

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



 **508_3**





























ORG 508 Anti-SS-A

INTENDED PURPOSE

Anti-SS-A is an ELISA test system for the quantitative measurement of IgG class autoantibodies against SS-A (52 and 60 kDa) in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

This test is useful for the differential diagnosis and monitoring of systemic rheumatic inflammatory autoimmune diseases. Autoantibodies against the two antigens SS-A 52 and SS-A 60 are predominantly found in cases of Sjogren's syndrome. Evaluation of a test result should always take into account all clinical and laboratory diagnostic findings.

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		TMB Substrate
	Electronic Instruction For Use: version		Stop solution
			Wash Buffer
			Ready to use

PRINCIPLE OF THE TEST

Highly purified SS-A (52 and 60 kDa) is bound to microwells.

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



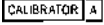
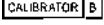
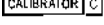
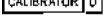
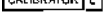
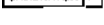








- All reagents of this kit are intended for professional in vitro diagnostic use only.
- Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based 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 508		Sufficient for 96 determinations
	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Color code on module
	1x 1.5 ml	Calibrator A 0 U/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Calibrator B 12.5 U/ml, containing SS-A antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Calibrator C 25 U/ml, containing SS-A antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Calibrator D 50 U/ml, containing SS-A antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Calibrator E 100 U/ml, containing SS-A antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Calibrator F 200 U/ml, containing SS-A antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Control positive, containing SS-A 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 SS-A 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 (5 x).
	15 ml	Enzyme Conjugate containing anti-human IgG 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, colorless. 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

 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.

 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										
B	B	P2										
C	C	P3										
D	D											
E	E											
F	F											
G	C+											
H	C-											

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 recognized reference sera from CDC, Atlanta USA.

Measuring range

The calculation range of this ELISA assay is 0 - 200 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 25 U/ml

Interpretation of results

Negative:	< 15 U/ml
Borderline:	15 - 25 U/ml
Positive:	> 25 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	Observed U/ml	Expected U/ml	O/E [%]
1	1:100	139.0	139.0	100
	1:200	67.9	69.5	98
	1:400	33.0	34.8	95
2	1:800	17.2	17.4	99
	1:100	161.6	161.6	100
	1:200	70.6	80.8	87
	1:400	39.2	40.4	97
	1:800	20.0	20.2	99

Limit of detection

Functional sensitivity was determined to be: 1 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		
Sample	Mean U/ml	CV %
1	32.2	2.7
2	73.2	2.6
3	134.0	3.6

Inter-Assay		
Sample	Mean U/ml	CV %
1	33.8	6.4
2	71.3	6.2
3	133.1	1.1

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	n Pos	%
Sjogren's syndrome	70	51	72.9
Normal human sera	100	7	7.0

		Clinical Diagnosis		
		POS	NEG	
ORG 508	POS	51	7	170
	NEG	19	93	
		70	100	

Sensitivity: 72.9 %

Specificity: 93.0 %

Overall agreement: 84.7 %

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.

Change Control

Former version: *ORG 508_IFU_EN_QM113135_2013-12-16_1.2* Reason for revision: *Introduction electronic IFU on homepage*

REFERENCES

1. Alba P, Bento L, Cuadrado MJ, Karim Y, Tungekar MF, Abbs I et al. Anti-dsDNA, anti-Sm antibodies, and the lupus anticoagulant: significant factors associated with lupus nephritis. *Ann Rheum Dis* 2003; 62(6):556-560.
2. Antico A, Platzgummer S, Bassetti D, Bizzaro N, Tozzoli R, Villalta D. Diagnosing systemic lupus erythematosus: new-generation immunoassays for measurement of anti-dsDNA antibodies are an effective alternative to the Farr technique and the Crithidia luciliae immunofluorescence test. *Lupus* 2010; 19(8):906-912.
3. Brouwer R, Hengstman GJ, Vree EW, Ehrfeld H, Bozic B, Ghirardello A et al. Autoantibody profiles in the sera of European patients with myositis. *Ann Rheum Dis* 2001; 60(2):116-123.
4. Castro C, Gourley M. Diagnostic testing and interpretation of tests for autoimmunity. *J Allergy Clin Immunol* 2010; 125(2 Suppl 2):S238-S247.
5. Defendenti C, Atzeni F, Spina MF, Grosso S, Cereda A, Guercilena G et al. Clinical and laboratory aspects of Ro/SSA-52 autoantibodies. *Autoimmun Rev* 2011; 10(3):150-154.
6. Eriksson C, Kokkonen H, Johansson M, Hallmans G, Wadell G, Rantapaa-Dahlqvist S. Autoantibodies predate the onset of Systemic Lupus Erythematosus in northern Sweden. *Arthritis Research & Therapy* 2011; 13(1):R30.
7. Haugbro K, Nossent JC, Winkler T, Figenschau Y, Rekvig OP. Anti-dsDNA antibodies and disease classification in antinuclear antibody positive patients: the role of analytical diversity. *Ann Rheum Dis JID* - 0372355 2004; 63(4):386-394.
8. Ippolito A, Wallace DJ, Gladman D, Fortin PR, Urowitz M, Werth V et al. Autoantibodies in systemic lupus erythematosus: comparison of historical and current assessment of seropositivity. *Lupus* 2011; 20(3):250-255.
9. Isenberg DA, Manson JJ, Ehrenstein MR, Rahman A. Fifty years of anti-ds DNA antibodies: are we approaching journey's end? *Rheumatology (Oxford)* 2007; 46(7):1052-1056.
10. Kattah NH, Kattah MG, Utz PJ. The U1-snRNP complex: structural properties relating to autoimmune pathogenesis in rheumatic diseases. *Immunol Rev* 2010; 233(1):126-145.
11. Kumar Y, Bhatia A, Minz RW. Antinuclear antibodies and their detection methods in diagnosis of connective tissue diseases: a journey revisited. *Diagn Pathol* 2009; 4:1.
12. Meroni PL, Schur PH. ANA screening: an old test with new recommendations. *Ann Rheum Dis* 2010; 69:1420-1422.
13. Petri M, Magder L. Classification criteria for systemic lupus erythematosus: a review. *Lupus* 2004; 13(11):829-837.
14. Poole BD, Schneider RI, Guthridge JM, Vette CA, Reichlin M, Harley JB et al. Early targets of nuclear RNP humoral autoimmunity in human systemic lupus erythematosus. *Arthritis Rheum* 2009; 60(3):848-859.
15. Putova I, Dostal C, Becvar R. Prevalence of antinucleosome antibodies by enzyme-linked immunosorbent assays in patients with systemic lupus erythematosus and other autoimmune systemic diseases. *Ann N Y Acad Sci* 2007; 1109:275-286.
16. Reveille JD. Predictive value of autoantibodies for activity of systemic lupus erythematosus. *Lupus JID* - 9204265 2004; 13(5):290-297.
17. Simon JA, Cabiedes J, Ortiz E, Alcocer-Varela J, Sanchez-Guerrero J. Anti-nucleosome antibodies in patients with systemic lupus erythematosus of recent onset. Potential utility as a diagnostic tool and disease activity marker. *Rheumatology (Oxford)* 2004; 43(2):220-224.
18. Sinclair D, Saas M, Williams D, Hart M, Goswami R. Can an ELISA replace immunofluorescence for the detection of anti-nuclear antibodies?--The routine use of anti-nuclear antibody screening ELISAs. *Clin Lab* 2007; 53(3-4):183-191.
19. Tozzoli R, Bizzaro N, Tonutti E, Villalta D, Bassetti D, Manoni F et al. Guidelines for the laboratory use of autoantibody tests in the diagnosis and monitoring of autoimmune rheumatic diseases. *Am J Clin Pathol* 2002; 117(2):316-324.
20. Maidhof W., Hiliak O. Lupus: an overview of the disease and management options. *P T* 2012; 37(4):240-9.
21. Hahn BH, McMahon MA, Wilkinson A, Wallace WD, Daikh DI, Fitzgerald JD et al. American College of Rheumatology guidelines for screening, treatment, and management of lupus nephritis. *Arthritis Care Res (Hoboken)* 2012; 64(6):797-808.

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.

- 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**

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



 **509_3**

ORG 509 Anti-SS-B

INTENDED PURPOSE

Anti-SS-B is an ELISA test system for the quantitative measurement of IgG class autoantibodies against SS-B (La) in human serum or plasma. This product is intended for professional in vitro diagnostic use only.


Antibodies against SS-B are used for the differential diagnosis of systemic inflammatory autoimmune diseases. Autoantibodies against the SS-B protein are usually found together with anti-SS-A in cases of Sjogren's syndrome. Evaluation of a test result should always take into account all clinical and laboratory diagnostic findings.

SYMBOLS USED ON LABELS

 In vitro diagnostic medical device

 Manufacturer


 Catalogue number

 Sufficient for 96 determinations


 Batch code


 Use by


 Temperature limitation

 Keep away from sunlight

 Do not reuse


 Date of manufacture

 CE marked according to 98/79/EC

 Consult instructions for use

 Electronic Instruction For Use: version

 Microplate

 Calibrator


 Calibrator

 Calibrator


 Calibrator

 Calibrator


 Calibrator

 Control positive

 Control negative

 Sample Buffer P

 Enzyme Conjugate

 TMB Substrate

 Stop solution

 Wash Buffer

 Ready to use

PRINCIPLE OF THE TEST

Highly purified SS-B is bound to microwells.

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



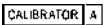
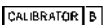
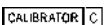
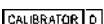
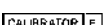
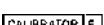








- All reagents of this kit are intended for professional in vitro diagnostic use only.
- Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based 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 509		Sufficient for 96 determinations
	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Color code on module
	1x 1.5 ml	Calibrator A 0 U/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Calibrator B 12.5 U/ml, containing SS-B antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Calibrator C 25 U/ml, containing SS-B antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Calibrator D 50 U/ml, containing SS-B antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Calibrator E 100 U/ml, containing SS-B antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Calibrator F 200 U/ml, containing SS-B antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Control positive, containing SS-B 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 SS-B 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 (5 x).
	15 ml	Enzyme Conjugate containing anti-human IgG 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, colorless. 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										
B	B	P2										
C	C	P3										
D	D											
E	E											
F	F											
G	C+											
H	C-											

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 recognized reference sera from CDC, Atlanta USA.

Measuring range

The calculation range of this ELISA assay is 0 - 200 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 25 U/ml

Interpretation of results

Negative:	< 15 U/ml
Borderline:	15 - 25 U/ml
Positive:	> 25 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	Observed U/ml	Expected U/ml	O/E [%]
1	1:100	124.2	127.1	98
	1:200	62.4	34.8	98
	1:400	33.2	17.4	104
2	1:800	16.1	8.7	101
	1:100	104.4	104.4	100
	1:200	53.1	52.2	102
	1:400	27.6	26.1	106
	1:800	13.9	13.1	107

Limit of detection

Functional sensitivity was determined to be: 1 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		
Sample	Mean U/ml	CV %
1	28.8	5.6
2	67.1	5.8
3	143.2	5.2

Inter-Assay		
Sample	Mean U/ml	CV %
1	24.5	11.0
2	70.5	6.9
3	157.6	4.1

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	n Pos	%
Sjoegren's syndrome	70	43	61.4
Rheumatoid arthritis	20	1	5.0
Normal human sera	100	4	4.0

		Clinical Diagnosis		
		POS	NEG	
ORG 509	POS	43	5	
	NEG	27	115	
		70	120	190

Sensitivity: 61.4 %

Specificity: 95.8 %

Overall agreement: 83.2 %

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

1. Alba P, Bento L, Cuadrado MJ, Karim Y, Tungekar MF, Abbs I et al. Anti-dsDNA, anti-Sm antibodies, and the lupus anticoagulant: significant factors associated with lupus nephritis. *Ann Rheum Dis* 2003; 62(6):556-560.
2. Antico A, Platzgummer S, Bassetti D, Bizzaro N, Tozzoli R, Villalta D. Diagnosing systemic lupus erythematosus: new-generation immunoassays for measurement of anti-dsDNA antibodies are an effective alternative to the Farr technique and the Crithidia luciliae immunofluorescence test. *Lupus* 2010; 19(8):906-912.
3. Brouwer R, Hengstman GJ, Vree EW, Ehrfeld H, Bozic B, Ghirardello A et al. Autoantibody profiles in the sera of European patients with myositis. *Ann Rheum Dis* 2001; 60(2):116-123.
4. Castro C, Gourley M. Diagnostic testing and interpretation of tests for autoimmunity. *J Allergy Clin Immunol* 2010; 125(2 Suppl 2):S238-S247.
5. Defendenti C, Atzeni F, Spina MF, Grosso S, Cereda A, Guercilena G et al. Clinical and laboratory aspects of Ro/SSA-52 autoantibodies. *Autoimmun Rev* 2011; 10(3):150-154.
6. Eriksson C, Kokkonen H, Johansson M, Hallmans G, Wadell G, Rantapaa-Dahlqvist S. Autoantibodies predate the onset of Systemic Lupus Erythematosus in northern Sweden. *Arthritis Research & Therapy* 2011; 13(1):R30.
7. Haugbro K, Nossent JC, Winkler T, Figenschau Y, Rekvig OP. Anti-dsDNA antibodies and disease classification in antinuclear antibody positive patients: the role of analytical diversity. *Ann Rheum Dis* 2004; 63(4):386-394.
8. Ippolito A, Wallace DJ, Gladman D, Fortin PR, Urowitz M, Werth V et al. Autoantibodies in systemic lupus erythematosus: comparison of historical and current assessment of seropositivity. *Lupus* 2011; 20(3):250-255.
9. Isenberg DA, Manson JJ, Ehrenstein MR, Rahman A. Fifty years of anti-ds DNA antibodies: are we approaching journey's end? *Rheumatology (Oxford)* 2007; 46(7):1052-1056.
10. Kattah NH, Kattah MG, Utz PJ. The U1-snRNP complex: structural properties relating to autoimmune pathogenesis in rheumatic diseases. *Immunol Rev* 2010; 233(1):126-145.
11. Kumar Y, Bhatia A, Minz RW. Antinuclear antibodies and their detection methods in diagnosis of connective tissue diseases: a journey revisited. *Diagn Pathol* 2009; 4:1.
12. Meroni PL, Schur PH. ANA screening: an old test with new recommendations. *Ann Rheum Dis* 2010; 69:1420-1422.
13. Petri M, Magder L. Classification criteria for systemic lupus erythematosus: a review. *Lupus* 2004; 13(11):829-837.
14. Poole BD, Schneider RI, Guthridge JM, Vette CA, Reichlin M, Harley JB et al. Early targets of nuclear RNP humoral autoimmunity in human systemic lupus erythematosus. *Arthritis Rheum* 2009; 60(3):848-859.
15. Putova I, Dostal C, Becvar R. Prevalence of antinucleosome antibodies by enzyme-linked immunosorbent assays in patients with systemic lupus erythematosus and other autoimmune systemic diseases. *Ann N Y Acad Sci* 2007; 1109:275-286.
16. Reveille JD. Predictive value of autoantibodies for activity of systemic lupus erythematosus. *Lupus* 2004; 13(5):290-297.
17. Simon JA, Cabiedes J, Ortiz E, Alcocer-Varela J, Sanchez-Guerrero J. Anti-nucleosome antibodies in patients with systemic lupus erythematosus of recent onset. Potential utility as a diagnostic tool and disease activity marker. *Rheumatology (Oxford)* 2004; 43(2):220-224.
18. Sinclair D, Saas M, Williams D, Hart M, Goswami R. Can an ELISA replace immunofluorescence for the detection of anti-nuclear antibodies?--The routine use of anti-nuclear antibody screening ELISAs. *Clin Lab* 2007; 53(3-4):183-191.
19. Tozzoli R, Bizzaro N, Tonutti E, Villalta D, Bassetti D, Manoni F et al. Guidelines for the laboratory use of autoantibody tests in the diagnosis and monitoring of autoimmune rheumatic diseases. *Am J Clin Pathol* 2002; 117(2):316-324.
20. Moidhof W., Hilius O. Lupus: an overview of the disease and management options. *P T* 2012; 37(4):240-9.
21. Hahn BH, McMahon MA, Wilkinson A, Wallace WD, Daikh DI, Fitzgerald JD et al. American College of Rheumatology guidelines for screening, treatment, and management of lupus nephritis. *Arthritis Care Res (Hoboken)* 2012; 64(6):797-808.

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 509_IFU_EN_QM113136_2013-12-16_1.2* 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**

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



 **511_3**





























ORG 511 Anti-RNP/Sm

INTENDED PURPOSE

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

Antibodies against the RNP/Sm complex are useful in the diagnosis of mixed connective tissue disorder (MCTD, Sharp syndrome) and related autoimmune diseases. Antibodies against the 70 kDa protein of this complex are a very specific marker for Sharp syndrome. The Sm proteins are recognised by antibodies that may occur in cases of mixed connective tissue disorder and systemic lupus erythematosus. Evaluation of a test result should always take into account all clinical and laboratory diagnostic findings.

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		TMB Substrate
	Electronic Instruction For Use: version		Stop solution
			Wash Buffer
			Ready to use

PRINCIPLE OF THE TEST

Highly purified RNP/Sm is bound to microwells.

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



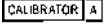


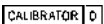
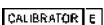
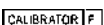








- All reagents of this kit are intended for professional in vitro diagnostic use only.
- Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based 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 511		Sufficient for 96 determinations
	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Color code on module
	1x 1.5 ml	Calibrator A 0 U/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Calibrator B 12.5 U/ml, containing RNP/Sm antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Calibrator C 25 U/ml, containing RNP/Sm antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Calibrator D 50 U/ml, containing RNP/Sm antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Calibrator E 100 U/ml, containing RNP/Sm antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Calibrator F 200 U/ml, containing RNP/Sm antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Control positive, containing RNP/Sm 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 RNP/Sm 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 (5 x).
	15 ml	Enzyme Conjugate containing anti-human IgG 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, colorless. 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										
B	B	P2										
C	C	P3										
D	D											
E	E											
F	F											
G	C+											
H	C-											

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 recognized reference sera from CDC, Atlanta USA.

Measuring range

The calculation range of this ELISA assay is 0 - 200 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 25 U/ml

Interpretation of results

Negative:	< 15 U/ml
Borderline:	15 - 25 U/ml
Positive:	> 25 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	Observed U/ml	Expected U/ml	O/E [%]
1	1:100	161.4	161.4	100
.	1:200	78.0	80.7	97
.	1:400	39.7	40.4	98
.	1:800	20.1	20.2	100
2	1:100	167.2	167.2	100
.	1:200	83.7	83.6	100
.	1:400	41.5	41.8	99
.	1:800	20.8	20.9	100

Limit of detection

Functional sensitivity was determined to be: 1 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		
Sample	Mean U/ml	CV %
1	65.6	4.1
2	101.9	5.9
3	182.0	1.8

Inter-Assay		
Sample	Mean U/ml	CV %
1	33.3	4.2
2	109.0	3.1
3	176.8	2.9

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	n Pos	%
SLE	70	37	52.9
MCTD	30	29	96.7
Rheumatoid arthritis	20	3	15.0
Normal human sera	100	2	2.0

	Clinical Diagnosis		
	POS	NEG	
ORG 511 POS	66	5	100 120 220
NEG	34	115	
Sensitivity:	66.0 %		
Specificity:	95.8 %		
Overall agreement:	82.3 %		

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

1. Alba P, Bento L, Cuadrado MJ, Karim Y, Tungekar MF, Abbs I et al. Anti-dsDNA, anti-Sm antibodies, and the lupus anticoagulant: significant factors associated with lupus nephritis. *Ann Rheum Dis* 2003; 62(6):556-560.
2. Antico A, Platzgummer S, Bassetti D, Bizzaro N, Tozzoli R, Villalta D. Diagnosing systemic lupus erythematosus: new-generation immunoassays for measurement of anti-dsDNA antibodies are an effective alternative to the Farr technique and the Crithidia luciliae immunofluorescence test. *Lupus* 2010; 19(8):906-912.
3. Brouwer R, Hengstman GJ, Vree EW, Ehrfeld H, Bozic B, Ghirardello A et al. Autoantibody profiles in the sera of European patients with myositis. *Ann Rheum Dis* 2001; 60(2):116-123.
4. Castro C, Gourley M. Diagnostic testing and interpretation of tests for autoimmunity. *J Allergy Clin Immunol* 2010; 125(2 Suppl 2):S238-S247.
5. Defendenti C, Atzeni F, Spina MF, Grosso S, Cereda A, Guercilena G et al. Clinical and laboratory aspects of Ro/SSA-52 autoantibodies. *Autoimmun Rev* 2011; 10(3):150-154.
6. Eriksson C, Kokkonen H, Johansson M, Hallmans G, Wadell G, Rantapaa-Dahlqvist S. Autoantibodies predate the onset of Systemic Lupus Erythematosus in northern Sweden. *Arthritis Research & Therapy* 2011; 13(1):R30.
7. Haugbro K, Nossent JC, Winkler T, Figenschau Y, Rekvig OP. Anti-dsDNA antibodies and disease classification in antinuclear antibody positive patients: the role of analytical diversity. *Ann Rheum Dis JID* - 0372355 2004; 63(4):386-394.
8. Ippolito A, Wallace DJ, Gladman D, Fortin PR, Urowitz M, Werth V et al. Autoantibodies in systemic lupus erythematosus: comparison of historical and current assessment of seropositivity. *Lupus* 2011; 20(3):250-255.
9. Isenberg DA, Manson JJ, Ehrenstein MR, Rahman A. Fifty years of anti-ds DNA antibodies: are we approaching journey's end? *Rheumatology (Oxford)* 2007; 46(7):1052-1056.
10. Kattah NH, Kattah MG, Utz PJ. The U1-snRNP complex: structural properties relating to autoimmune pathogenesis in rheumatic diseases. *Immunol Rev* 2010; 233(1):126-145.
11. Kumar Y, Bhatia A, Minz RW. Antinuclear antibodies and their detection methods in diagnosis of connective tissue diseases: a journey revisited. *Diagn Pathol* 2009; 4:1.
12. Meroni PL, Schur PH. ANA screening: an old test with new recommendations. *Ann Rheum Dis* 2010; 69:1420-1422.
13. Petri M, Magder L. Classification criteria for systemic lupus erythematosus: a review. *Lupus* 2004; 13(11):829-837.
14. Poole BD, Schneider RI, Guthridge JM, Velte CA, Reichlin M, Harley JB et al. Early targets of nuclear RNP humoral autoimmunity in human systemic lupus erythematosus. *Arthritis Rheum* 2009; 60(3):848-859.
15. Putova I, Dostal C, Becvar R. Prevalence of antinucleosome antibodies by enzyme-linked immunosorbent assays in patients with systemic lupus erythematosus and other autoimmune systemic diseases. *Ann N Y Acad Sci* 2007; 1109:275-286.
16. Reveille JD. Predictive value of autoantibodies for activity of systemic lupus erythematosus. *Lupus JID* - 9204265 2004; 13(5):290-297.
17. Simon JA, Cabiedes J, Ortiz E, Alcocer-Varela J, Sanchez-Guerrero J. Anti-nucleosome antibodies in patients with systemic lupus erythematosus of recent onset. Potential utility as a diagnostic tool and disease activity marker. *Rheumatology (Oxford)* 2004; 43(2):220-224.
18. Sinclair D, Saas M, Williams D, Hart M, Goswami R. Can an ELISA replace immunofluorescence for the detection of anti-nuclear antibodies?--The routine use of anti-nuclear antibody screening ELISAs. *Clin Lab* 2007; 53(3-4):183-191.
19. Tozzoli R, Bizzaro N, Tonutti E, Villalta D, Bassetti D, Manoni F et al. Guidelines for the laboratory use of autoantibody tests in the diagnosis and monitoring of autoimmune rheumatic diseases. *Am J Clin Pathol* 2002; 117(2):316-324.
20. Maidhof W., Hilius O. Lupus: an overview of the disease and management options. *P T* 2012; 37(4):240-9.
21. Hahn BH, McMahon MA, Wilkinson A, Wallace WD, Daikh DI, Fitzgerald JD et al. American College of Rheumatology guidelines for screening, treatment, and management of lupus nephritis. *Arthritis Care Res (Hoboken)* 2012; 64(6):797-808.

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 511_IFU_EN_QM113138_2013-12-16_1.2* 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**

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



 **512_3**





























ORG 512 Anti-Sci-70

INTENDED PURPOSE

Anti-Sci-70 is an ELISA test system for the quantitative measurement of IgG class autoantibodies against Sci-70 in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

Antibodies against Sci-70 (DNA topoisomerase I) are an accepted marker for progressive systemic scleroderma. They contribute to the differential diagnosis of scleroderma. Evaluation of a test result should always take into account all clinical and laboratory diagnostic findings.

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		TMB Substrate
	Electronic Instruction For Use: version		Stop solution
			Wash Buffer
			Ready to use

PRINCIPLE OF THE TEST

Highly purified Sci-70 is bound to microwells.

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



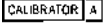
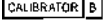
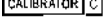
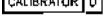
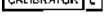
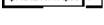








- All reagents of this kit are intended for professional in vitro diagnostic use only.
- Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based 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 512		Sufficient for 96 determinations
	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Color code on module
	1x 1.5 ml	Calibrator A 0 U/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Calibrator B 12.5 U/ml, containing Scl-70 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Calibrator C 25 U/ml, containing Scl-70 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Calibrator D 50 U/ml, containing Scl-70 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Calibrator E 100 U/ml, containing Scl-70 antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Calibrator F 200 U/ml, containing Scl-70 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Control positive, containing Scl-70 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 Scl-70 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 (5 x).
	15 ml	Enzyme Conjugate containing anti-human IgG 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, colorless. 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

 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.

 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										
B	B	P2										
C	C	P3										
D	D											
E	E											
F	F											
G	C+											
H	C-											

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 recognized reference sera from CDC, Atlanta USA.

Measuring range

The calculation range of this ELISA assay is 0 - 200 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 25 U/ml

Interpretation of results

Negative:	< 15 U/ml
Borderline:	15 - 25 U/ml
Positive:	> 25 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	Observed U/ml	Expected U/ml	O/E [%]
1	1:100	146.9	146.9	100
	1:200	76.3	73.5	104
	1:400	38.1	36.7	104
2	1:800	18.8	18.4	102
	1:100	122.3	122.3	100
	1:200	60.4	61.2	99
	1:400	29.6	30.6	97
	1:800	14.8	15.3	97

Limit of detection

Functional sensitivity was determined to be: 1 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		
Sample	Mean U/ml	CV %
1	45.7	4.0
2	90.4	3.2
3	184.1	3.4

Inter-Assay		
Sample	Mean U/ml	CV %
1	41.1	2.8
2	89.9	2.8
3	157.4	2.3

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	n Pos	%
Scleroderma	25	19	76.0
Rheumatoid arthritis	20	0	0.0
Normal human sera	80	1	1.3

		Clinical Diagnosis		
		POS	NEG	
ORG 512	POS	19	1	25
	NEG	6	99	
		25	100	125

Sensitivity: 76.0 %

Specificity: 99.0 %

Overall agreement: 94.4 %

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

1. Alba P, Bento L, Cuadrado MJ, Karim Y, Tungekar MF, Abbs I et al. Anti-dsDNA, anti-Sm antibodies, and the lupus anticoagulant: significant factors associated with lupus nephritis. *Ann Rheum Dis* 2003; 62(6):556-560.
2. Antico A, Platzgummer S, Bassetti D, Bizzaro N, Tozzoli R, Villalta D. Diagnosing systemic lupus erythematosus: new-generation immunoassays for measurement of anti-dsDNA antibodies are an effective alternative to the Farr technique and the Crithidia luciliae immunofluorescence test. *Lupus* 2010; 19(8):906-912.
3. Brouwer R, Hengstman GJ, Vree EW, Ehrfeld H, Bozic B, Ghirardello A et al. Autoantibody profiles in the sera of European patients with myositis. *Ann Rheum Dis* 2001; 60(2):116-123.
4. Castro C, Gourley M. Diagnostic testing and interpretation of tests for autoimmunity. *J Allergy Clin Immunol* 2010; 125(2 Suppl 2):S238-S247.
5. Defendenti C, Atzeni F, Spina MF, Grosso S, Cereda A, Guercilena G et al. Clinical and laboratory aspects of Ro/SSA-52 autoantibodies. *Autoimmun Rev* 2011; 10(3):150-154.
6. Eriksson C, Kokkonen H, Johansson M, Hallmans G, Wadell G, Rantapaa-Dahlqvist S. Autoantibodies predate the onset of Systemic Lupus Erythematosus in northern Sweden. *Arthritis Research & Therapy* 2011; 13(1):R30.
7. Haugbro K, Nossent JC, Winkler T, Figenschau Y, Rekvig OP. Anti-dsDNA antibodies and disease classification in antinuclear antibody positive patients: the role of analytical diversity. *Ann Rheum Dis JID* - 0372355 2004; 63(4):386-394.
8. Ippolito A, Wallace DJ, Gladman D, Fortin PR, Urowitz M, Werth V et al. Autoantibodies in systemic lupus erythematosus: comparison of historical and current assessment of seropositivity. *Lupus* 2011; 20(3):250-255.
9. Isenberg DA, Manson JJ, Ehrenstein MR, Rahman A. Fifty years of anti-ds DNA antibodies: are we approaching journey's end? *Rheumatology (Oxford)* 2007; 46(7):1052-1056.
10. Kattah NH, Kattah MG, Utz PJ. The U1-snRNP complex: structural properties relating to autoimmune pathogenesis in rheumatic diseases. *Immunol Rev* 2010; 233(1):126-145.
11. Kumar Y, Bhatia A, Minz RW. Antinuclear antibodies and their detection methods in diagnosis of connective tissue diseases: a journey revisited. *Diagn Pathol* 2009; 4:1.
12. Meroni PL, Schur PH. ANA screening: an old test with new recommendations. *Ann Rheum Dis* 2010; 69:1420-1422.
13. Petri M, Magder L. Classification criteria for systemic lupus erythematosus: a review. *Lupus* 2004; 13(11):829-837.
14. Poole BD, Schneider RI, Guthridge JM, Vette CA, Reichlin M, Harley JB et al. Early targets of nuclear RNP humoral autoimmunity in human systemic lupus erythematosus. *Arthritis Rheum* 2009; 60(3):848-859.
15. Putova I, Dostal C, Becvar R. Prevalence of antinucleosome antibodies by enzyme-linked immunosorbent assays in patients with systemic lupus erythematosus and other autoimmune systemic diseases. *Ann N Y Acad Sci* 2007; 1109:275-286.
16. Reveille JD. Predictive value of autoantibodies for activity of systemic lupus erythematosus. *Lupus JID* - 9204265 2004; 13(5):290-297.
17. Simon JA, Cabiedes J, Ortiz E, Alcocer-Varela J, Sanchez-Guerrero J. Anti-nucleosome antibodies in patients with systemic lupus erythematosus of recent onset. Potential utility as a diagnostic tool and disease activity marker. *Rheumatology (Oxford)* 2004; 43(2):220-224.
18. Sinclair D, Saas M, Williams D, Hart M, Goswami R. Can an ELISA replace immunofluorescence for the detection of anti-nuclear antibodies?--The routine use of anti-nuclear antibody screening ELISAs. *Clin Lab* 2007; 53(3-4):183-191.
19. Tozzoli R, Bizzaro N, Tonutti E, Villalta D, Bassetti D, Manoni F et al. Guidelines for the laboratory use of autoantibody tests in the diagnosis and monitoring of autoimmune rheumatic diseases. *Am J Clin Pathol* 2002; 117(2):316-324.
20. Moidhof W., Hiliak O. Lupus: an overview of the disease and management options. *P T* 2012; 37(4):240-9.
21. Hahn BH, McMahon MA, Wilkinson A, Wallace WD, Daikh DI, Fitzgerald JD et al. American College of Rheumatology guidelines for screening, treatment, and management of lupus nephritis. *Arthritis Care Res (Hoboken)* 2012; 64(6):797-808.

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 512_IFU_EN_QM113139_2013-12-16_1.2* 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**



REF ORG 515 Anti-Cardiolipin IgG/IgM

INTENDED PURPOSE

Anti-Cardiolipin 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
	Manufacturer
	Catalogue number
	Sufficient for ... determinations
	Batch code
	Use by
	Temperature limitation
	Keep away from sunlight
	Do not reuse
	Date of manufacture
	CE marked according to 98/79/EC
	Consult electronic Instructions For Use
	Electronic Instruction For Use: version

	Microplate
	Calibrator
	Calibrator
	Calibrator
	Calibrator
	Calibrator
	Calibrator
	Control positive
	Control negative
	Sample Buffer P
	Enzyme Conjugate
	Enzyme Conjugate
	TMB Substrate
	Stop solution
	Wash Buffer
	Ready to use
	50 x concentrate

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
MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Product code on module: CLP
CALIBRATOR A	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.
CALIBRATOR B	1x 1.5 ml	Calibrator B 7.5 GPL-U/ml / 5 MPL-U/ml, containing Cardioliipin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR C	1x 1.5 ml	Calibrator C 15 GPL-U/ml / 10 MPL-U/ml, containing Cardioliipin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR D	1x 1.5 ml	Calibrator D 30 GPL-U/ml / 20 MPL-U/ml, containing Cardioliipin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR E	1x 1.5 ml	Calibrator E 60 GPL-U/ml / 40 MPL-U/ml, containing Cardioliipin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR F	1x 1.5 ml	Calibrator F 120 GPL-U/ml / 80 MPL-U/ml, containing Cardioliipin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing cardioliipin 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.
CONTROL -	1x 1.5 ml	Control negative, containing cardioliipin 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.
DILUENT	20 ml	Sample Buffer P; containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate 5x.
CONJUGATE G	15 ml	Enzyme Conjugate IgG; containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
CONJUGATE M	15 ml	Enzyme Conjugate IgM; containing anti-human IgM antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
TMB	15 ml	TMB Substrate, containing 3,3', 5,5'- Tetramethylbenzidin. Ready to use.
STOP	15 ml	Stop solution; contains acid. Ready to use.
WASH	20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.

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 dessicated 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 90 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 GPL/MPL-U/ml	Expected GPL/MPL-U/ml	O/E [%]
IgG 1	1	73.0	73.0	100
	2	37.1	36.5	102
	4	19.6	18.3	107
IgG 2	1	80.5	80.5	100
	2	42.0	40.3	104
	4	22.2	20.1	111
IgG 3	1	66.2	64.4	103
	2	34.5	32.2	107
	4	16.2	16.1	101
IgM 1	1	70.9	70.9	100
	2	34.1	35.5	96
	4	18.2	17.7	103
IgM 2	1	114.0	114.0	100
	2	50.6	57.0	89
	4	27.3	28.5	96
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

ORG 515 IgG	Clinical Diagnosis		
	POS	NEG	
POS	63	6	223
NEG	10	144	
	73	150	

Sensitivity: 86.3 %
Specificity: 96.0 %
Overall agreement: 92.8 %

ORG 515 IgM	Clinical Diagnosis		
	Pos	Neg	
Pos	30	3	223
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 anti-cardiolipin antibody testing and reporting. *Pathology* 2004; 36(1):63-8.
- Higgins V, Augustin R, Kulasingam V, Taher J. Sample stability of autoantibodies: A tool for laboratory quality initiatives 2021. *Clin Biochem* 96: 43-8

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_2018-01-02_3*

Reason for revision: *Definition of symbols used and symbols updated.*

- ① **100 µl** Standards, Kontrollen und verdünnte Patientenproben pipettieren
→ **30 Minuten** bei Raumtemperatur inkubieren
→ Inhalt der Platte verwerfen und
3 mal mit **300 µl** Waschpuffer waschen
- ② **100 µl** Enzymkonjugatlösung pipettieren
→ **15 Minuten** bei Raumtemperatur inkubieren
→ Inhalt der Platte verwerfen und
3 mal mit **300 µl** Waschpuffer waschen
- ③ **100 µl** Substratlösung pipettieren
→ **15 Minuten** bei Raumtemperatur inkubieren
- ④ **100 µl** Stopplösung zugeben
→ Platte **5 Minuten** stehenlassen
→ Bei **450 nm** messen

ORGENTEC Diagnostika GmbH

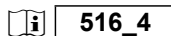
Carl-Zeiss-Straße 49-51

55129 Mainz - Germany

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

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

Internet: www.orgentec.com



ORG 516 AMA-M2

INTENDED PURPOSE

AMA-M2 is an ELISA test system for the quantitative measurement of IgG class autoantibodies against mitochondrial M2 subtype antigen in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

The test is used as an aid in the differential diagnosis of primary biliary cirrhosis (PBC). In patients with other autoimmune diseases occurrence of AMA antibodies may be related to the development or association of PBC. Evaluation of a test result should always take into account all clinical and laboratory diagnostic findings.

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		TMB Substrate
	Electronic Instruction For Use: version		Stop solution
			Wash Buffer
			Ready to use



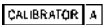
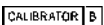
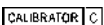
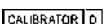
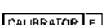
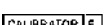








PRINCIPLE OF THE TEST

Highly purified mitochondrial M2 subtype (PDC-E2, BCOADC-E2, OGDC-E2) antigen is bound to microwells. 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 516		Sufficient for 96 determinations
	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Product code on module: AMA
	1x 1.5 ml	Calibrator A 0 IU/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Calibrator B 12.5 IU/ml, containing AMA-M2 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Calibrator C 25 IU/ml, containing AMA-M2 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Calibrator D 50 IU/ml, containing AMA-M2 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Calibrator E 100 IU/ml, containing AMA-M2 antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Calibrator F 200 IU/ml, containing AMA-M2 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Control positive, containing AMA-M2 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 AMA-M2 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 (5 x).
	15 ml	Enzyme Conjugate containing anti-human IgG 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, colorless. 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

 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.

 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										
B	B	P2										
C	C	P3										
D	D											
E	E											
F	F											
G	C+											
H	C-											

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 international reference preparation WHO 67/183 for AMA-M2 as 100 IU/ml.

Measuring range

The calculation range of this ELISA assay is 0 - 200 IU/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 10 IU/ml

Interpretation of results

Negative: < 10 IU/ml
Positive: ≥ 10 IU/ml

Linearity

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	Observed IU/ml	Expected IU/ml	O/E [%]
WHO	1:100	108.5	100.0	109
.	1:200	51.2	50.0	102
.	1:400	25.2	25.0	101
.	1:800	12.8	12.5	102
.	1:1600	6.1	6.3	98
.	1:3200	3.1	3.1	99
1	1:100	49.5	49.5	100
.	1:200	25.0	24.8	101
.	1:400	12.2	12.4	99
.	1:800	5.9	6.2	95

Limit of detection

Functional sensitivity was determined to be: 1 IU/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		
Sample	Mean IU/ml	CV %
1	39.8	7.0
2	81.3	3.8
3	177.3	3.6

Inter-Assay		
Sample	Mean IU/ml	CV %
1	40.1	6.2
2	84.6	11.8
3	180.4	3.8

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	n Pos	%
Primary biliary cirrhosis (PBC)	143	139	97.2
Rheumatoid Arthritis	60	1	1.7
Normal human sera	267	18	6.7

		Clinical Diagnosis		
		POS	NEG	
ORG 516	POS	139	19	470
	NEG	4	308	
		143	327	

Sensitivity: 97.2 %

Specificity: 94.2 %

Overall agreement: 95.1 %

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

1. Berg, P.A. and Klein, R. Diagnose der primär-biliären Zirrhose. IVD Nachrichten 1990; 1/1: 6 -7.
2. Berg, P.A. and Klein, R. Heterogeneity of anti-mitochondrial antibodies. Sem. Liver Dis. 1989; 9: 103 - 116.
3. Berg, P.A. and Klein, R. Immunology of primary biliary cirrhosis. Ballière's Clin.Gastroenterol. 1987; 1: 675 - 706.
4. Baum, H. and Palmer, C. The PBC specific antigen. Mol. Aspects Med. 1985; 8: 201 - 234.
5. Fussey, S.P.M., Guest, J.R., James, O.F W. et al. Identification and analysis of the major M2 autoantigens in primary biliary cirrhosis. PNAS, USA 1988; 85: 8654 - 8658.

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 516_IFU_EN_QM113145_2013-12-16_2.1* 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**

ORGENTEC Diagnostika GmbH

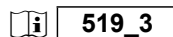
Carl-Zeiss-Straße 49-51

55129 Mainz - Germany

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

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

Internet: www.orgentec.com



ORG 519 Anti-MPO (pANCA)

INTENDED PURPOSE

Anti-MPO is an ELISA test system for the quantitative measurement of IgG class autoantibodies against myeloperoxidase (MPO) in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

Anti-neutrophil cytoplasmic antibodies (ANCA) are diagnostic markers for ANCA-associated vasculitides. Anti-MPO differentiates microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis (EGPA) The test supports differential diagnosis of vasculitis, when used in conjunction with other clinical and laboratory findings.

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		TMB Substrate
	Electronic Instruction For Use: version		Stop solution
			Wash Buffer
			Ready to use

PRINCIPLE OF THE TEST

Highly purified myeloperoxidase (MPO) is bound to microwells.

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



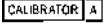
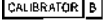
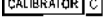
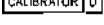
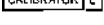
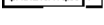








- All reagents of this kit are intended for professional in vitro diagnostic use only.
- Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based 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 519		Sufficient for 96 determinations
	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Product code on module: MPO
	1x 1.5 ml	Calibrator A 0 U/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Calibrator B 5 U/ml, containing MPO antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Calibrator C 10 U/ml, containing MPO antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Calibrator D 20 U/ml, containing MPO antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Calibrator E 40 U/ml, containing MPO antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Calibrator F 100 U/ml, containing MPO antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
	1x 1.5 ml	Control positive, containing MPO 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 MPO 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 (5 x).
	15 ml	Enzyme Conjugate containing anti-human IgG 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, colorless. 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

 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.

 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										
B	B	P2										
C	C	P3										
D	D											
E	E											
F	F											
G	C+											
H	C-											

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

This assay system is calibrated in relative arbitrary units, since no international reference preparation is available for this assay.

Measuring range

The calculation range of this ELISA assay is 0 - 100 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 5 U/ml

Interpretation of results

Negative: < 5 U/ml
Positive: ≥ 5 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	Observed U/ml	Expected U/ml	O/E [%]
1	1:100	87.3	87.3	100
.	1:200	44.1	43.7	101
.	1:400	21.5	21.8	99
.	1:800	9.7	10.9	89
.	1:1600	5.0	5.5	91
2	1:100	79.9	79.9	100
.	1:200	39.3	40.0	98
.	1:400	19.0	20.0	95
.	1:800	8.5	10.0	85
.	1:1600	4.3	5.0	86

Limit of detection

Functional sensitivity was determined to be: 0.5 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		
Sample	Mean U/ml	CV %
1	7.5	6.4
2	30.2	4.1
3	59.9	3.1

Inter-Assay		
Sample	Mean U/ml	CV %
1	7.0	5.0
2	33.8	4.9
3	78.3	6.3

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	n Pos	%
Crescendic glomerulonephritis	55	53	96.4
Morbus Wegener (cANCA pos)	20	1	5.0
Non-ANCA kidney disease	10	1	10.0
Normal human sera	120	3	2.5

		Immunological Diagnosis		
		POS	NEG	
ORG 519	POS	54	5	205
	NEG	1	145	
		55	150	

Sensitivity: 98.2 %

Specificity: 96.7 %

Overall agreement: 97.1 %

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

1. Jennette, J. C. and Falk, R.J . Antineutrophil Cytoplasmic Autoantibodies and Associated Diseases: a Review. Am. J. Kidney Dis. 1990, Vol. XV, No. 6: 517 - 529.
2. Gross, W. L. et al. Antineutrophil Cytoplasmic Autoantibody-Associated Diseases: A Rheumatologist's Perspective. Am. J. Kidney Dis. 1991, Vol. XVIII, No. 2: 175 - 179.
3. Wieslander, J. How are Antineutrophil Cytoplasmic Autoantibodies Detected ? Am. J. Kidney Dis. 1991, Vol. XVIII, No. 2: 154 - 158.
4. Lesavre, P. Antineutrophil cytoplasmic antibodies antigen specificity. Am. J. Kidney Dis. 1991, Vol. XVIII, No. 2: 159 - 163.
5. Hagen, E. C. et al. Antineutrophil cytoplasmic autoantibodies: a review of the antigens involved, the assays, and the clinical and possible pathogenic consequences. Blood 1993, Vol.81: 1996 - 2000.
6. Gross, W .L. et al. Immunodiagnostische und immunopathogenetische Bedeutung von Anti-Neutrophilen-Cytoplasma-Antikörpern. Deutsche Medizinische Wochenschrift 1993, Vol. 118: 191 - 199.

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 519_IFU_EN_QM113148_2016-05-03_1.3* 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**



529_5
www.orgentec.com

REF ORG 529 Anti-Phospholipid Screen IgG/IgM

INTENDED PURPOSE

Anti-Phospholipid Screen IgG/IgM is an ELISA test system to screen for the presence of IgG and IgM class autoantibodies against cardiolipin, phosphatidyl serine, phosphatidyl inositol, phosphatidic acid and beta-2-glycoprotein I 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 ... 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 electronic Instructions For Use		Enzyme Conjugate
	Electronic Instruction For Use: version		TMB Substrate
			Stop solution
			Wash Buffer
			Ready to use
			50 x concentrate

PRINCIPLE OF THE TEST

A mixture of highly purified cardiolipin, phosphatidyl serine, phosphatidyl inositol, phosphatidic acid and human beta-2-Glycoprotein I is bound to microwells. 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 529	▽ 96	Sufficient for 96 determinations
MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Product code on module: PSC
CALIBRATOR A	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.
CALIBRATOR B	1x 1.5 ml	Calibrator B 6.3 GPL-U/ml / 6.3 MPL-U/ml, containing phospholipid antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR C	1x 1.5 ml	Calibrator C 12.5 GPL-U/ml / 12.5 MPL-U/ml, containing phospholipid antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR D	1x 1.5 ml	Calibrator D 25 GPL-U/ml / 25 MPL-U/ml, containing phospholipid antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR E	1x 1.5 ml	Calibrator E 50 GPL-U/ml / 50 MPL-U/ml, containing phospholipid antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR F	1x 1.5 ml	Calibrator F 100 GPL-U/ml / 100 MPL-U/ml, containing phospholipid antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing phospholipid 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.
CONTROL -	1x 1.5 ml	Control negative, containing phospholipid 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.
DILUENT	20 ml	Sample Buffer P, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate (5 x).
CONJUGATE G	15 ml	Enzyme Conjugate; containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
CONJUGATE M	15 ml	Enzyme Conjugate; containing anti-human IgM antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
TMB	15 ml	TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.
STOP	15 ml	Stop solution; contains acid. Ready to use.
WASH	20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.

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 90 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

Calibration is related to the internationally recognised reference sera from E.N. Harris, Louisville and to IRP 97/656 (IgG) and HCAL (IgG) / EY2C9 (IgM).

Measuring range

The calculation range of this ELISA assay is IgG: 0 - 100 GPL-U/ml IgM: 0 - 100 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: 10 MPL-U/ml

Interpretation of results

Negative: IgG < 10 GPL-U/ml IgM < 10 MPL-U/ml
Positive: ≥ 10 GPL-U/ml ≥ 10 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	Observed GPL/MPL-U/ml	Expected GPL/MPL-U/ml	O/E [%]
IgG 1	1:100	98.0	98.4	100
	1:200	49.6	49.2	101
	1:400	24.3	24.6	99
	1:800	12.0	12.3	98
IgG 2	1:1600	5.8	6.2	94
	1:100	92.4	92.4	100
	1:200	45.9	46.2	99
	1:400	22.7	23.1	98
IgM 1	1:800	11.4	11.6	99
	1:1600	5.4	5.8	94
	1:100	92.7	92.7	100
	1:200	45.7	46.4	99
IgM 2	1:400	22.8	23.2	98
	1:800	11.2	11.6	97
	1:1600	5.4	5.8	93
	1:100	72.4	74.2	100
	1:200	36.5	37.1	98
	1:400	18.7	18.6	101
	1:800	8.9	9.3	96
	1:1600	4.4	4.6	95

Limit of detection

Functional sensitivity was determined to be: IgG: 0.5 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	10.4	5.1
2	18.7	3.4
3	59.9	5.2

Inter-Assay IgG		
Sample	Mean GPL-U/ml	CV %
1	10.0	3.6
2	17.7	5.4
3	57.9	4.9

Intra-Assay IgM		
Sample	Mean MPL-U/ml	CV %
1	12.8	4.1
2	30.8	3.5
3	63.8	3.7

Inter-Assay IgM		
Sample	Mean MPL-U/ml	CV %
1	12.6	5.3
2	31.9	4.1
3	62.1	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	7	87.5	6	75.0
Secondary APS	65	60	92.3	33	50.8
Normal human sera	150	4	2.7	5	3.3

	Clinical Diagnosis			Clinical Diagnosis		
	POS	NEG		Pos	Neg	
ORG 529 POS IgG	67	4	ORG 529 Pos IgM	39	5	
NEG	6	146	Neg	34	145	
	73	150	223	73	150	223
Sensitivity:	91.8 %		Sensitivity:	53.4 %		
Specificity:	97.3 %		Specificity:	96.7 %		
Overall agreement:	95.5 %		Overall agreement:	82.5 %		

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, Krihis 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 anti-cardiolipin antibody testing and reporting. *Pathology* 2004; 36(1):63-8.
- Higgins V, Augustin R, Kulusingam V, Taher J. Sample stability of autoantibodies: A tool for laboratory quality initiatives 2021. *Clin Biochem* 96: 43-8

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 529_IFU_EN_QM113163_2018-01-02_4*

Reason for revision: *Definition of symbols used and symbols updated*

- ① **100 µl** Standards, Kontrollen und verdünnte Patientenproben pipettieren
→ **30 Minuten** bei Raumtemperatur inkubieren
→ Inhalt der Platte verwerfen und
3 mal mit **300 µl** Waschpuffer waschen
- ② **100 µl** Enzymkonjugatlösung pipettieren
→ **15 Minuten** bei Raumtemperatur inkubieren
→ Inhalt der Platte verwerfen und
3 mal mit **300 µl** Waschpuffer waschen
- ③ **100 µl** Substratlösung pipettieren
→ **15 Minuten** bei Raumtemperatur inkubieren
- ④ **100 µl** Stopplösung zugeben
→ Platte **5 Minuten** stehenlassen
→ Bei **450 nm** messen

ORGENTEC Diagnostika GmbH

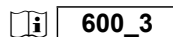
Carl-Zeiss-Straße 49-51

55129 Mainz - Germany

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

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

Internet: www.orgentec.com



ORG 600 ANA Detect

INTENDED PURPOSE

ANA Detect is an ELISA test system for the qualitative measurement of IgG class autoantibodies against SS-A-52 (Ro-52), SS-A-60 (Ro-60), SS-B (La), RNP/Sm, RNP-70, RNP-A, RNP-C, Sm-BB, Sm-D, Sm-E, Sm-F, Sm-G, Scl-70, Jo-1, dsDNA, ssDNA, polynucleosomes, mononucleosomes, histone complex, histone H1, histone H2A, histone H2B, histone 3, histone H4, Pm-Scl-100 and centromere B in human serum or plasma samples. This product is intended for professional in vitro diagnostic use only.

The test is used for screening of patients with suspected autoimmune connective tissue diseases, e.g. systemic lupus erythematosus, mixed connective tissue disease, Sjogren's syndrome, scleroderma, and polymyositis/dermatomyositis. Evaluation of a test result should always take into account all clinical and laboratory diagnostic findings.

SYMBOLS USED ON LABELS



In vitro diagnostic medical device



Manufacturer



Catalogue number



Sufficient for 96 determinations



Batch code



Use by



Temperature limitation



Keep away from sunlight



Do not reuse



Date of manufacture



CE marked according to 98/79/EC



Consult instructions for use



Electronic Instruction For Use: version



Microplate



Control



Control



Control



Sample Buffer P



Enzyme Conjugate



TMB Substrate



Stop solution



Wash Buffer



Ready to use

PRINCIPLE OF THE TEST

A mixture of purified antigens SS-A-52 (Ro-52), SS-A-60 (Ro-60), SS-B (La), RNP/Sm, RNP-70, RNP-A, RNP-C, Sm-BB, Sm-D, Sm-E, Sm-F, Sm-G, Scl-70, Jo-1, dsDNA, ssDNA, polynucleosomes, mononucleosomes, histone complex, histone H1, histone H2A, histone H2B, histone 3, histone H4, Pm-Scl-100 and centromere B is coated on to microwells.

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 600	▽ 96	Sufficient for 96 determinations
MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Product code on module: ANA
CONTROL A	1x 1.5 ml	Control A (negative), containing ANA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL B	1x 1.5 ml	Control B (cut-off), containing ANA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL C	1x 1.5 ml	Control C (positive), containing ANA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
DILUENT	20 ml	Sample Buffer P, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate (5 x).
CONJUGATE	15 ml	Enzyme Conjugate containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
TMB	15 ml	TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.
STOP	15 ml	Stop solution; contains acid. Ready to use.
WASH	20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.
CA	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											
B	B											
C	C											
D	P1											
E	P2											
F	P3											
G												
H												

P1, ... patient sample A-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 qualitative results the optical density (OD) of a sample is compared to the optical density of Control B:

Negative: OD sample < OD Control B

Positive: OD sample ≥ OD Control B

For detailed results the optical density of a sample is expressed as Index value:

Index = OD sample / OD Control B

PERFORMANCE CHARACTERISTICS

Calibration

The assay system is calibrated against the internationally recognised reference sera from CDC, Atlanta, USA and furthermore against the reference preparation WHO Wo/80 for human anti-dsDNA.

Measuring range

not applicable

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 Index 1.0

Interpretation of results

Negative: Index < 1.0
Borderline: Index 1.0 - 1.2
Positive: Index > 1.2

Linearity

Patient samples containing high levels of specific antibody were serially diluted in sample buffer. Activity for each dilution step was calculated as Index-Value.

Sample	Dilution	Observed Index	Expected Index	O/E [%]
1	1:100	4.8	4.8	100
	1:200	2.2	2.4	92
	1:400	1.3	1.2	108
2	1:800	0.6	0.6	100
	1:100	2.8	2.8	100
	1:200	1.5	1.4	107
3	1:400	0.8	0.7	114
	1:800	0.4	0.4	111
	1:100	3.5	3.5	100
	1:200	1.7	1.8	94
	1:400	0.8	0.9	89
	1:800	0.5	0.5	96

Limit of detection (not applicable)

n.a.

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		
Sample	Mean Index	CV %
1	1.8	6.9
2	2.4	9.1
3	2.8	10.4

Inter-Assay		
Sample	Mean Index	CV %
1	1.6	9.9
2	3.7	10.4
3	4.1	11.2

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	n Pos	%
SLE	63	62	98.4
Sjogren's syndrome	2	2	100.0
MCTD	9	9	100.0
Poly-/dermatomyositis	8	8	100.0
Scleroderma	3	3	100.0
CREST syndrome	9	9	100.0
Normal human sera	148	3	2.0

		Clinical Diagnosis		
		POS	NEG	
ORG 600	POS	93	3	242
	NEG	1	145	
		94	148	
Sensitivity:		98.9 %		
Specificity:		98.0 %		
Overall agreement:		98.3 %		

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

- Alba P, Bento L, Cuadrado MJ, Karim Y, Tungekar MF, Abbs I et al. Anti-dsDNA, anti-Sm antibodies, and the lupus anticoagulant: significant factors associated with lupus nephritis. *Ann Rheum Dis* 2003; 62(6):556-560.
- Antico A, Platzgummer S, Bassetti D, Bizzaro N, Tozzoli R, Villalta D. Diagnosing systemic lupus erythematosus: new-generation immunoassays for measurement of anti-dsDNA antibodies are an effective alternative to the Farr technique and the Crithidia luciliae immunofluorescence test. *Lupus* 2010; 19(8):906-912.
- Brouwer R, Hengstman GJ, Vree EW, Ehrfeld H, Bozic B, Ghirardello A et al. Autoantibody profiles in the sera of European patients with myositis. *Ann Rheum Dis* 2001; 60(2):116-123.
- Castro C, Gourley M. Diagnostic testing and interpretation of tests for autoimmunity. *J Allergy Clin Immunol* 2010; 125(2 Suppl 2):S238-S247.
- Defendenti C, Atzeni F, Spina MF, Grosso S, Cereda A, Guercilena G et al. Clinical and laboratory aspects of Ro/SSA-52 autoantibodies. *Autoimmun Rev* 2011; 10(3):150-154.
- Eriksson C, Kokkonen H, Johansson M, Hallmans G, Wadell G, Rantapaa-Dahlqvist S. Autoantibodies predate the onset of Systemic Lupus Erythematosus in northern Sweden. *Arthritis Research & Therapy* 2011; 13(1):R30.
- Haugbro K, Nossent JC, Winkler T, Figenschau Y, Rekvig OP. Anti-dsDNA antibodies and disease classification in antinuclear antibody positive patients: the role of analytical diversity. *Ann Rheum Dis JID* - 0372355 2004; 63(4):386-394.
- Ippolito A, Wallace DJ, Gladman D, Fortin PR, Urowitz M, Werth V et al. Autoantibodies in systemic lupus erythematosus: comparison of historical and current assessment of seropositivity. *Lupus* 2011; 20(3):250-255.
- Isenberg DA, Manson JJ, Ehrenstein MR, Rahman A. Fifty years of anti-ds DNA antibodies: are we approaching journey's end? *Rheumatology (Oxford)* 2007; 46(7):1052-1056.
- Kattah NH, Kattah MG, Utz PJ. The U1-snRNP complex: structural properties relating to autoimmune pathogenesis in rheumatic diseases. *Immunol Rev* 2010; 233(1):126-145.
- Kumar Y, Bhatia A, Minz RW. Antinuclear antibodies and their detection methods in diagnosis of connective tissue diseases: a journey revisited. *Diagn Pathol* 2009; 4:1.
- Meroni PL, Schur PH. ANA screening: an old test with new recommendations. *Ann Rheum Dis* 2010; 69:1420-1422.
- Petri M, Magder L. Classification criteria for systemic lupus erythematosus: a review. *Lupus* 2004; 13(11):829-837.
- Poole BD, Schneider RI, Guthridge JM, Velte CA, Reichlin M, Harley JB et al. Early targets of nuclear RNP humoral autoimmunity in human systemic lupus erythematosus. *Arthritis Rheum* 2009; 60(3):848-859.
- Putova I, Dostal C, Becvar R. Prevalence of antinucleosome antibodies by enzyme-linked immunosorbent assays in patients with systemic lupus erythematosus and other autoimmune systemic diseases. *Ann N Y Acad Sci* 2007; 1109:275-286.
- Reveille JD. Predictive value of autoantibodies for activity of systemic lupus erythematosus. *Lupus JID* - 9204265 2004; 13(5):290-297.
- Simon JA, Cabiedes J, Ortiz E, Alcocer-Varela J, Sanchez-Guerrero J. Anti-nucleosome antibodies in patients with systemic lupus erythematosus of recent onset. Potential utility as a diagnostic tool and disease activity marker. *Rheumatology (Oxford)* 2004; 43(2):220-224.
- Sinclair D, Saas M, Williams D, Hart M, Goswami R. Can an ELISA replace immunofluorescence for the detection of anti-nuclear antibodies?--The routine use of anti-nuclear antibody screening ELISAs. *Clin Lab*

2007; 53(3-4):183-191.

- Tozzoli R, Bizzaro N, Tonutti E, Villalta D, Bassetti D, Manoni F et al. Guidelines for the laboratory use of autoantibody tests in the diagnosis and monitoring of autoimmune rheumatic diseases. *Am J Clin Pathol* 2002; 117(2):316-324.
- Maidhof W., Hilius O. Lupus: an overview of the disease and management options. *P T* 2012; 37(4):240-9.
- Hahn BH, McMahon MA, Wilkinson A, Wallace WD, Daikh DI, Fitzgerald JD et al. American College of Rheumatology guidelines for screening, treatment, and management of lupus nephritis. *Arthritis Care Res (Hoboken)* 2012; 64(6):797-808.

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 600_IFU_EN_QM113200_2013-12-16_1.2* 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**

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



 **601_3**





























ORG 601 Anti-CCP hs® (high sensitive)

INTENDED PURPOSE

Anti-CCP hs® (high sensitive) is an ELISA test system for the quantitative measurement of IgG class autoantibodies against cyclic citrullinated peptides (CCP) in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

Measurement of anti-CCP antibodies may aid in the diagnosis of rheumatoid arthritis (RA), where anti-CCP antibody levels represent one parameter of a multi-criterion diagnostic process, encompassing both clinical and laboratory-based assessments.

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		TMB Substrate
	Electronic Instruction For Use: version		Stop solution
			Wash Buffer
			Ready to use

PRINCIPLE OF THE TEST

Highly purified cyclic citrullinated vimentin peptides (CCP) is bound to microwells.

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 601	▽ 96	Sufficient for 96 determinations
MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Product code on module: CCP
CALIBRATOR A	1x 1.5 ml	Calibrator A 0 U/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR B	1x 1.5 ml	Calibrator B 20 U/ml, containing CCP antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR C	1x 1.5 ml	Calibrator C 40 U/ml, containing CCP antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR D	1x 1.5 ml	Calibrator D 100 U/ml, containing CCP antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR E	1x 1.5 ml	Calibrator E 300 U/ml, containing CCP antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR F	1x 1.5 ml	Calibrator F 1000 U/ml, containing CCP antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing CCP 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.
CONTROL -	1x 1.5 ml	Control negative, containing CCP 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.
DILUENT	20 ml	Sample Buffer P, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate (5 x).
CONJUGATE	15 ml	Enzyme Conjugate containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
TMB	15 ml	TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.
STOP	15 ml	Stop solution; contains acid. Ready to use.
WASH	20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.
i	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										
B	B	P2										
C	C	P3										
D	D											
E	E											
F	F											
G	C+											
H	C-											

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

This assay system is calibrated in relative arbitrary units. It is calibrated against an external anti-CCP Assay, since no international reference sera for RA diagnostic are available so far.

Measuring range

The calculation range of this ELISA assay is 0 - 1000 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 20 U/ml

Interpretation of results

Negative: < 20 U/ml
Positive: ≥ 20 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	Observed U/ml	Expected U/ml	O/E [%]
1	1:100	950.2	950.2	100
	1:200	467.3	475.1	98
	1:400	245.4	237.6	103
	1:800	115.6	118.8	97
2	1:100	120.0	120.0	100
	1:200	60.5	60.0	101
	1:400	31.4	30.0	105
	1:800	14.2	15.0	95
3	1:1600	7.3	7.5	97
	1:100	321.3	321.3	100
	1:200	157.9	160.7	98
	1:400	96.4	80.3	120
	1:800	48.2	40.2	120

Limit of detection

Functional sensitivity was determined to be: 1 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		
Sample	Mean U/ml	CV %
1	13.0	7.8
2	144.5	9.9
3	250.6	13.6

Inter-Assay		
Sample	Mean U/ml	CV %
1	12.3	6.1
2	134.9	7.1
3	262.2	9.3

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	n Pos	%
Rheumatoid arthritis	259	237	91.5
Other arthritis	22	6	27.3
Other rheumatic disease	37	1	2.7
Healthy controls	118	1	0.8

		Clinical Diagnosis		
		POS	NEG	
ORG 601	POS	237	8	
	NEG	22	169	
		259	177	436
Sensitivity:		91.5 %		
Specificity:		95.5 %		
Overall agreement:		93.1 %		

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

- Bang H, Egerer K, Gauliard A, et al. Mutation and citrullination modifies vimentin to a novel autoantigen for rheumatoid arthritis. *Arthritis Rheum.* 56(8):2503, 2007.
- Aletaha D, et al. Rheumatoid arthritis classification criteria: An American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis & Rheumatism* 62 (9):2569-2581, 2010.
- Prujin GJ, Wiik A, van Venrooij WJ. The use of citrullinated peptides and proteins for the diagnosis of rheumatoid arthritis. *Arthritis Res Ther* 12 (1):203, 2010.
- Snir O, Widhe M, Hermansson M, von Spee C, Lindberg J, Hensen S, Lundberg K, Engstrom A, Venables PJ, Toes RE, Holmdahl R, Klareskog L, Malmstrom V. Antibodies to several citrullinated antigens are enriched in the joints of rheumatoid arthritis patients. *Arthritis Rheum* 62 (1):44-52, 2009.
- Van Steendam K, Tillemans K, Deforce D. The relevance of citrullinated vimentin in the production of antibodies against citrullinated proteins and the pathogenesis of rheumatoid arthritis. *Rheumatology (Oxford)*, 2011.
- Van Steendam K, Tillemans K, De Ceuleneer M, De Keyser F, Elewaut D, Deforce D. Citrullinated vimentin as an important antigen in immune complexes from synovial fluid of rheumatoid arthritis patients with antibodies against citrullinated proteins. *Arthritis Res Ther* 12 (4):R132, 2010.
- Syversen SW, Goll GL, van der Heijde D, Landewe R, Lie BA, Odegard S, Uhlig T, Gaarder PI, Kvien TK. Prediction of radiographic progression in rheumatoid arthritis and the role of antibodies against mutated citrullinated vimentin: results from a ten-year prospective study. *Ann.Rheum.Dis.* 69 (2):345-351, 2009.
- Innala L, Kokkonen H, Eriksson C, Jidell E, Berglin E, Dahlqvist SR. Antibodies against mutated citrullinated vimentin are a better predictor of disease activity at 24 months in early rheumatoid arthritis than antibodies against cyclic citrullinated peptides. *J Rheumatol.* 35(6):1002, 2008.
- Mathsson L, Mullazehi M, Wick MC, et al. Antibodies against citrullinated vimentin in rheumatoid arthritis: higher sensitivity and extended prognostic value concerning future radiographic progression as compared with antibodies against cyclic citrullinated peptides. *Arthritis Rheum.* 58(1):36, 2008.
- Soós L, Szekanez Z, Szabó Z, et al. Clinical evaluation of anti-mutated citrullinated vimentin by ELISA in rheumatoid arthritis. *J Rheumatol.* 34(8):1658, 2007.
- Bizzaro N, Tonutti E, Tozzoli R, Villalta D. Analytical and diagnostic characteristics of 11 2nd- and 3rd-generation immunoenzymatic methods for the detection of antibodies to citrullinated proteins. *Clin Chem.* 53 (8):1527, 2007.
- Coenen D, Verschuere P, Westhovens R, Bossuyt X. Technical and diagnostic performance of 6 assays for the measurement of citrullinated protein/peptide antibodies in the diagnosis of rheumatoid arthritis. *Clin Chem.* 53(3):498, 2007.
- Dejaco C, Klotz W, Larcher H, Duftner C, Schirmer M, Herold M. Diagnostic value of antibodies against a modified citrullinated vimentin in rheumatoid arthritis. *Arthritis Res Ther.* 8(4):R119, 2006.
- Keskin G, Inal A, Keskin D, et al. Diagnostic utility of anti-cyclic citrullinated peptide and anti-modified citrullinated vimentin antibodies in rheumatoid arthritis. *Protein Pept Lett.* 15(3):314 2008.
- Poulsom H, Charles PJ. Antibodies to citrullinated vimentin are a specific and sensitive marker for the diagnosis of rheumatoid arthritis. *Clin Rev Allergy Immunol.* 34(1):4 2008.
- Song JS, Park GB, Park AJ. Comparison of anti-mutated citrullinated vimentin with anti-cyclic citrullinated peptide and rheumatoid factors for the diagnostic value of rheumatoid arthritis [in Korean]. *J Korean Rheum Assoc.* 14(3):235 2007.

- Ursum J, Nielsen MM, van Schaardenburg D, et al. Antibodies to mutated citrullinated vimentin and disease activity score in early arthritis: a cohort study. *Arthritis Res Ther.* 10(1):R12, 2008.
- Besada E, Nikolaisen C, Nossent H. Diagnostic value of antibodies against mutated citrullinated vimentin for rheumatoid arthritis. *Clin Exp Rheumatol.* 29(1):85 2011.

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 601_IFU_EN_QM113201_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**

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



 **604_4**

ORG 604 Anti-dsDNA

INTENDED PURPOSE

Anti-dsDNA is an ELISA test system for the quantitative measurement of IgG class autoantibodies against double-stranded DNA in human serum or plasma. This product is intended for professional in vitro diagnostic use only.


The test is used as an aid in the differential diagnosis of inflammatory autoimmune diseases, especially systemic lupus erythematosus (SLE). Autoantibodies to dsDNA are diagnostic markers for SLE and levels may be elevated during active disease. Evaluation of a test result should always take into account all clinical and laboratory diagnostic findings.

SYMBOLS USED

 In vitro diagnostic medical device

 Manufacturer


 Catalogue number

 Sufficient for 96 determinations


 Batch code


 Use by


 Temperature limitation

 Keep away from sunlight

 Do not reuse

 Date of manufacture

 CE marked according to 98/79/EC

 Consult instructions for use

 Electronic Instruction For Use: version

 Microplate

 Calibrator


 Calibrator

 Calibrator

 Calibrator


 Calibrator


 Calibrator

 Control positive

 Control negative

 Sample Buffer

 Enzyme Conjugate

 TMB Substrate

 Stop solution

 Wash Buffer

 Ready to use

PRINCIPLE OF THE TEST

Highly purified double-stranded DNA (dsDNA) is bound to microwells.

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 604	▽ ⁹⁶	Sufficient for 96 determinations
MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Product code on module: dsD
CALIBRATOR A	1x 1.5 ml	Calibrator A 0 IU/ml, containing no serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR B	1x 1.5 ml	Calibrator B 12.5 IU/ml, containing dsDNA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR C	1x 1.5 ml	Calibrator C 25 IU/ml, containing dsDNA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR D	1x 1.5 ml	Calibrator D 50 IU/ml, containing dsDNA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR E	1x 1.5 ml	Calibrator E 100 IU/ml, containing dsDNA antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR F	1x 1.5 ml	Calibrator F 200 IU/ml, containing dsDNA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing dsDNA 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.
CONTROL -	1x 1.5 ml	Control negative, containing dsDNA 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.
DILUENT	20 ml	Sample Buffer PD, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate (5 x).
CONJUGATE	15 ml	Enzyme Conjugate containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
TMB	15 ml	TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.
STOP	15 ml	Stop solution; contains acid. Ready to use.
WASH	20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.

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 PD 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.

1. 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.
2. 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.
3. Dispense **100 µl** of TMB substrate solution into each well.
Incubate for **15 minutes** at room temperature
4. **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										
B	B	P2										
C	C	P3										
D	D											
E	E											
F	F											
G	C+											
H	C-											

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 international reference preparation WHO Wo/80 for human anti-dsDNA IgG antibodies as 200 IU/ml.

Measuring range

The calculation range of this ELISA assay is 0 - 200 IU/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 20 IU/ml

Interpretation of results

Negative: < 20 IU/ml
Positive: ≥ 20 IU/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	Observed IU/ml	Expected IU/ml	O/E [%]
1	1:100	104.2	104.2	100
.	1:200	50.6	52.1	97
.	1:400	24.9	26.1	95
.	1:800	11.2	13.0	86
2	1:100	135.3	135.3	100
.	1:200	68.9	67.7	102
.	1:400	35.2	33.8	104
.	1:800	18.2	16.9	108

Limit of detection

Functional sensitivity was determined to be: 1 IU/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		
Sample	Mean IU/ml	CV %
1	26.0	4.5
2	61.0	3.1
3	114.0	6.4

Inter-Assay		
Sample	Mean IU/ml	CV %
1	29.0	12.4
2	68.0	7.3
3	138.0	5.2

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

<u>Study population</u>	<u>n</u>	<u>n_pos</u>	<u>%</u>
SLE	202	164	81.2
Other autoimmune diseases	33	1	3.0
Normal human sera	115	1	0.9

Clinical Diagnosis

		POS	NEG		
ORG 604	POS	164	2		
	NEG	38	146		
		202	148	350	

Sensitivity: 81.2 %
Specificity: 98.6 %
Overall agreement: 88.6 %

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

- Alba P, Bento L, Cuadrado MJ, Karim Y, Tungekar MF, Abbs I et al. Anti-dsDNA, anti-Sm antibodies, and the lupus anticoagulant: significant factors associated with lupus nephritis. *Ann Rheum Dis* 2003; 62(6):556-560.
- Antico A, Platzgummer S, Bassetti D, Bizzaro N, Tozzoli R, Villalta D. Diagnosing systemic lupus erythematosus: new-generation immunoassays for measurement of anti-dsDNA antibodies are an effective alternative to the Farr technique and the Crithidia luciliae immunofluorescence test. *Lupus* 2010; 19(8):906-912.
- Brouwer R, Hengstman GJ, Vree EW, Ehrfeld H, Bozic B, Ghirardello A et al. Autoantibody profiles in the sera of European patients with myositis. *Ann Rheum Dis* 2001; 60(2):116-123.
- Castro C, Gourley M. Diagnostic testing and interpretation of tests for autoimmunity. *J Allergy Clin Immunol* 2010; 125(2 Suppl 2):S238-S247.
- Defendenti C, Atzeni F, Spina MF, Grosso S, Cereda A, Guercilena G et al. Clinical and laboratory aspects of Ro/SSA-52 autoantibodies. *Autoimmun Rev* 2011; 10(3):150-154.
- Eriksson C, Kokkonen H, Johansson M, Hallmans G, Wadell G, Rantapaa-Dahlqvist S. Autoantibodies predate the onset of Systemic Lupus Erythematosus in northern Sweden. *Arthritis Research & Therapy* 2011; 13(1):R30.
- Haugbro K, Nossent JC, Winkler T, Figenschau Y, Rekvig OP. Anti-dsDNA antibodies and disease classification in antinuclear antibody positive patients: the role of analytical diversity. *Ann Rheum Dis JID* - 0372355 2004; 63(4):386-394.
- Ippolito A, Wallace DJ, Gladman D, Fortin PR, Urowitz M, Werth V et al. Autoantibodies in systemic lupus erythematosus: comparison of historical and current assessment of seropositivity. *Lupus* 2011; 20(3):250-255.
- Isenberg DA, Manson JJ, Ehrenstein MR, Rahman A. Fifty years of anti-ds DNA antibodies: are we approaching journey's end? *Rheumatology (Oxford)* 2007; 46(7):1052-1056.
- Kattah NH, Kattah MG, Utz PJ. The U1-snRNP complex: structural properties relating to autoimmune pathogenesis in rheumatic diseases. *Immunol Rev* 2010; 233(1):126-145.
- Kumar Y, Bhatia A, Minz RW. Antinuclear antibodies and their detection methods in diagnosis of connective tissue diseases: a journey revisited. *Diagn Pathol* 2009; 4:1.
- Meroni PL, Schur PH. ANA screening: an old test with new recommendations. *Ann Rheum Dis* 2010; 69:1420-1422.
- Petri M, Magder L. Classification criteria for systemic lupus erythematosus: a review. *Lupus* 2004; 13(11):829-837.
- Poole BD, Schneider RI, Guthridge JM, Velte CA, Reichlin M, Harley JB et al. Early targets of nuclear RNP humoral autoimmunity in human systemic lupus erythematosus. *Arthritis Rheum* 2009; 60(3):848-859.
- Putova I, Dostal C, Becvar R. Prevalence of antinucleosome antibodies by enzyme-linked immunosorbent assays in patients with systemic lupus erythematosus and other autoimmune systemic diseases. *Ann N Y Acad Sci* 2007; 1109:275-286.
- Reveille JD. Predictive value of autoantibodies for activity of systemic lupus erythematosus. *Lupus JID* - 9204265 2004; 13(5):290-297.
- Simon JA, Cabiedes J, Ortiz E, Alcocer-Varela J, Sanchez-Guerrero J. Anti-nucleosome antibodies in patients with systemic lupus erythematosus of recent onset. Potential utility as a diagnostic tool and disease activity marker. *Rheumatology (Oxford)* 2004; 43(2):220-224.
- Sinclair D, Saas M, Williams D, Hart M, Goswami R. Can an ELISA replace immunofluorescence for the detection of anti-nuclear antibodies?--The routine use of anti-nuclear antibody screening ELISAs. *Clin Lab*

2007; 53(3-4):183-191.

- Tozzoli R, Bizzaro N, Tonutti E, Villalta D, Bassetti D, Manoni F et al. Guidelines for the laboratory use of autoantibody tests in the diagnosis and monitoring of autoimmune rheumatic diseases. *Am J Clin Pathol* 2002; 117(2):316-324.
- Maidhof W., Hilius O. Lupus: an overview of the disease and management options. *P T* 2012; 37(4):240-9.
- Hahn BH, McMahon MA, Wilkinson A, Wallace WD, Daikh DI, Fitzgerald JD et al. American College of Rheumatology guidelines for screening, treatment, and management of lupus nephritis. *Arthritis Care Res (Hoboken)* 2012; 64(6):797-808.

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 604_IFU_DE_QM112991_2018-01-02_3*

Reason for revision: *updated description of coating material.*

- ① **100 µl** Standards, Kontrollen und verdünnte Patientenproben pipettieren
→ **30 Minuten** bei Raumtemperatur inkubieren
→ Inhalt der Platte verwerfen und
3 mal mit **300 µl** Waschpuffer waschen
- ② **100 µl** Enzymkonjugatlösung pipettieren
→ **15 Minuten** bei Raumtemperatur inkubieren
→ Inhalt der Platte verwerfen und
3 mal mit **300 µl** Waschpuffer waschen
- ③ **100 µl** Substratlösung pipettieren
→ **15 Minuten** bei Raumtemperatur inkubieren
- ④ **100 µl** Stopplösung zugeben
→ Platte **5 Minuten** stehenlassen
→ Bei **450 nm** messen

ORGENTEC Diagnostika GmbH

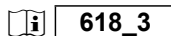
Carl-Zeiss-Straße 49-51

55129 Mainz - Germany

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

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

Internet: www.orgentec.com



ORG 618 Anti-PR3 hs

INTENDED PURPOSE

Anti-PR3 hs is an ELISA test system for the quantitative measurement of IgG class autoantibodies against proteinase 3 (PR3) in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

Anti-neutrophil cytoplasmic antibodies (ANCA) are diagnostic markers for ANCA-associated vasculitides. Anti-PR3 characterises granulomatosis with polyangiitis (GPA, formerly: Wegener's granulomatosis). The test supports the differential diagnosis of vasculitis when used in combination with other laboratory and clinical findings.

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		TMB Substrate
	Electronic Instruction For Use: version		Stop solution
			Wash Buffer
			Ready to use

PRINCIPLE OF THE TEST

Highly purified proteinase 3 (PR3) is bound to microwells.

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 618	▽ 96	Sufficient for 96 determinations
MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Product code on module: PR3
CALIBRATOR A	1x 1.5 ml	Calibrator A 0 U/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR B	1x 1.5 ml	Calibrator B 10 U/ml, containing PR3 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR C	1x 1.5 ml	Calibrator C 20 U/ml, containing PR3 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR D	1x 1.5 ml	Calibrator D 40 U/ml, containing PR3 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR E	1x 1.5 ml	Calibrator E 80 U/ml, containing PR3 antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR F	1x 1.5 ml	Calibrator F 200 U/ml, containing PR3 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing PR3 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.
CONTROL -	1x 1.5 ml	Control negative, containing PR3 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.
DILUENT	20 ml	Sample Buffer P, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate (5 x).
CONJUGATE	15 ml	Enzyme Conjugate containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
TMB	15 ml	TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.
STOP	15 ml	Stop solution; contains acid. Ready to use.
WASH	20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.
i	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 all patient samples **1:200** with sample buffer prior to use in the assay: combine **5 µl** of sample with 995 µl of sample buffer in a polystyrene tube. Mix well. Note: Calibrators / Controls need no dilution.

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										
B	B	P2										
C	C	P3										
D	D											
E	E											
F	F											
G	C+											
H	C-											

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

This assay system is calibrated in relative arbitrary units, since no international reference preparation is available for this assay.

Measuring range

The calculation range of this ELISA assay is 0 - 200 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 10 U/ml

Interpretation of results

Negative: < 10 U/ml
Positive: ≥ 10 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	Observed U/ml	Expected U/ml	O/E [%]
1	1:100	168.2	168.2	100
.	1:200	84.6	84.1	101
.	1:400	41.3	42.1	98
.	1:800	20.1	21.0	96
.	1:1600	9.7	10.5	92
2	1:100	122.1	122.1	100
.	1:200	60.7	61.1	99
.	1:400	29.8	30.5	98
.	1:800	14.6	15.3	95
.	1:1600	6.7	7.6	88

Limit of detection

Functional sensitivity was determined to be: 0.5 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		
Sample	Mean U/ml	CV %
1	11.9	3.5
2	44.7	2.0
3	132.8	2.5

Inter-Assay		
Sample	Mean U/ml	CV %
1	12.6	3.8
2	46.8	4.1
3	122.7	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	n Pos	%
Morbus Wegener (cANCA pos)	51	49	96.1
inflam./Non-inflammatory disorders	450	8	1.8
Healthy controls	80	0	0.0

		Immunological Diagnosis		
		POS	NEG	
ORG 618	POS	49	8	581
	NEG	2	522	
		51	530	

Sensitivity: 96.1 %

Specificity: 98.5 %

Overall agreement: 98.3 %

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

1. Cohen Tervaert J.W., Goldschmeding, R., Elema, J.D., Limburg, P.C. et al Association of autoantibodies to myeloperoxidase with different forms of vasculitis. *Arthritis Rheum.* 33 (8):1264-1272, 1990
2. Cotch, M.F., Hoffman, G.S., Yerg, D.E., et.al. The epidemiology of Wegener's granulomatosis. Estimates of the five-year period prevalence, annual mortality, and geographic disease distribution from population-based data sources. *Arthritis Rheum.*, Vol 39, 87-92, 1996
3. de Grot, K., Schnabel, A., Gross, W. L. ANCA-assoziierte Vaskulitiden (Wegener Granulomatose, Churg-Strauß Syndrom, Mikroskopische Polyangiitis) - 1. Diagnostisches Procedere. *Z. Rheumatol.*, Vol 54, 291 - 302, 1995
4. Gross, W.L. Systemic necrotizing vasculitis. *Baillieres.Clin.Rheumatol.*, Vol. 11, 259-284, 1997
5. Gross, W.L., Csernok, E., Helmchen, U. Antineutrophil cytoplasmic autoantibodies, autoantigens, and systemic vasculitis. *APMIS* , Vol. 103, 81-97, 1995
6. Jenette, J. C. Falk, R. J. Antineutrophil cytoplasmic autoantibodies and associated diseases. A review. *Am. J. Kidney Dis.*, Vol 15, 517 - 529, 1990
7. Jennette, C.J., Falk, R.J., Andrassy, K., Bacon, P.A. et al. Nomenclature of systemic vasculitides. Proposal of an international consensus conference. *Arthritis Rheum.* Vol. 37, 187-192, 1994
8. Kallenberg, C.G Vasculitis 1995: new concepts in pathophysiology. *Isr.J Med Sci.*, Vol. 32, 7-12, 1996
9. Kallenberg, C.G. Laboratory findings in the vasculitides. *Baillieres Clin Rheumatol.*, Vol 11, 395-421, 1997
10. Schmitt, W. H., Gross, W. L. Antineutrophile zytoplasmatische Antikörper (ANCA). *Internist*, Vol. 36, 282 - 290, 1995
11. Wiik, A., Stumman, L., Kjeldsen, L., Borregaard, N., et al. The diversity of perinuclear antineutrophil cytoplasmic antibodies (pANCA) antigens. *Clin. Exp. Immunol.*, Vol. 101, Suppl. I, 15-17, 1995

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 618_IFU_EN_QM113207_2013-12-16_1.2* 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**

ORGENTEC Diagnostika GmbH

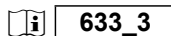
Carl-Zeiss-Straße 49-51

55129 Mainz - Germany

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

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

Internet: www.orgentec.com



ORG 633 Anti-Centromere B

INTENDED PURPOSE

Anti-Centromere B is an ELISA test system for the quantitative measurement of IgG class autoantibodies against centromere B in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

The test is used as an aid in the differential diagnosis of inflammatory autoimmune diseases, e.g. CREST syndrome. Evaluation of a test result should always take into account all clinical and laboratory diagnostic findings.

SYMBOLS USED ON LABELS

In vitro diagnostic medical device

Manufacturer

Catalogue number

Sufficient for 96 determinations

Batch code

Use by

Temperature limitation

Keep away from sunlight

Do not reuse

Date of manufacture

CE marked according to 98/79/EC

Consult instructions for use

Electronic Instruction For Use: version

Microplate

Calibrator

Calibrator

Calibrator

Calibrator

Calibrator

Control positive

Control negative

Sample Buffer P

Enzyme Conjugate

TMB Substrate

Stop solution

Wash Buffer

Ready to use

PRINCIPLE OF THE TEST

Recombinant centromere protein B is bound to microwells.

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 633	▽ 96	Sufficient for 96 determinations
MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Product code on module: CEN
CALIBRATOR A	1x 1.5 ml	Calibrator A 0 U/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR B	1x 1.5 ml	Calibrator B 10 U/ml, containing centromere B antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR C	1x 1.5 ml	Calibrator C 30 U/ml, containing centromere B antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR D	1x 1.5 ml	Calibrator D 100 U/ml, containing centromere B antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR E	1x 1.5 ml	Calibrator E 300 U/ml, containing centromere B antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing centromere B 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.
CONTROL -	1x 1.5 ml	Control negative, containing centromere B 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.
DILUENT	20 ml	Sample Buffer P, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate (5 x).
CONJUGATE	15 ml	Enzyme Conjugate containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
TMB	15 ml	TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.
STOP	15 ml	Stop solution; contains acid. Ready to use.
WASH	20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.
i	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	P2										
B	B	P3										
C	C											
D	D											
E	E											
F	C+											
G	C-											
H	P1											

P1, ... patient sample A-E 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

This assay system is calibrated in relative arbitrary units, since no international reference preparation is available for this assay.

Measuring range

The calculation range of this ELISA assay is 0 - 300 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 10 U/ml

Interpretation of results

Negative: < 10 U/ml
Positive: ≥ 10 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	Observed U/ml	Expected U/ml	O/E [%]
1	1:100	136.8	136.8	100
.	1:200	67.1	68.4	98
.	1:400	35.2	34.2	103
.	1:800	16.9	17.1	99
2	1:100	285.0	285.0	100
.	1:200	139.2	142.5	98
.	1:400	73.5	71.3	103
.	1:800	37.0	35.6	104

Limit of detection

Functional sensitivity was determined to be: 1 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		
Sample	Mean U/ml	CV %
1	15.2	5.4
2	122.0	4.4
3	220.0	4.7

Inter-Assay		
Sample	Mean U/ml	CV %
1	16.4	5.4
2	125.6	5.0
3	225.4	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	n Pos	%
CREST syndrome	32	31	96.9
Rheumatoid arthritis	20	1	5.0
Normal human sera	100	6	6.0

		Clinical Diagnosis		
		POS	NEG	
ORG 633	POS	31	7	
	NEG	1	113	
		32	120	152

Sensitivity: 96.9 %

Specificity: 94.2 %

Overall agreement: 94.7 %

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

- 1 Alba P, Bento L, Cuadrado MJ, Karim Y, Tungekar MF, Abbs I et al. Anti-dsDNA, anti-Sm antibodies, and the lupus anticoagulant: significant factors associated with lupus nephritis. *Ann Rheum Dis* 2003; 62(6):556-560.
- 2 Antico A, Platzgummer S, Bassetti D, Bizzaro N, Tozzoli R, Villalta D. Diagnosing systemic lupus erythematosus: new-generation immunoassays for measurement of anti-dsDNA antibodies are an effective alternative to the Farr technique and the Crithidia luciliae immunofluorescence test. *Lupus* 2010; 19(8):906-912.
- 3 Brouwer R, Hengstman GJ, Vree EW, Ehrfeld H, Bozic B, Ghirardello A et al. Autoantibody profiles in the sera of European patients with myositis. *Ann Rheum Dis* 2001; 60(2):116-123.
- 4 Castro C, Gourley M. Diagnostic testing and interpretation of tests for autoimmunity. *J Allergy Clin Immunol* 2010; 125(2 Suppl 2):S238-S247.
- 5 Defendenti C, Atzeni F, Spina MF, Grosso S, Cereda A, Guercilena G et al. Clinical and laboratory aspects of Ro/SSA-52 autoantibodies. *Autoimmun Rev* 2011; 10(3):150-154.
- 6 Eriksson C, Kokkonen H, Johansson M, Hallmans G, Wadell G, Rantapaa-Dahlqvist S. Autoantibodies predate the onset of Systemic Lupus Erythematosus in northern Sweden. *Arthritis Research & Therapy* 2011; 13(1):R30.
- 7 Haugbro K, Nossent JC, Winkler T, Figenschau Y, Rekvig OP. Anti-dsDNA antibodies and disease classification in antinuclear antibody positive patients: the role of analytical diversity. *Ann Rheum Dis JID* - 0372355 2004; 63(4):386-394.
- 8 Ippolito A, Wallace DJ, Gladman D, Fortin PR, Urowitz M, Werth V et al. Autoantibodies in systemic lupus erythematosus: comparison of historical and current assessment of seropositivity. *Lupus* 2011; 20(3):250-255.
- 9 Isenberg DA, Manson JJ, Ehrenstein MR, Rahman A. Fifty years of anti-ds DNA antibodies: are we approaching journey's end? *Rheumatology (Oxford)* 2007; 46(7):1052-1056.
10. Kattah NH, Kattah MG, Utz PJ. The U1-snRNP complex: structural properties relating to autoimmune pathogenesis in rheumatic diseases. *Immunol Rev* 2010; 233(1):126-145.
- 11.Kumar Y, Bhatia A, Minz RW. Antinuclear antibodies and their detection methods in diagnosis of connective tissue diseases: a journey revisited. *Diagn Pathol* 2009; 4:1.
12. Meroni PL, Schur PH. ANA screening: an old test with new recommendations. *Ann Rheum Dis* 2010; 69:1420-1422.
13. Petri M, Magder L. Classification criteria for systemic lupus erythematosus: a review. *Lupus* 2004; 13(11):829-837.
14. Poole BD, Schneider RI, Guthridge JM, Velte CA, Reichlin M, Harley JB et al. Early targets of nuclear RNP humoral autoimmunity in human systemic lupus erythematosus. *Arthritis Rheum* 2009; 60(3):848-859.
- 15.Putova I, Dostal C, Becvar R. Prevalence of antinucleosome antibodies by enzyme-linked immunosorbent assays in patients with systemic lupus erythematosus and other autoimmune systemic diseases. *Ann N Y Acad Sci* 2007; 1109:275-286.
16. Reveille JD. Predictive value of autoantibodies for activity of systemic lupus erythematosus. *Lupus JID* - 9204265 2004; 13(5):290-297.
17. Simon JA, Cabiedes J, Ortiz E, Alcocer-Varela J, Sanchez-Guerrero J. Anti-nucleosome antibodies in patients with systemic lupus erythematosus of recent onset. Potential utility as a diagnostic tool and disease activity marker. *Rheumatology (Oxford)* 2004; 43(2):220-224.
18. Sinclair D, Saas M, Williams D, Hart M, Goswami R. Can an ELISA replace immunofluorescence for the detection of anti-nuclear antibodies?--The routine use of anti-nuclear antibody screening ELISAs. *Clin Lab* 2007; 53(3-4):183-191.
19. Tozzoli R, Bizzaro N, Tonutti E, Villalta D, Bassetti D, Manoni F et al. Guidelines for the laboratory use of autoantibody tests in the diagnosis and monitoring of autoimmune rheumatic diseases. *Am J Clin Pathol* 2002; 117(2):316-324.
20. Maidhof W., Hiliias O. Lupus: an overview of the disease and management options. *P T* 2012; 37(4):240-9.

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 633_IFU_EN_QM113209_2016-08-16_1.3* 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**