

## 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



Instruction For Use  
2014-01

US Market: For Research Use Only

## ORG 605 Anti-ssDNA

### NAME AND INTENDED USE

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

### SYMBOLS USED ON LABELS

IVD	In vitro diagnostic medical device	MICROPLATE	Microplate
Manufacturer		CALIBRATOR A	Calibrator
REF	Catalogue number	CALIBRATOR B	Calibrator
96	Sufficient for 96 determinations	CALIBRATOR C	Calibrator
LOT	Batch code	CALIBRATOR D	Calibrator
Use by		CALIBRATOR E	Calibrator
Temperature limitation		CALIBRATOR F	Calibrator
Consult instructions for use		CONTROL +	Control positive
Keep away from sunlight		CONTROL -	Control negative
Do not reuse		DILUENT	Sample Buffer P
Date of manufacture		CONJUGATE	Enzyme Conjugate
CE	conform to European directive 98/79/EC	TMB	TMB Substrate
		WASH	Stop solution
		STOP	Wash Buffer
		RTU	Ready to use

### PRINCIPLE OF THE TEST

Recombinant single-stranded DNA (ssDNA) 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.

### SUMMARY AND EXPLANATION OF THE TEST

Autoimmune diseases are characterized by the occurrence of antibodies against own antigenic structures - so-called autoantibodies. Presence of autoantibodies to native Desoxyribonucleic acids (n-DNA, dsDNA, double-stranded DNA) is typical for the clinical picture of Systemic Lupus erythematosus (SLE).

Antibodies against dsDNA belong to the group of Anti Nuclear Antibodies (ANA), which are directed against various structures of the nucleus of the cell. They appear in a variety of rheumatoid diseases. Besides the ANA antibodies another group of autoantibodies is of interest, which are directed against the so-called Extractable Nuclear Antigens (ENA). The ARA criteria of the American Rheumatism Association provide an extensive diagnostic scheme for the diagnosis of Systemic Lupus erythematosus (SLE). In case that at least 4 of the eleven ARA criteria are fulfilled, SLE is highly predictive [8].

Antibodies to dsDNA are found during the active phases of SLE, where the serum concentration exhibits positive correlation to the severity of the disease. An ongoing therapy may be monitored by the aid of autoantibody determination. Diagnostic sensitivity of the anti-dsDNA determination in cases of SLE is approximately 91 % combined with a diagnostic specificity of nearly 96 percent.

Antibodies against DNA can be differentiated into two groups:

1. antibodies, that bind only to native double-stranded DNA (dsDNA) and
2. antibodies recognizing single-stranded DNA (ssDNA) too.

Measurement of anti nuclear antibodies (ANA, or anti nuclear factor (ANF)) by indirect immunofluorescence test (IFT) is widely accepted as screening method in suspected SLE. Since in some stages of the diseases or during therapy IFT sometimes gives false results, a more specific test system is needed. Negative IFT for anti nuclear antibodies does not exclude the presence of anti-dsDNA antibodies, since the antigenic structures may be masked by other structures. Furthermore the ANA titers determined by IF test show only weak correlation to the severity of the disease.

Most antibodies against dsDNA are directed against the phosphate units of DNA. Thus, these autoantibodies also bind to DNA single strains. For quantitation of anti-dsDNA it has to be proven, that the antigen preparation exhibits no contamination with single stranded DNA.

Autoantibodies against single-stranded DNA are mainly directed against its basic compound, which in the native DNA is masked inside the helical structure. In serum of SLE patients anti-ssDNA antibodies are found with a frequency of up to 87 percent during acute phases and 43 percent during inactive phases. SLE like diseases are caused by some drugs. For differential diagnosis of drug-induced LE the determination of anti-ssDNA is a valuable diagnostic tool. In drug-induced LE anti-ssDNA is elevated in more the 50 percent of alle cases. Furthermore elevated anti-ssDNA serum concentrations have been reported in Mononucleosis, Hepatitis and various forms of Leukemia.

Autoantibody prevalence to (values in %)

Diseases	dsDNA	ssDNA	Histone	SS-A	SS-B	Sm	RNP/Sm	Sci-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					
Sjogren's syndrome	10-30	10-30		> 90	> 90				
Sclerodema	10-30	10-30		10-30				> 90	
Photosensitive dermatitis, dermatomyositis	10-30	10-30							50-90

## CONTENTS OF THE KIT

ORG 605	▽ 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>SSD</b>
<b>CALIBRATOR A</b>	1x 1.5 ml	Calibrator A 0 U/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN <sub>3</sub> 0.09%), yellow. Ready to use.
<b>CALIBRATOR B</b>	1x 1.5 ml	Calibrator B 12.5 U/ml, containing ssDNA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN <sub>3</sub> 0.09%), yellow. Ready to use.
<b>CALIBRATOR C</b>	1x 1.5 ml	Calibrator C 25 U/ml, containing ssDNA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN <sub>3</sub> 0.09%), yellow. Ready to use.
<b>CALIBRATOR D</b>	1x 1.5 ml	Calibrator D 50 U/ml, containing ssDNA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN <sub>3</sub> 0.09%), yellow. Ready to use.
<b>CALIBRATOR E</b>	1x 1.5 ml	Calibrator E 100 U/ml, containing ssDNA antibodies in a serum/buffer matrix (PBS, BSA, NaN <sub>3</sub> 0.09%), yellow. Ready to use.
<b>CALIBRATOR F</b>	1x 1.5 ml	Calibrator F 200 U/ml, containing ssDNA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN <sub>3</sub> 0.09%), yellow. Ready to use.
<b>CONTROL +</b>	1x 1.5 ml	Control positive, containing ssDNA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN <sub>3</sub> 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
<b>CONTROL -</b>	1x 1.5 ml	Control negative, containing ssDNA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN <sub>3</sub> 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
<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>WASH</b>	20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.
<b>STOP</b>	15 ml	Stop solution; contains acid. Ready to use.
<b>i</b>	1	Instruction for Use: ELISA Mini-DVD
<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.

## 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.

## 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, 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 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	189.2	189.2	100
.	1:200	90.3	94.6	95
.	1:400	50.1	47.3	106
.	1:800	21.9	23.7	92
2	1:100	163.2	163.2	100
.	1:200	77.9	81.6	95
.	1:400	39.2	40.8	96
.	1:800	21.1	20.4	103

### 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	16.0	5.6
2	65.0	4.2
3	135.0	6.4

Inter-Assay		
Sample	Mean U/ml	CV %
1	15.0	6.3
2	63.0	4.4
3	132.0	6.8

### 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	%
drug induced lupus	79	28	35.4
Normal human sera	315	5	1.6

		Clinical Diagnosis	
		Pos	Neg
ORG 605	Pos	28	5
	Neg	51	310
		79	315
		394	

Sensitivity: 35.4 %  
Specificity: 98.4 %  
Overall agreement: 85.8 %

#### 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

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8201 Central Ave NE, Suite P  
Minneapolis, MN 55432, USA  
[info@ibl-america.com](mailto:info@ibl-america.com)  
(888) 523 1246

- 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