IgG **Negative control**, 1.5 ml, #3300-242NC	1 vial
Anti- Annexin V IgG **Sample Buffer** (5X), 20 ml, #3300-243	1 bottle
Anti-hIgG **HRP Conjugate**, 15 ml, #3300-244	1 bottle
Wash buffer (50X), 20 ml, # WB-3300-240 **dilute 1:50 with distilled water**	1 bottle
HRP Substrate Solution, 15 ml, #TMB-3300-240	1 bottle
Stop solution, 15 ml, #ST-3300-240	1 bottle
Complete Instruction Manual; M-3300-240-AVA	1

Intended Use:

Human Anti-Annexin V IgG kit is for Quantitative Determination of IgG class autoantibodies to anti- Annexin V in human serum or plasma. **For In Vitro Research Use Only (RUO).**

Introduction

Anti phospholipid syndrome (APS, Hughes Syndrome) is a systemic autoimmune disease that causes thromboses, recurrent miscarriage, and intrauterine foetal death. Clinical symptoms are accompanied by the occurrence of specific autoantibodies that are detectable in the blood of patients with APS. These antibodies bind to phospholipids like cardiolipin, or phospholipid-binding proteins like beta-2-glycoprotein I. The clinical symptoms of APS alone are not sufficiently specific to make a definitive diagnosis. Laboratory tests thus play an important role in the diagnosis of the disease.

The diagnosis of APS is considered as confirmed when at least one clinical and one of the laboratory criteria are fulfilled. In primary APS autoantibodies against phospholipids appear independently, while in secondary APS phospholipid antibodies are detected in conjunction with other autoimmune diseases, such as lupus erythematosus, rheumatoid arthritis, or Sjögren's syndrome. Phospholipid antibodies are detectable in only 1-5 % of healthy individuals, but they are found in 16-35 % of lupus patients.

PERFORMANCE CHARACTERISTICS

PRECISION

Inter-assay precision for IgG:

Sample	Mean (U/ml)	**CV%**
1	13.0	5.4
2	27.9	4.8
3	59.4	5.5

Intra-assay precision for IgG:

Sample	Mean (U/ml)	**CV%**
1	12.7	2.5
2	28.5	3.2
3	58.7	2.9

Sensitivity:

Functional sensitivity was determined to be 1 U/ml.

Parallelism:

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

Measuring range:

The calculation range of this ELISA assay is 0 - 100 U/ml

Interfering substances:

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.

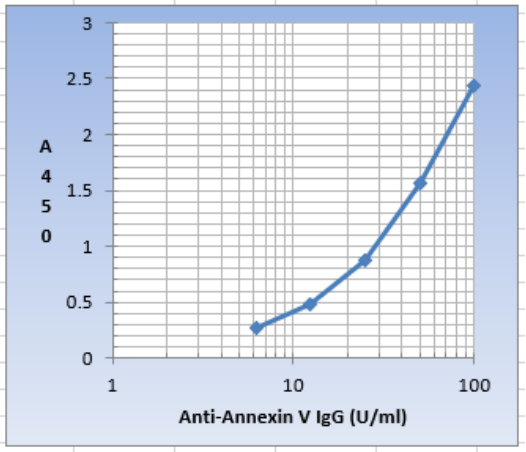
References:

1. Giannakopoulos B, Passam F, Ioannou Y, Krilis SA. How we diagnose the antiphospholipid syndrome. Blood 2009; 113(5):985-94.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds (U/ml)	Mean $A_{450\ nm}$
A1, A2	0	0.033
B1, B2	6.3	0.309
C1, C2	12.5	0.563
D1, D2	25	0.944
E1, E2	50	1.423
F1, F2	100	1.981

NOTE: These data are for demonstration purpose only. A complete set of negative, positive, and calibrator standards set must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



*3_ADI_XL-

VALIDATION

Test results are valid if the optical densities at 450 nm for calibrators / controls and the results for controls comply with the reference ranges indicated on the Certificate of Analysis enclosed in each test kit. If these quality control criteria are not met the assay run is invalid and should be repeated.

CALCULATION OF 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

Human Annexin V is bound to microwells. The determination is based on an indirect enzyme linked immune reaction where Specific antibodies in the patient sample bind to the antigen coated on the surface of the reaction wells. After incubation, a washing step removes unbound and unspecifically bound serum or plasma components. Subesquently added enzyme conjugate binds to the immobilized antibody-antigen-complexes. After incubation, a second washing step removes unbound enzyme conjugate. After addition of substrate solution the bound enzyme conjugate hydrolyses the substrate forming a blue coloured product. Addition of an acid stops the reaction generating a yellow end-product. The intensity of the yellow color correlates with the concentration of the antibody-antigen-complex and can be measured photometrically at 450 nm.

MATERIALS AND EQUIPMENT REQUIRED

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

PRECAUTIONS

The Alpha Diagnostic International Human Anti-Annexin V IgG ELISA Kit is intended for *in vitro research* use only. The Negative, Positive and Calibrator 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

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow to clot, and separate the serum or plasma by centrifugation at room temperature. Do not heat inactivate the serum.. If sera cannot 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.

REAGENTS PREPARATIONS:

Wash buffer is supplied as **50x stock**. Dilute **20 ml into 980** ml de-ionized or distilled water, mix, and store at room temp for 1-2 weeks. It can be stored at 4^{o}C for long term storage.

Sample Diluent (5X): **Dilute 20 ml into 80 ml de-ionized or distilled water.**

Dilute serum sample 1:100 in 1x sample diluent (5 ul sample in 495 ul buffer) .

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. Store microplate sealed and dessicated in the clip bag provided. Shelf life of the unopended test kit is 18 months from day of production. Diluted Wash Buffer and Sample Buffer are stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.

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

Label or mark the microtiter well strips to be used on the plate. **Dilute serum samples 1:100** (5 µl of sample in a total volume of 500 µl of sample diluents). **Dilute wash buffer (1:50) with distilled water (20 ml stock in total of 1-liter). Dilute Sample Diluent (5X)**: **Dilute 20 ml into 80 ml de-ionized or distilled water.**

Note: Standards and controls are supplied ready to use.

1. Pipet **100 µl of ready-to-use standards, controls** and prediluted patient samples into the wells in *duplicate*. Cover the plate and incubate for **30 minutes** at **room temperature** (20-28^{o}C).

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 or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

3. Add **100 µl** of antibody-enzyme conjugate into **each well.** Mix gently. Cover the plate and incubate for **15 minutes** at room temperature(20-28^{o}C).

4. Aspirate and wash the wells **3 times** with 300 µl of diluted wash buffer, as above.

5. Dispense **100 ul TMB substrate per well**. Mix the plate gently for 5-10 seconds. Cover the plate and incubate for **15 minutes** at room temperature. Blue color develops into standards and positive samples.

6. Stop the reaction by adding **100 µl of stopping solution** to **all wells** at the same timed intervals as in step 8. Mix gently. Blue color turns yellow.

7. 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. The unused strips should be stored in a sealed bag at 4^{o}C. 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. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

Calculation of results

For the Anti- Annexin V test a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is recommended. Spline Approximation and log-log coordinates are also suitable.

Recommended Lin-Log Plot

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 with the Anti- Annexin V test:
Anti- Annexin V [U/ml]
negative: <5
borderline: 5-8
positive: > 8
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- Annexin V antibodies. The above reference ranges should be regarded as guidelines only.

LIMITATIONS OF PROCEDURE

This assay 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 concerning the entire clinical picture of the patient. Also every decision for therapy should be taken individually. The above pathological and normal reference ranges for antibodies in patient samples should be regarded as recommendations only. Each laboratory should establish its own ranges according to ISO 15189 or other applicable laboratory guidelines.