

## ORGENTEC Diagnostika GmbH

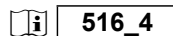
Carl-Zeiss-Straße 49-51

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
<b>MICROPLATE</b>	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Product code on module: <b>AMA</b>
<b>CALIBRATOR A</b>	1x 1.5 ml	Calibrator A 0 IU/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
<b>CALIBRATOR B</b>	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.
<b>CALIBRATOR C</b>	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.
<b>CALIBRATOR D</b>	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.
<b>CALIBRATOR E</b>	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.
<b>CALIBRATOR F</b>	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.
<b>CONTROL +</b>	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.
<b>CONTROL -</b>	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.
<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.
	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 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	470

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

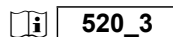
Carl-Zeiss-Straße 49-51

55129 Mainz - Germany

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

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

Internet: www.orgentec.com



## ORG 520 Anti-Insulin

### INTENDED PURPOSE

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

Anti-Insulin antibodies are found in a large part of patients with autoimmune diabetes (type 1). These antibodies may be directed against endogenous insulin or against therapeutic insulin preparations of human or animal origin.

### 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

A mixture of highly purified preparations of bovine, porcine and recombinant human insulin 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 520		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>INS</b>
<b>CALIBRATOR A</b>	1x 1.5 ml	Calibrator A 0 U/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
<b>CALIBRATOR B</b>	1x 1.5 ml	Calibrator B 6.3 U/ml, containing insulin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
<b>CALIBRATOR C</b>	1x 1.5 ml	Calibrator C 12.5 U/ml, containing insulin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
<b>CALIBRATOR D</b>	1x 1.5 ml	Calibrator D 25 U/ml, containing insulin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
<b>CALIBRATOR E</b>	1x 1.5 ml	Calibrator E 50 U/ml, containing insulin antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
<b>CALIBRATOR F</b>	1x 1.5 ml	Calibrator F 100 U/ml, containing insulin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
<b>CONTROL +</b>	1x 1.5 ml	Control positive, containing insulin 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.
<b>CONTROL -</b>	1x 1.5 ml	Control negative, containing insulin 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.
<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.
	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, 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 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	77.6	77.6	100
.	1:200	41.7	38.8	107
.	1:400	21.1	19.4	109
.	1:800	10.3	9.7	106
.	1:1600	4.7	4.9	96
2	1:100	100.7	100.7	100
.	1:200	50.7	50.4	101
.	1:400	23.7	25.2	94
.	1:800	11.1	12.6	88
.	1:1600	5.3	6.3	84

## 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	11.2	2.5
2	27.6	2.9
3	59.7	4.0

Inter-Assay		
Sample	Mean U/ml	CV %
1	11.6	6.0
2	31.2	5.2
3	69.5	4.3

## 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	%
Diabetes mellitus Type I	100	72	72.0
Normal human sera	160	2	1.3

		Clinical Diagnosis		
		POS	NEG	
ORG 520	POS	72	2	100 160 260
	NEG	28	158	

Sensitivity: 72.0 %

Specificity: 98.8 %

Overall agreement: 88.5 %

## 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

1. Reeves, W. G. Insulin Antibody Determination: Theoretical and Practical Considerations. *Diabetologia* 24, 399 (1983).
2. Atkinson, M. A., Fisk, D. D., Spillar, R. P. and MacLaren, N. K. Insulin antibodies as markers for Insulin dependent diabetes mellitus (IDD). *Diabetes* 34, 926 - 930 (1985).
3. Willein, T., Nicholson, S. and Casey, C. A Micro Enzyme-Linked Immunosorbent Assay for Insulin Antibodies in Serum. *J. Imm. Methods* 76, 185 (1985).
4. Kobayashi, N. et al. A Solid-Phase Enzyme Immunoassay for Anti-Insulin Antibody in Diabetes Mellitus Patients. *J. Imm. Methods* 84, 245 (1985).
5. Wisslein, T. et al. Value of Insulin Antibodies as Serum marker for Insulin-Dependent Diabetes Mellitus. *Lancet* 1, 480 (1985).
6. Soeldner, J. S. et al. Insulin-Dependent Diabetes Mellitus and Autoimmunity: Islet-Cell Autoantibodies and beta-Cell Failure. *New Engl. J. Med.* 313, 893 (1985).
7. Torfs, C. P. et al. Long Term Frozen Sera for Epidemiological Studies of Antibodies. *Lancet* 1, 503 (1986).
8. Seino, S. et al. Characterisation of Circulating Insulin in Insulin Autoimmune Syndrome. *Clin. Endo. & Metab.* 62, 64 (1986).
9. Boitard, C. H. and McDewitt, H. O. Immunology of insulin-dependent diabetes mellitus. In: Cohen, J. R., (ed.) *Perspectives on autoimmunity*, 39-58 (1987). CRC Press, Boca Raton, FL. Nouvo, J. A., Baker, Jr., J. R., Wartowsky, I. et al. Autoantibodies to insulin are present in sera of patients with autoimmune thyroid diseases. *Diabetes*, 37, 317-320 (1988).
10. Witkin, T. J. Insulin Autoantibodies as markers for type I diabetes. *Endocrine Reviews*, 11, 92-104 (1990).

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 520\_IFU\_EN\_QM113149\_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



 **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

**604\_4**

Electronic Instruction For Use: version

MICROPLATE

Microplate

CALIBRATOR A

Calibrator

CALIBRATOR B

Calibrator

CALIBRATOR C

Calibrator

CALIBRATOR D

Calibrator

CALIBRATOR E

Calibrator

CALIBRATOR F

Calibrator

CONTROL +

Control positive

CONTROL -

Control negative

DILUENT

Sample Buffer

CONJUGATE

Enzyme Conjugate

TMB

TMB Substrate

STOP

Stop solution

WASH

Wash Buffer

RTU

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	▽ <sup>96</sup>	Sufficient for 96 determinations
MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Product code on module: <b>dsD</b>
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