

ORGENTEC Diagnostika GmbH

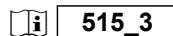
Carl-Zeiss-Straße 49-51

55129 Mainz - Germany

Phone: +49 (0) 61 31 / 92 58-0

Fax: +49 (0) 61 31 / 92 58-58

Internet: www.orgentec.com



ORG 515 Anti-Cardioliipin IgG/IgM

INTENDED PURPOSE

Anti-Cardioliipin IgG/IgM is an ELISA test system for the quantitative measurement of IgG and IgM class autoantibodies against cardiolipin in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

Antiphospholipid syndrome (APS, Hughes Syndrome) is a systemic autoimmune disease that causes thromboses, recurrent miscarriage or stillbirths, and stroke. Clinical symptoms are accompanied by specific autoantibodies in the blood, which bind to phospholipids like cardiolipin, or phospholipid-binding proteins like beta-2-glycoprotein I. Autoantibodies against proteins of the coagulation cascade, e.g. prothrombin or annexin V may also be found in patients with APS with otherwise negative phospholipid antibody results. 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.

SYMBOLS USED ON LABELS

	In vitro diagnostic medical device		Microplate
	Manufacturer		Calibrator
	Catalogue number		Calibrator
	Sufficient for 96 determinations		Calibrator
	Batch code		Calibrator
	Use by		Calibrator
	Temperature limitation		Calibrator
	Keep away from sunlight		Control positive
	Do not reuse		Control negative
	Date of manufacture		Sample Buffer P
	CE marked according to 98/79/EC		Enzyme Conjugate
	Consult instructions for use		Enzyme Conjugate
	Electronic Instruction For Use: version		TMB Substrate
			Stop solution
			Wash Buffer
			Ready to use

PRINCIPLE OF THE TEST

Highly purified cardiolipin is coated on microwells saturated with beta-2-glycoprotein I.

The determination is based on an indirect enzyme linked immune reaction with the following steps:

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. Subsequently 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.

WARNINGS AND PRECAUTIONS







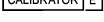
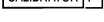



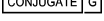
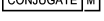




- All reagents of this kit are intended for professional in vitro diagnostic use only.
- Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
- Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
- Avoid contact with the substrate TMB (3,3',5,5'-Tetramethyl-benzidine).
- Stop solution contains acid, classification is non-hazardous. Avoid contact with skin.
- Control, sample buffer and wash buffer contain sodium azide 0.09% as preservative. This concentration is classified as non-hazardous.
- Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.

During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:

- First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
- Personal precautions, protective equipment and emergency procedures:
Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.
- Exposure controls / personal protection: Wear protective gloves of nitril rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.
- Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
- For disposal of laboratory waste the national or regional legislation has to be observed.

Observe the guidelines for performing quality control in medical laboratories by assaying control sera.

CONTENTS OF THE KIT

ORG 515		96	Sufficient for 96 determinations
	1		One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Product code on module: CLP
	1x 1.5 ml		Calibrator A 0 GPL-U/ml / 0 MPL-U/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml		Calibrator B 7.5 GPL-U/ml / 5 MPL-U/ml, containing Cardiolipin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml		Calibrator C 15 GPL-U/ml / 10 MPL-U/ml, containing Cardiolipin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml		Calibrator D 30 GPL-U/ml / 20 MPL-U/ml, containing Cardiolipin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml		Calibrator E 60 GPL-U/ml / 40 MPL-U/ml, containing Cardiolipin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml		Calibrator F 120 GPL-U/ml / 80 MPL-U/ml, containing Cardiolipin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml		Control positive, containing cardiolipin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
	1x 1.5 ml		Control negative, containing cardiolipin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
	20 ml		Sample Buffer P; containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate 5x.
	15 ml		Enzyme Conjugate IgG; containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
	15 ml		Enzyme Conjugate IgM; containing anti-human IgM antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
	15 ml		TMB Substrate, containing 3,3', 5,5'- Tetramethylbenzidin. Ready to use.
	15 ml		Stop solution; contains acid. Ready to use.
	20 ml		Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.
	1		Certificate of Analysis

MATERIALS REQUIRED

- Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm
- Data reduction software
- Multi-channel dispenser or repeatable pipette for 100 µl
- Vortex mixer
- Pipettes for 10 µl, 100 µl and 1000 µl
- Laboratory timing device
- Distilled or deionised water
- Measuring cylinder for 1000 ml and 100 ml
- Plastic container for storage of the wash solution

This ELISA assay is suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system. Detailed information is provided upon request.

SPECIMEN COLLECTION, STORAGE AND HANDLING

- Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
- Allow blood to clot and separate the serum or plasma by centrifugation.
- Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
- Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
- Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.
- Testing of heat-inactivated sera is not recommended.

STORAGE AND STABILITY

- Store test kit at 2-8°C in the dark.
- Do not expose reagents to heat, sun, or strong light during storage and usage.
- Store microplate sealed and desiccated in the clip bag provided.
- Shelf life of the unopened test kit is 18 months from day of production.
Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- 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.

PROCEDURAL NOTES

- Do not use kit components beyond their expiration dates.
- Do not interchange kit components from different lots and products.
- All materials must be at room temperature (20-28°C) prior to use.
- Prepare all reagents and samples. Once started, perform the test without interruption.
- Double determinations may be done. By this means pipetting errors may become obvious.
- Perform the assay steps only in the order indicated.
- Always use fresh sample dilutions.
- Pipette all reagents and samples into the bottom of the wells.
- To avoid carryover or contamination, change the pipette tip between samples and different kit controls.
- Wash microwells thoroughly and remove the last droplets of wash buffer.
- All incubation steps must be accurately timed.
- Do not re-use microplate wells.

PREPARATION OF REAGENTS

WASH

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.

DILUENT

Sample Buffer P: Prior to use dilute the contents (20 ml) of one vial of sample buffer 5x concentrate with distilled or deionised water to a final volume of 100 ml.

Preparation of samples

Dilute patient samples 1:100 before the assay: Put 990 µl of prediluted sample buffer in a polystyrene tube and add 10 µl of sample. Mix well. Note: Calibrators / Controls are ready to use and need not be diluted.

TEST PROCEDURE

Prepare enough microplate modules for all calibrators / controls and patient samples.

- Pipette **100 µl** of calibrators, controls and prediluted patient samples into the wells.
Incubate for **30 minutes** at room temperature (20-28 °C).
Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
- Dispense **100 µl** of enzyme conjugate into each well.
Incubate for **15 minutes** at room temperature.
Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
- Dispense **100 µl** of TMB substrate solution into each well.
Incubate for **15 minutes** at room temperature
- Add 100 µl** of stop solution to each well of the modules
Incubate for **5 minutes** at room temperature.
Read the optical density at 450 nm (reference 600-690nm) and calculate the results.
The developed colour is stable for at least 30 minutes. Read during this time.

Example for a pipetting scheme:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	P1	A	P1								
B	B	P2	B	P2								
C	C	P3	C	P3								
D	D	P4	D	P4								
E	E	P5	E	P5								
F	F	P6	F	P6								
G	C+	P7	C+	P7								
H	C-	P8	C-	P8								

IgG IgG IgM IgM

P1, ... patient sample A-F calibrators C+, C- controls

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.

PERFORMANCE CHARACTERISTICS

Calibration

The assay system is calibrated against the internationally recognised reference sera from E.N. Harris, Louisville and the specific reference material IRP 97/656 (IgG) and HCAL (IgG) / EY2C9 (IgM).

Measuring range

The calculation range of this ELISA assay is IgG: 0 - 120 GPL-U/ml IgM: 0 - 80 MPL-U/ml

Expected values

In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off IgG: 10 GPL-U/ml IgM: 7 MPL-U/ml

Interpretation of results

Negative: IgG < 10 GPL-U/ml IgM < 7 MPL-U/ml
Positive: ≥ 10 GPL-U/ml ≥ 7 MPL-U/ml

Linearity

Patient samples containing high levels of specific antibody were serially diluted in sample buffer to demonstrate the dynamic range of the assay and the upper / lower end of linearity. Activity for each dilution was calculated from the calibration curve using a 4-Parameter-Fit with lin-log coordinates.

Sample	Dilution Factor	Observed	Expected	O/E [%]
		GPL/MPL-U/ml	GPL/MPL-U/ml	
IgG 1	1	73.0	73.0	100
	2	37.1	36.5	102
	4	19.6	18.3	107
	8	10.9	9.1	120
IgG 2	1	80.5	80.5	100
	2	42.0	40.3	104
	4	22.2	20.1	111
	8	12.1	10.1	120
IgG 3	1	66.2	64.4	103
	2	34.5	32.2	107
	4	16.2	16.1	101
	8	8.1	8.1	101
IgM 1	1	70.9	70.9	100
	2	34.1	35.5	96
	4	18.2	17.7	103
	8	10.1	8.9	114
IgM 2	1	114.0	114.0	100
	2	50.6	57.0	89
	4	27.3	28.5	96
	8	14.8	14.3	104
IgM 3	1	48.2	48.2	100
	2	24.7	24.1	102
	4	12.7	12.1	105
	8	7.1	6.0	118

Limit of detection

Functional sensitivity was determined to be: IgG: 1 GPL-U/ml IgM: 0.5 MPL-U/ml

Reproducibility

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.

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.

Intra-Assay IgG		
Sample	Mean GPL-U/ml	CV %
1	10.9	5.5
2	20.5	5.4
3	73.0	5.4

Inter-Assay IgG		
Sample	Mean GPL-U/ml	CV %
1	11.8	5.3
2	21.1	3.7
3	70.5	6.3

Intra-Assay IgM		
Sample	Mean MPL-U/ml	CV %
1	12.8	3.7
2	30.7	4.1
3	65.2	3.8

Inter-Assay IgM		
Sample	Mean MPL-U/ml	CV %
1	12.2	3.5
2	31.4	3.5
3	64.9	4.2

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.

Study results

Study population	n	Pos IgG	%	Pos IgM	%
Primary APS	8	6	75.0	4	50.0
Secondary APS	65	57	87.7	26	40.0
Normal human serum	150	6	4.0	3	2.0

		Clinical Diagnosis		
		POS	NEG	
ORG 515	POS	63	6	223
IgG	NEG	10	144	
		73	150	
Sensitivity:		86.3 %		
Specificity:		96.0 %		
Overall agreement:		92.8 %		

		Clinical Diagnosis		
		Pos	Neg	
ORG 515	Pos	30	3	223
IgM	Neg	43	147	
		73	150	
Sensitivity:		41.1 %		
Specificity:		98.0 %		
Overall agreement:		79.4 %		

LIMITATIONS OF THE 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.

REFERENCES

- Banzato A, Pozzi N, Frasson R, De F, V, Ruffatti A, Bison E et al. Antibodies to Domain I of beta(2)Glycoprotein I are in close relation to patients risk categories in Antiphospholipid Syndrome (APS). *Thromb Res* 2011; 128 (6):583-6.
- Bertolaccini ML, Amengual O, Atsumi T, Binder WL, de LB, Forastiero R et al. 'Non-criteria' aPL tests: report of a task force and preconference workshop at the 13th International Congress on Antiphospholipid Antibodies, Galveston, TX, USA, April 2010. *Lupus* 2011; 20(2):191-205.
- de Laat B, de Groot PG. Autoantibodies directed against domain I of beta2-glycoprotein I. *Curr Rheumatol Rep* 2011; 13(1):70-6.
- de Laat B, Mertens K, de Groot PG. Mechanisms of disease: antiphospholipid antibodies-from clinical association to pathologic mechanism. *Nat Clin Pract Rheumatol* 2008; 4(4):192-9.
- de Laat B, Pengo V, Pabinger I, Musial J, Voskuyl AE, Bultink IE et al. The association between circulating antibodies against domain I of beta2-glycoprotein I and thrombosis: an international multicenter study. *J Thromb Haemost* 2009; 7(11):1767-73.
- Espinosa G, Cervera R. Antiphospholipid syndrome. *Arthritis Res Ther* 2008; 10(6):230.
- Favaloro EJ, Wong RC. Laboratory testing for the antiphospholipid syndrome: making sense of antiphospholipid antibody assays. *Clin Chem Lab Med* 2011; 49(3):447-61.
- Fischer MJ, Rauch J, Levine JS. The antiphospholipid syndrome. *Arthritis Rheum* 2007; 27(1):35-46.
- Giannakopoulos B, Passam F, Ioannou Y, Krilis SA. How we diagnose the antiphospholipid syndrome. *Blood* 2009; 113(5):985-94.
- Greaves M, Cohen H, Machin SJ, Mackie I. Guidelines on the investigation and management of the antiphospholipid syndrome. *Br J Haematol* 2000; 109(4):704-15.
- Hughes GR. Hughes syndrome: antiphospholipid syndrome. *J R Coll Physicians Lond* 1998; 32(3):260-4.
- Hughes GR. Hughes Syndrome (the antiphospholipid syndrome): ten clinical lessons. *Autoimmun Rev* 2008; 7(3):262-6.
- Hughes GR. Antiphospholipid syndrome, migraine and stroke. *Lupus* 2010; 19(5):555-6.
- Hughes GR, Harris NN, Gharavi AE. The anticardiolipin syndrome. *J Rheumatol* 1986; 13(3):486-9.

- Koike T, Bohgaki M, Amengual O, Atsumi T. Antiphospholipid antibodies: lessons from the bench. *J Autoimmun* 2007; 28(2-3):129-33.
- Lakos G, Favaloro EJ, Harris EN, Meroni PL, Tincani A, Wong RC et al. International consensus guidelines on anticardiolipin and anti-beta2-glycoprotein I testing: report from the 13th International Congress on Antiphospholipid Antibodies. *Arthritis Rheum* 2012; 64(1):1-10.
- Mackworth-Young C. Primary antiphospholipid syndrome: a distinct entity? *Autoimmun Rev* 2006; 5(1):70-5.
- Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost* 2006; 4(2):295-306.
- Molina JF, Gutierrez-Urena S, Molina J, Uribe O, Richards S, De CC et al. Variability of anticardiolipin antibody isotype distribution in 3 geographic populations of patients with systemic lupus erythematosus. *J Rheumatol* 1997; 24(2):291-6.
- Oku K, Atsumi T, Amengual O, Koike T. Antiprothrombin antibody testing: detection and clinical utility. *Semin Thromb Hemost* 2008; 34(4):335-9.
- Pengo V, Biasiolo A, Bison E, Chantarangkul V, Tripodi A. Antiphospholipid antibody ELISAs: survey on the performance of clinical laboratories assessed by using lyophilized affinity-purified IgG with anticardiolipin and anti-beta2-Glycoprotein I activity. *Thromb Res* 2007; 120(1):127-33.
- Pierangeli SS, de Groot PG, Dlott J, Favaloro E, Harris EN, Lakos G et al. 'Criteria' aPL tests: report of a task force and preconference workshop at the 13th International Congress on Antiphospholipid Antibodies, Galveston, Texas, April 2010. *Lupus* 2011; 20(2):182-90.
- Pierangeli SS, Favaloro EJ, Lakos G, Meroni PL, Tincani A, Wong RC et al. Standards and reference materials for the anticardiolipin and anti-beta-2-glycoprotein I assays: a report of recommendations from the APL Task Force at the 13th International Congress on Antiphospholipid Antibodies. *Clin Chim Acta* 2012; 413(1-2):358-60.
- Sinico RA, Bollini B, Sabadini E, Di Toma L, Radice A. The use of laboratory tests in diagnosis and monitoring of systemic lupus erythematosus. *J Nephrol JID - 9012268* 2002; 15 Suppl 6:S20-S27.
- Tincani A, Andreoli L, Casu C, Cattaneo R, Meroni P. Antiphospholipid antibody profile: implications for the evaluation and management of patients. *Lupus* 2010; 19(4):432-5.
- Tincani A, Morozzi G, Afeltra A, Alessandri C, Allegri F, Bistoni O et al. Antiprothrombin antibodies: a comparative analysis of homemade and commercial methods. A collaborative study by the Forum Interdisciplinare per la Ricerca nelle Malattie Autoimmuni (FIRMA). *Clin Exp Rheumatol* 2007; 25(2):268-74.
- Wilson WA, Gharavi AE, Koike T, Lockshin MD, Branch DW, Piette JC et al. International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome: report of an international workshop. *Arthritis Rheum* 1999; 42(7):1309-11.
- Wong RC, Favaloro EJ, Adelstein S, Baumgart K, Bird R, Brighton TA et al. Consensus guidelines on anti-beta 2 glycoprotein I testing and reporting. *Pathology* 2008; 40(1):58-63.
- Wong RC, Gillis D, Adelstein S, Baumgart K, Favaloro EJ, Hendle MJ et al. Consensus guidelines on anticardiolipin antibody testing and reporting. *Pathology* 2004; 36(1):63-8.

Notice to the user (European Union):

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State in which the user and/or the patient is established .

Change Control

Former version: *ORG 515_IFU_EN_QM113142_2016-04-18_2*

Reason for revision: *Introduction electronic IFU on homepage*

- 1 Pipet **100 µl** calibrator, control or patient sample
 - Incubate for **30 minutes** at room temperature
 - Discard the contents of the wells and wash 3 times with **300 µl** wash solution
- 2 Pipet **100 µl** enzyme conjugate
 - Incubate for **15 minutes** at room temperature
 - Discard the contents of the wells and wash 3 times with **300 µl** wash solution
- 3 Pipet **100 µl** substrate solution
 - Incubate for **15 minutes** at room temperature
- 4 Add **100 µl** stop solution
 - Leave untouched for **5 minutes**
 - Read at **450 nm**