

## ORGENTEC Diagnostika GmbH

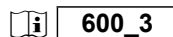
Carl-Zeiss-Straße 49-51

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