INSTRUCTION MANUAL

REF 4015

February 18, 2008

Anti-dsDNA

- 96 determinations -

IVD *In vitro* diagnostic device

Enzyme immunoassay for the determination of IgG antibodies to dsDNA in human serum and plasma

Symbol	Meaning	Symbol	Meaning
REF	Catalogue number	LOT	Batch code
	Consult accompanying documents		Manufactured by
	Temperature limitation		Use by
	Consult operating instruction		Biological risk

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INTENDED USE

Anti-dsDNA is used for the quantitative determination of IgG antibodies to double-stranded desoxyribonucleic acid (dsDNA) in human serum for the diagnosis of systemic lupus erythematosus (SLE).

Systemic autoimmune diseases such as SLE are characterized by the appearance of a variety of autoantibodies directed against cell components of the nucleus or plasma.
Although significance and pathological relevance of some autoantibodies are not completely revealed yet, the detection of autoantibodies is widely established and plays an important role in the diagnostics of systemic autoimmune diseases.

SLE has an unknown etiology and is characterized by multiorgan pathology. SLE has a female predominance. The onset of the disease occurs usually during childbearing age.

Antibodies to dsDNA are the hallmark for SLE diagnostics and are included in the diagnostic criteria of the American College of Rheumatology for SLE (1,2).

Generic Assays offers a complete range of serological markers for systemic autoimmune diseases. All assays employ the same assay scheme and predilution maximizing laboratory efficiency.

(1) Tan EM: Antibodies to nuclear antigens (ANA) and their immunobiology and medicine. Adv Immunol 1982 33:167-240
(2) Tan EM, Cohen AS, Fries JF et al.: The 1982 revised criteria for the classification of systemic lupus erythematosus. Athritis Rheum 1982 25:1271-7

PRINCIPLE OF THE TEST

Anti-dsDNA is an enzyme immunoassay for the quantitative determination of IgG antibodies to dsDNA.

The antibodies of the calibrators, control and diluted patient samples react with dsDNA immobilized on the solid phase of microtiter plates. Highly purified dsDNA coated on the microtiter plate guarantees the specific binding of dsDNA IgG antibodies of the specimen under investigation. Following an incubation period of 60 min at room temperature (RT), unbound sample components are removed by a wash step.

The bound IgG antibodies react specifically with anti-human-IgG conjugated to horseradish peroxidase (HRP). Within the incubation period of 30 min at RT, excessive conjugate is separated from the solid-phase immune complexes by the following wash step.

HRP converts the colorless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution into the wells after 15 min at room temperature turning the solution from blue to yellow.

The optical density (OD) of the solution at 450 nm is directly proportional to the amount of specific antibodies bound. The standard curve is established by plotting the antibody concentrations of the calibrators (x-axis) and their corresponding OD values (y-axis) measured. The antibody concentration of the specimen is directly read off the standard curve.

Alternatively, results can be calculated by a semi-quantitative method too using calibrator 2 as cut-off calibrator.

PATIENT SAMPLES

Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can be used, too. Lipaemic, hemolytic or contaminated samples should not be run.

Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at - 20 °C.

Preparation before use

Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

Note: ***Patient samples have to be diluted 1 + 100 (v/v), e.g. 10 µl sample + 1.0 ml sample diluent (C), prior to assay.***

The samples may be kept at 2 - 8 °C for up to three days. Long-term storage requires - 20 °C.

TEST COMPONENTS FOR 96 DETERMINATIONS

A Ag 96	**Microtiter plate**, 12 breakable strips per 8 wells (total 96 individual wells) coated with purified dsDNA	1 vacuum sealed with desiccant
B BUF WASH	**Concentrated wash buffer** sufficient for 1000 ml solution 10x	100 ml concentrate capped white
C DIL	**Sample diluent**	100 ml ready for use capped black
D CONJ	**Conjugate** containing anti-human-IgG- (sheep) coupled with HRP	15 ml ready for use capped red
E SOLN TMB	**Substrate** 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	15 ml ready for use capped blue
F H2SO4	**Stop solution** 0.25 M sulfuric acid 0.25M	15 ml ready for use capped yellow
0 - 4 CAL	**Calibrators** (human serum diluted) conc.: 1, 10, 30, 100, 300 IU/ml	1 ml each ready for use
P CONTROL	**Control** (human serum diluted) conc.: see leaflet enclosed	1 ml ready for use

Materials required in addition

- micropipette 100 - 1000 µl
- micropipette 10 - 100 µl
- multi-channel pipette 50 - 200 µl
  trough for multi-channel pipette
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- glassware
- microplate reader with optical filters for 450 nm and 620 nm or 690 nm
- distilled or de-ionized water

Size and storage

Anti-dsDNA has been designed for 96 determinations.

The expiry date of each component is reported on its respective label that of the complete kit on the box labels.

Upon receipt, all components of the Anti-dsDNA have to be kept at 2 - 8 °C, preferably in the original kit box.

After opening all kit components are stable for at least 2 months, provided proper storage.

Preparation before use

Allow all components to reach room temperature prior to use in the assay.

The microtiter plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed microplate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of wash solution by diluting the concentrated wash buffer 10 times (1 + 9) with de-ionized or distilled water. For example, dilute 8 ml of the concentrate with 72 ml of distilled water per strip. The wash solution prepared is stable at 2 - 8 °C up to 30 days.
Crystallization of the undiluted washing buffer may occur and can be dissolved by warming up at 37°C.

Avoid exposure of the TMB substrate solution to light!

ASSAY PROCEDURE

- **Dilute patient sera with sample diluent (C) 1 + 100 (v/v), e.g. 10 µl serum + 1.0 ml sample diluent (C)**.
- **Avoid any time shift during pipetting of reagents and samples.**

1. Bring all reagents to room temperature (18-25°C) before use. Mix gently without causing foam.
2. Dispense
   100 µl calibrators 0 (optionally) 1 - 4 or
   100 µl calibrator 2 (semi-quantitative)
   100 µl control (P)
   100 µl diluted patient samples
   into the respective wells.
3. Cover plate, incubate **60 min** at room temperature (18…25°C).
4. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
5. Add **100 µl** of conjugate (D) solution to each well.
6. Cover plate, incubate **30 min** at room temperature (18…25°C).
7. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
8. Add **100 µl** of substrate (E) to each well.
9. Incubate **15 min protected from light** at room temperature (18…25°C).
10. Add **100 µl** of stop solution (F) to each well and mix gently.
11. Read the OD at **450 nm** versus 620 or 690 nm within 30 min after adding the stop solution.

DATA PROCESSING

Anti-dsDNA allows both quantitative and qualitative (semi-quantitative) evaluations of results.

Semi-Quantitative evaluation

Results are interpreted by calculating the binding index (BI) using calibrator 2 (**30 IU / ml**) as **cut-off control** according to the following formula:

$$BI = OD_{sample} / OD_{Calibrator\ 2\ (30\ IU/ml)}$$

This calculation can be done by the integrated evaluation software of the microplate reader used, too.

Quantitative evaluation

The standard curve is established by plotting the mean OD-values of the calibrators 0 (optionally) and 1 - 4 on the ordinate, y-axis, (lin. scale) versus their respective dsDNA-IgG concentrations on the abscissa, x-axis, (log. scale). Anti-dsDNA concentrations of the unknown samples are directly read off in IU/ml against the respective OD values.

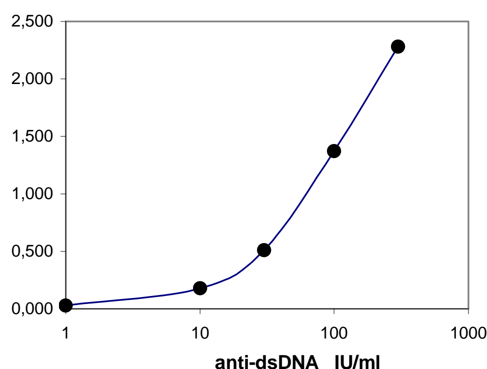
Anti-dsDNA may be used also with Computer Assisted Analysis using software able to plot log/lin curves with four-parameter fit.

Using the recommended dilution of 1 + 100 (v/v) for patient's sera, no correction factor is necessary, as all other components of the kit are supplied accordingly.

Example of Typical Assay Results

well	OD (a)	OD (b)	OD (mean)	IU/ml
Calibrator **0**	0.028	0.029	0.029	**1**
Calibrator **1**	0.175	0.184	0.180	**10**
Calibrator **2**	0.504	0.518	0.511	**30**
Calibrator **3**	1.350	1.394	1.372	**100**
Calibrator **4**	2.271	2.289	2.280	**300**
Patient **1**	1.179	1.159	1.169	**75**

TYPICAL STANDARD CURVE



Specimens with an OD > calibrator 4, should be diluted with dsDNA antibody negative serum and tested again. Results are multiplied with the dilution factor chosen.

Test validity

The test run is valid if:

- the mean OD of the calibrator 1 is ≤ 0.5
- the mean OD of the calibrator 4 is ≥ 1.2

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

REFERENCE VALUES

Anti-dsDNA	IU/ml	BI
positive	> 35	> 1.2
negative	< 30	< 1.0
grey zone	30 - 35	1.0 – 1.2

Specimens with concentrations detected in the grey zone should be tested again.

It is recommended that each laboratory establishes its own normal and pathological reference ranges for serum anti-dsDNA levels, as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

Limitations of Method

Healthy individuals should be tested negative by the Anti-dsDNA. However, dsDNA autoantibody positive apparently healthy persons do occur. Furthermore, autoimmune patients suffering from rheumatoid arthritis, Sjögren's syndrome or autoimmune hepatitis may exhibit positive dsDNA autoantibodies levels.
Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

CHARACTERISTIC ASSAY DATA

Calibration

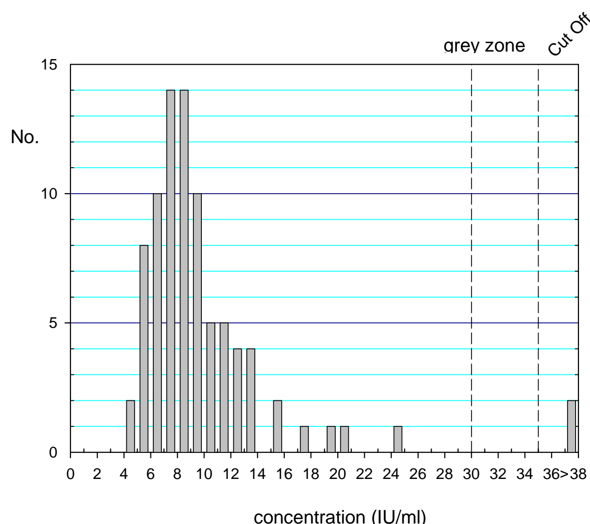
Anti-dsDNA is calibrated against the international reference serum preparation WO/80 (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam).

Sensitivity

Testing a group of 44 clinically defined SLE patients a sensitivity of > 95% was determined in Anti-dsDNA in compariosn to another commercially available anti-dsDNA Elisa.

Specificity

Testing 84 non-selected sera from blood donors a specificity of 98% was determined in Anti-dsDNA.



Precision

Intraassay (n=8)		Interassay (n=4x8)	
IU/ml	CV (%)	IU/ml	CV (%)
180	3.3	157	7.6
92	3.9	130	8.4
67	4.5	92	5.0
41	2.4	41	8.6

INCUBATION SCHEME

Anti-dsDNA (4015)

Dilute patients sample	**10 µl serum + 1.0 ml sample diluent (C)**

			Calibrators	Control	Patient sera
1	Bring all ready for use reagents to room temperature (18-25°C) before use.				
2	Pipette	Calibrators (0 - 4) or Calibrator 2	100 µl		
		Control (P)		100 µl	
		prediluted 1 + 100 patient sera			100 µl
3	Incubate		60 minutes at room temperature		
4	Wash		Decant, Dispense 3 x 300 µl (made of B)		
5	Pipette conjugate (D)		100 µl	100 µl	100 µl
6	Incubate		30 minutes at room temperature		
7	Wash		Decant, Dispense 3 x 300 µl (made of B)		
8	Pipette substrate (E)		100 µl	100 µl	100 µl
9	Incubate protected from light		15 minutes at room temperature		
10	Pipette stop solution (F)		100 µl	100 µl	100 µl
11	Measure 450 nm versus 620 (690) nm				

SAFETY PRECAUTIONS

- **This kit is for in vitro use only**. Follow the working instructions carefully. GA GENERIC ASSAYS GmbH and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated. The kit should be performed by trained technical staff only.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts of Thimerosal (< 0.1 % w/v) and Kathon (1.0 % v/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed:

- Do not smoke, eat or drink while handling kit material,
- Always use protective gloves,
- Never pipette material by mouth,
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.