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Instruction For Use
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ORG 590 DNase Activity

NAME AND INTENDED USE

DNase Activity is an ELISA test system for the quantitative measurement of DNase Activity in human serum or EDTA-plasma. This product is intended for professional in vitro diagnostic use only.

SYMBOLS USED ON LABELS

IVD	In vitro diagnostic medical device
	Manufacturer
REF	Catalogue number
96	Sufficient for 96 determinations
LOT	Batch code
	Use by
	Temperature limitation
	Consult instructions for use
	Keep away from sunlight
	Do not reuse
	Date of manufacture

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

Specific DNase substrate is bound to microwells.

DNase present in the sample will turn over the immobilised DNase substrate during incubation at 37 °C for exactly 60 minutes. Washing of the microwells removes sample thus stopping the substrate reaction. Remaining immobilised DNase substrate is now reacting with the horseradish peroxidase (HRP) conjugated DNase substrate antibody for exactly 15 minutes at room temperature. Washing of the microwells removes unbound conjugate. The HRP-substrate TMB will hydrolyze bound HRP-conjugate to form a blue color. The addition of acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrically at 450 nm. The amount of colour is inversely proportional to the DNase activity in the sample, that means no DNase activity in the sample results in 100% activity reduction.

SUMMARY AND EXPLANATION OF THE TEST

Systemic lupus erythematosus (SLE) is one of the most severe autoimmune disorders, particularly caused by the production of pathogenic autoantibodies to an extensive spectrum of nuclear antigens, including chromatin, ribonucleoprotein components (RNP), components of the spliceosome complex together with phospholipid components of the cell membrane. The diverse array of clinical symptoms involves multiple organ systems resulting in diseases such as glomerulonephritis, vasculitis, arthritis, stroke or disorder of the central nervous system 1.

Since the diagnosis of SLE is difficult, current diagnostic guidelines have been defined. Patients suffering from SLE have to fulfil any four of the eleven criteria established by the American College of Rheumatology(ACR). The determination of double stranded DNA antibodies together with antibodies against the nuclear Sm protein are diagnostic criteria of the ACR. Another diagnostic tool is the detection of autoantibodies directed against single stranded DNA, histone proteins and nulceosomal complexes.

Although the determination of SLE still remains unclear, it seems that a defect in the mechanism of apoptosis - a form of physiological cell death to remove unwanted cells - may promote autoimmune disease susceptibility. During apoptosis DNA from dying cells is automatically digested, necessarily as a biological defence against intracellular parasites such as viruses and bacteria 2. Thus failure to DNA fragmentation could affect tissue homeostasis and consequently influence the development of autoimmune diseases and cancer. This might be the reason, why patients with SLE often reveal high titers of circulating nucleosomes 3.

Desoxyribunuclease (DNAse), an enzyme potentially involved in chromatin metabolism has been implicated in degrading DNA during apoptosis. Recently, studies have shown that a DNase-deficient human cell line is resistant to drug-induced apoptosis. Furthermore, it has been described that transgenic mice, deficient in DNase cannot remove circulating nucleosomes. This failure in apoptosis leads to the development of a spontaneous lupus-like syndrome (e.g. glomerulonephritis) 3. A deficiency in DNase was also found in patients with SLE correlating with high titers of antibodies against nucleosomal antigens. The activity of DNase is often decreased in patients with SLE. It has been shown that a single nucleotide mutation in the DNase I gene reduces the total activity of this enzyme. B-cells of SLE patients with this mutation have only 30-50% the of DNase activity compared to healthy individuals. Accordingly, the IgG titer against nucleosomal antigens was 7-8 times higher in SLE patients than in SLE patients without this mutation and 70-80 times higher compared to healthy indi-viduals 4.

A hypothetical model describes various genetic pathways in the initiation of the SLE pathogenesis involving interactions between different genes like C1q and DNase. With references to this many first-degree relatives of SLE patients exhibit a similar seropositive phenotype without severe disease pathogenesis. This might be due to a decrease of DNase activity.

Today, human recombinant DNase I is used to treat patients with cystic fibrosis, whose airways become blocked by thick mucus, containing high concentrations of bacterial DNA. Recently, a phase Ib clinical trial was performed in which DNase I was administered to humans with SLE and indicated that the treatment was safe. Future aspects are the development of a therapy for SLE patients with DNase I 5, 6.

The DNase Activity ELISA of ORGENTEC GmbH is a novel and absolutely innovative assay for the determination of DNase activity. It is the first commercial assay worldwide for the measurement of the DNase enzymatic activity in a microtiterplate. It can prove to be a very useful diagnostic tool for the determination and monitoring as well as the prediction of many autoimmune disorders.

CONTENTS OF THE KIT

ORG 590	Σ 96	Sufficient for 96 determinations
MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Product code on module: ***DA***
CALIBRATOR A	1x 1.5 ml	Calibrator A 5 %AR/ml, containing DNase in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), green. Ready to use.
CALIBRATOR B	1x 1.5 ml	Calibrator B 15% AR/ml, containing DNase in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR C	1x 1.5 ml	Calibrator C 35% AR/ml, containing DNase in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR D	1x 1.5 ml	Calibrator D 50 %AR/ml, containing DNase in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), green. Ready to use.
CALIBRATOR E	1x 1.5 ml	Calibrator E 75% AR/ml, containing DNase in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR F	1x 1.5 ml	Calibrator F 100 % AR/ml, containing DNase in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), green. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing DNase in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), green. Ready to use. The concentration is specified on the certificate of analysis.
CONTROL −	1x 1.5 ml	Control negative, containing DNase in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), green. Ready to use. The concentration is specified on the certificate of analysis.
DILUENT	20 ml	Sample Buffer PN, containing PBS, BSA, detergent, preservative sodium azide 0.09%, green, Ready to use.
CONJUGATE	15 ml	Enzyme Conjugate containing anti-DNase substrate 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 WN, containing Tris, detergent, preservative sodium azide 0.09%, green; 50 x conc.
	1	Instruction for Use: ELISA Mini-CD
	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 dessicated in the clip bag provided.
- Shelf life of the unopended 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, performe 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, classifiaction 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

Wash buffer (WN) is specific for DNase assay. Dilute the contents of each 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 PN is specific for DNase assay. Ready to use.

Preparation of samples

Dilute patient samples 1:11 before the assay: add 10 µl of sample to 100 µl of sample buffer in a polystyrene tube. Mix well. Note: Controls are ready to use and need no dilution.

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 **60 minutes** at **37 °C.**
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
2. Dispense **100 µ**l 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 µ**l 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 quantitative calibration of the DNase Activity assay is performed with defined concentrations of DNase enzyme in Kunitz units (KuU). The calibration range of 6.5-82 % activity reduction (AR) corresponds to 16-0.04 mKuU/ml.

Measuring range

The calculation range of this ELISA assay is 5 - 100 % AR/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 % AR/ml

Interpretation of results

Negative: < 25 % AR/ml
Positive: ≥ 25 % AR/ml

Linearity

DNase Activity is a non-linear assay.
The figure below shows typical results for inactivated serum spiked with DNase-I.

Sample	Mean [%AR/ml]	DNase Activity [mKuU/ml]	DNase-I concentration (specific activity 4000 KuU/mg) [ng/ml]
1	6.5	16	4
2	23.5	4	1
3	42.2	1.6	0.4
4	53.0	1.0	0.25
5	79.5	0.2	0.05
6	82.6	0.04	0.01

Limit of detection

Lower detection limit for DNase Activity assay: 80 % AR, 0.2 mKuU/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 % AR/ml	CV %
1	15.0	5.1
2	58.0	4.6
3	66.0	7.8

Inter-Assay		
Sample	Mean % AR/ml	CV %
1	17.0	4.4
2	50.0	4.1
3	72.0	2.0

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 anticoagulant EDTA. However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Study results

Sample Description	n	n Pos	%
SLE	108	101	93.5
normal human sera	154	10	6.5

		Clinical Diagnosis		
		Pos	Neg	
ORG 590	Pos	101	10	
	Neg	7	144	
	.	108	154	262
Sensitivity:	93.5	%		
Specificity:	93.5	%		
Overall agreement:	93.5	%		

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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 in patient samples should be regarded as recommendations only. Each laboratory should establishe its own ranges according to ISO 15189 or other applicable laboratory guidelines.

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