

ORGENTEC Diagnostika GmbH

Carl-Zeiss-Straße 49-51

55129 Mainz - Germany

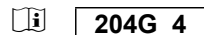
Phone: +49 (0) 61 31 / 92 58-0

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

Internet: www.orgentec.com



Electronic Instruction For Use: version



ORG 204G Anti-dsDNA IgG

INTENDED PURPOSE

Anti-dsDNA IgG is an ELISA-based test system for the quantitative measurement of IgG class autoantibodies against double-stranded DNA in human serum or plasma samples. 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 ... determinations
	Batch code
	Use by
	Temperature limitation
	Consult instructions for use
	Keep away from sunlight
	Do not reuse
	Date of manufacture
	CE marked according to 98/79/EC
	Electronic Instruction For Use: version

PRINCIPLE OF THE TEST

Human recombinant double-stranded DNA (dsDNA) is bound to microwells.

The Alegria[®] assay features barcoded 8-well-microstrips, called Alegria[®] Test Strips. Each strip is designed for a single determination of one patient sample. The Alegria[®] Test Strip holds a complete set of reagents. Included are enzyme conjugate, enzyme substrate, sample buffer and a test specific control. Furthermore each strip has two antigen-coated wells which serve as reaction wells for one control and one patient sample.

The determination is based on an indirect enzyme linked immune reaction with the following steps: Antibodies present in positive samples bind to the antigen coated on the surface of the two reaction wells forming an antibody antigen complex. After incubation, a first washing step removes unbound and unspecific bound molecules. Subsequently added enzyme conjugate binds to the immobilized antibody-antigen complex. After incubation, a second washing step removes unbound enzyme conjugate. Addition of enzyme substrate solution results in hydrolysis and color development during incubation. The intensity of the blue color correlates with the concentration of the antibody-antigen-complex and can be measured photometrically at 650 nm.

The Alegria[®] Test Strip is based on the proprietary SMC[®]-Technology (Sensotronic Memorized Calibration); information about the assay, analysis and evaluation, and the lot-specific expiry date is contained on the barcode printed on each Alegria[®] Test Strip.

The Alegria[®] Test Strip can be used with the diagnostic instrument Alegria[®] - a fully automated Random Access Analyser. By means of SMC[®]-Technology data encoded on the barcode are transferred from the Alegria[®] Test Strip to the instrument and the assay is automatically processed and evaluated. The instrument reads the date of expiry and rejects further processing if the Alegria[®] Test Strip is out of date.

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).
- System fluid 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, control and sample buffer contain 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 Alegria[®] strips 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 controls and/or pooled sera.

CONTENTS OF THE KIT

▽ 24 ORG 204G-24

Sufficient for 24 determinations

ALEGRIA TEST STRIPS 24

Alegria® Test Strips are modules of 8 wells each composed of:

Wells 1 + 2: empty and not coated (wells for the sample dilution)

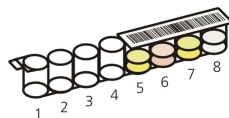
Wells 3 + 4: coated with antigen (reaction wells)

Well 5: Control; yellow; containing test specific antibodies, PBS, BSA, detergent, preservative sodium azide 0.09% and ProClin 300 0.05%.

Well 6: Enzyme Conjugate; light red; containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative ProClin 300 0.05%.

Well 7: Sample Buffer: yellow; containing PBS, BSA, detergent, preservative sodium azide 0.09% and ProClin 300 0.05%.

Well 8: TMB Substrate: clear; containing 3,3', 5,5'- Tetramethylbenzidin.



Code on barcode: **dsDNA IgG** on printout: **ds-DNA-G**

WASH

1x 20 ml Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.

SYSTEM FLUID

1x 2.5 ml System Fluid, contains acid; 1000 x concentrate



1 Certificate of Analysis

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 Alegria® Test Strips sealed and desiccated in the clip bag provided.
- Shelf life of the unopened test kit is 15 months from day of production.
- Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- Diluted Wash Buffer and System Fluid are stable for at least 30 days when stored at 2-8°C.
- Once transferred to the reagent container we recommend consumption on the same day.

MATERIALS REQUIRED

- Vortex mixer
- Pipettes for 10 µl
- Measuring cylinder for 1000 ml and 2500 ml
- Distilled or deionized water

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.

PROCEDURAL NOTES

- Do not use kit components beyond their expiration dates.
- All materials must be at room temperature (20-28°C) prior to use.
- To avoid carryover or contamination, change the pipette tip between samples.

PREPARATION OF REAGENTS

WASH

Dilute the content of the Wash Buffer concentrate (50x) with distilled or deionized water to a final volume of 1000 ml prior to use. Transfer the diluted Wash Buffer into the instrument reagent container. If only one Alegria run is to be performed on one day we recommend transferring only 500 ml diluted Wash Buffer.

SYSTEM FLUID

Dilute the content of the System Fluid concentrate (1000x) with distilled or deionized water to a final volume of 2500 ml prior to use. Transfer the diluted System Fluid into the instrument reagent container.

ALEGRIA TEST STRIPS

Take the required number of Alegria® Test Strips out of the clip bag and let them reach room temperature (20-28°C). Do not remove foil covering the empty wells until you are ready to start the assay.

TEST PROCEDURE

Alegria® Test Strips with SMC® technology are used with the diagnostic instrument Alegria®. Detailed information about operating the instrument can be taken from the Instrument User Manual.

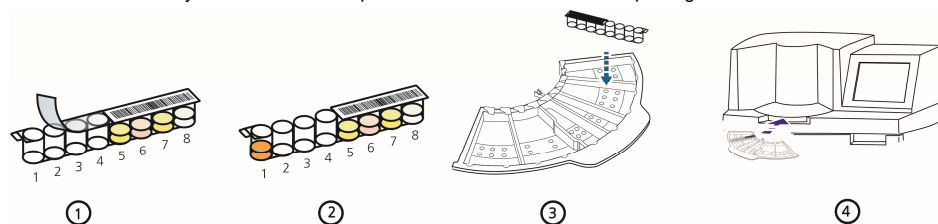
(1) Remove the foil from the empty wells 1 to 4 of the Alegria® Test Strip.

Do not remove foil with printed barcode, covering wells 5 to 8.

(2) Pipette 10 µl undiluted sample at the bottom of well 1.

(3) Insert the strip into the SysTray.

(4) Place loaded SysTrays into the correct position in the Alegria® instrument and start run. All further steps will be done automatically. The test run is completed when the instrument starts printing the results.



CALIBRATION

The assay system is calibrated against the international reference preparation WHO Wo/80 for human anti-dsDNA IgG antibodies as 200 IU/ml.

CALCULATION OF RESULTS

By means of SMC® Technology (Sensotronic Memorized Calibration), all test data are transferred to the system through individual barcodes on the Alegria® Test Strip. Calculation and interpretation of results will be performed automatically.

PERFORMANCE CHARACTERISTICS

Measuring range

The calculation range of this Alegria® 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 Alegria® assay: Cut-off 20 IU/ml

Interpretation of results

Normal: < 20 IU/ml
Elevated: ≥ 20 IU/ml

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.

Linearity

Three patient samples containing high levels of specific antibody were serially diluted in sample buffer to demonstrate the dynamic range of the assay. Activity for each dilution was calculated by means of SMC® Technology.

Sample	Dilution	Observed IU/ml	Expected IU/ml	O/E [%]
1	1:100	126.8	126.8	100
	1:200	65.4	63.4	103
	1:400	31.2	31.7	98
	1:800	15.9	15.9	100
2	1:100	143.7	143.7	100
	1:200	74.9	71.9	104
	1:400	38.6	35.9	107
	1:800	19.3	18.0	107
3	1:100	89.5	89.5	100
	1:200	46.9	44.8	105
	1:400	21.2	22.4	95
	1:800	10.1	11.2	90

Sensitivity

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	29.1	5.6
2	46.0	6.2
3	114.7	0.9

Inter-Assay		
Sample	Mean IU/ml	% CV
1	27.7	6.7
2	44.4	6.4
3	123.8	6.6

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	202	164	81.2
Other autoimmune diseases	33	1	3.0
Normal human sera	115	1	0.9

		Clinical Diagnosis		
		Pos	Neg	
ORG 204G	Pos	164	2	350
Anti-dsDNA IgG	Neg	38	146	
		202	148	

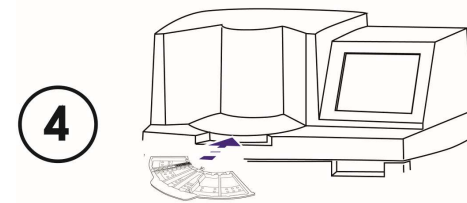
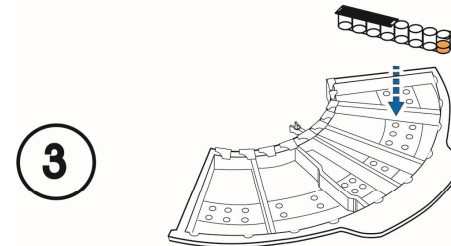
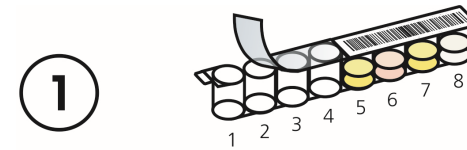
Sensitivity: 81.2 %
 Specificity: 98.6 %
 Overall agreement: 88.6 %

Diseases	Autoantibody prevalence to (values in %)									
	dsDNA	ssDNA	Histone	SS-A	SS-B	Sm	RNP/Sm	Scl-70	Jo-1	
Systemic lupus erythematosus (SLE)	> 90	> 90	30-50	10-30	30-50	10-30	10-30			
Drug-induced lupus (DIL)		30-50	50-90							
Sharp-syndrome / mixed connective tissue disease	10-30	10-30					> 90			
Rheumatoid arthritis	10-30	30-50	30-50	10-30						
Sjögren's syndrome	10-30	10-30		> 90	> 90					
Scleroderma	10-30	10-30		10-30				> 90		
Photosensitive dermatitis, dermatomyositis	10-30	10-30								50-90

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.

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 .