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Instruction For Use  
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## ORG 509 Anti-SS-B

### NAME AND INTENDED USE

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.

### 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
Consult instructions for use	Control positive
Keep away from sunlight	Control negative
Do not reuse	Sample Buffer P
Date of manufacture	Enzyme Conjugate
conform to European directive 98/79/EC	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.

### SUMMARY AND EXPLANATION OF THE TEST

Connective tissue diseases (CTD) are a group of autoimmune disorders which are characterized by presence of antinuclear antibodies (ANA) in the blood of patients. ANA are a specific class of autoantibodies that have the capability of binding and destroying certain structures within the nucleus of the cells. These antibodies are involved in the disease pathogenesis, and they also constitute the basis for diagnosis and treatment of CTD.

ANA have been categorized into two main groups:

1. Autoantibodies to DNA and histones
2. Autoantibodies to extractable nuclear antigens (ENA): Sm, ribonucleoproteins (RNP), SSA/Ro, SSB/La, Scl-70, Jo-1 and PM1

Autoantibodies to DNA and histones include antibodies against single and double stranded DNA (ssDNA and dsDNA). Significant levels of anti-dsDNA antibodies are considered to be confirmatory in the diagnosis of systemic lupus erythematosus (SLE). Anti-histone antibodies are indicative of drug-induced lupus.

Besides DNA and histones, autoantibodies may also target other nuclear antigens. These nuclear antigens were named extractable nuclear antigens (ENA), as originally they were extracted from the nuclei with saline solution. Autoantibodies to Smith antigen (Sm) which is also considered to be highly specific for SLE were the first anti-ENA detected. Thereafter, further subtypes of ENA i.e. ribonucleoproteins (RNP), Sjögren antigen A or B (SSA/Ro or SSB/La), Scl-70, Jo-1 and PM1 were identified.

Although most of these ENA are disease specific, a significant overlap exists. Sensitivity and specificity may also vary depending upon the type of underlying CTD.

Presence of autoantibodies in the sera of patients constitutes one of the criteria used for diagnosis of CTD. Together with the clinical diagnosis ANA subtyping helps in identifying a specific CTD.

Indirect immunofluorescence tests (IF) and enzyme immunoassays (ELISA) are commonly used for ANA detection in day to day practice. Initially, screening is carried out by IF-ANA or a generic ELISA which detects ANA of a broad specificity similar to IF-ANA. If positive, more specific tests are performed based on clinical findings and the IF-ANA staining pattern.

These antigen specific ELISA assays react with single autoantigens e.g. dsDNA, SS-A/Ro, SS-B/La, Scl-70, Sm, Sm/RNP etc.

Autoantibodies to dsDNA are specific and diagnostic for SLE and levels are elevated during active disease. Recently published ACR Guidelines for Screening, Treatment, and Management of Lupus Nephritis recommend the testing of antibodies to dsDNA for monitoring of lupus nephritis, ranging from monthly intervals in pregnant patients with active glomerulonephritis at onset of treatment to every three months in patients with active nephritis at onset of treatment or pregnant patients with previous but not current nephritis, up to six-monthly testing in patients with previous active nephritis or no prior or current nephritis.

SLE-Patients without antibodies against dsDNA often produce antibodies against ssDNA. Similarly anti-Sm is highly specific for SLE but is present in only 10 % to 30 % of SLE cases.

Antibodies against dsDNA, histones, the 70 kD protein of the U1-snRNP complex (RNP70) and anti Sm are closely associated with SLE. Anti-SSA/Ro and anti-SSB/La antibodies are indicative for Sjögren's syndrome, but can also be found in up to 30 % cases of SLE with cutaneous involvement.



Anti-SS-A/Ro antibodies pass the placenta and may cause the development of SLE in neonates. Anti-SSA/Ro antibodies are almost always present in sera of mothers with babies with neonatal lupus syndrome and with complete congenital heart block.

Antinucleolar antibodies are a group of autoantibodies which give a nucleolar IF-staining pattern. Most common of these are anti-PM-Scl, anti-RNA polymerase I-III and anti-U3-RNP They are found in scleroderma and polymyositis (PM). Antibodies against RNP and the complex RNP/Sm are linked to mixed connective tissue disease (MCTD, Sharp syndrome) and to SLE. Serologically MCTD is characterized by the presence of autoantibodies directed against the 70 kD protein of the U1-snRNP-complex. Up to 100% of MCTD patients manifest high titers of Anti-RNP-70 antibodies.

Autoantibody prevalence to (values in %)

Diseases	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					
Sjogren'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

## CONTENTS OF THE KIT

ORG 509	▽ 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>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 SS-B 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 SS-B 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 SS-B 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 SS-B 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 SS-B 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 SS-B 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 SS-B 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.
	1	Instruction for Use: ELISA Mini-DVD
	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

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
.	1:800	16.1	8.7	101
2	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	%
Sjögren'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
		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.

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- 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



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