



## **Anti-ds-DNA-G ELISA**

For the quantitative and qualitative determination of IgG antibodies against double-stranded DNA (dsDNA) in human serum

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 35-DSGHU-E01  
Size: 96 wells  
Version: 009: 2004-11-11 – ALPCO 11/19/09

### **ALPCO Diagnostics**

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## 1. Intended Use

**Anti-ds-DNA-G ELISA** is a solid phase enzyme linked immunosorbent assay with human recombinant double-stranded DNA (dsDNA) for the quantitative and qualitative detection of IgG antibodies against dsDNA in human serum. Anti-dsDNA antibodies mainly recognize the phosphate units of DNA; these autoantibodies also bind single stranded DNA (ssDNA). To ensure correct quantitation of anti-dsDNA antibodies the antigen used in the kit has been proven to be free of contamination with ssDNA. The assay is an aid in the study of systemic lupus erythematosus (SLE).

## 2. Application and Principle of the Assay

Antibodies binding DNA belong to the group of anti-nuclear antibodies (ANA) that have been observed in several autoimmune diseases. Antibodies reacting with native double-stranded (ds) DNA are regarded as being specific for systemic lupus erythematosus (SLE) and have been observed in approximately 50-80% of patients.

Antibodies against dsDNA are found during active phases of SLE. The amount of the serum concentration is positively correlated with the severity of the disease. Thus, detection of these autoantibodies is important for the study of SLE.

Most patients with SLE display IgG class antibodies against dsDNA. These autoantibodies are associated with lupus nephritis. Approximately 30% of the SLE patients additionally develop IgA class anti-dsDNA antibodies. It has been suggested that the presence of these IgA class anti-dsDNA antibodies may define a certain subset of SLE patients. Studies demonstrated the association of this subclass with certain parameters of disease activity, such as elevated erythrocyte sedimentation rate, or the consumption of complement component C3, as well as the clinical parameters of cutaneous vasculitis, acral necrosis, and erythema. No association was found for nephritis and arthritis.

IgM class anti-dsDNA antibodies were found in 52% of the sera from patients with SLE. In contrast to IgG and IgA class autoantibodies, the subclass IgM antibodies do not correlate with disease activity. However, a highly significant negative correlation between IgM anti-dsDNA antibodies and lupus nephritis, including its laboratory parameters, was demonstrated. Therefore, IgM class anti-dsDNA antibodies may indicate a subset of lupus patients being protected against the risk of developing nephritis.

### ***Principle of the test***

Serum samples diluted 1:101 are incubated in the microplates coated with the specific antigen. Antibodies, if present in the sample, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards, anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples in the microplates. Unbound conjugate is washed off in the following step. Addition of TMB substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The rate of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective antibodies in the sample.

### 3. Kit Contents

#### ***To be diluted:***

5X Sample Buffer	1 vial, 20 ml - 5X concentrated (capped white: yellow solution) Containing: Tris, NaCl, BSA, sodium azide < 0.1% and thimerosal 0.01% (preservatives)
50X Wash Buffer	1 vial, 20 ml - 50X concentrated (capped white: green solution) Containing: Tris, NaCl, Tween, sodium azide < 0.1% and thimerosal 0.01% (preservatives)

#### ***Ready to use:***

Negative Control	1 vial, 1.5 ml (capped green: yellow solution) Containing: Human serum (diluted), sodium azide < 0.1% (preservative)
Positive Control	1 vial, 1.5 ml (capped red: yellow solution) Containing: Human serum (diluted), sodium azide < 0.1% (preservative)
Cut-off Control	1 vial, 1.5 ml (capped blue: yellow solution) Containing: Human serum (diluted), sodium azide < 0.1% (preservative)
Calibrators	6 vials, 1.5 ml each 0, 3, 10, 30, 100, 300 IU/ml (color increasing with concentration: yellow solutions) Containing: Human serum (diluted), sodium azide < 0.1% (preservative)
Conjugate	1 vial, 15 ml IgG (capped blue: blue solution) Containing: Anti-human immunoglobulins conjugated to horseradish peroxidase, thimerosal 0.01% (preservative)
TMB Substrate	1 vial, 15 ml (capped black) Containing: Stabilized TMB/H <sub>2</sub> O <sub>2</sub>
Stop Solution	1 vial, 15 ml (capped white: colorless solution) Containing: 1 M hydrochloric acid
Microtiterplate	12 x 8 well strips with breakapart microwells Coating, see paragraph 1

#### ***Materials required but not provided:***

Microtiter plate reader 450 nm reading filter and optional 620 nm reference filter (600-690 nm). Glassware, test tubes for dilutions. Vortex mixer, precision pipettes (10; 100; 200; 500; 1,000 µl) or multipipette. Microplate washing device (multi-channel pipette or automated system), absorbent paper.

These tests are designed to be used with purified water according to the definition of the United States Pharmacopeia (USP 26 - NF 21) and the European Pharmacopeia (Eur.Ph. 4th ed.).

### 4. Storage and Shelf Life

Store all reagents and the microplate at 2-8°C/35-46°F, in their original containers. Once prepared, reconstituted solutions are stable for at least 1 month at 4°C/39°F. ***Reagents and the microplate shall be used only within the expiry date indicated on each component. Avoid intense exposure of TMB substrate to light. Store microplates in designated foil, including the desiccant, and seal tightly.***

## 5. Precautions of Use

### 5.1 Health hazard data

This product is for research use only. Although this product is not considered particularly toxic or dangerous in conditions of normal use, refer to the following for maximum safety:

#### ***Recommendations and precautions***

This kit contains potentially hazardous components. Though kit reagents are not classified as being irritants for eyes and skin it is recommended to avoid contact with eyes and skin and wear disposable gloves.

Do not smoke, eat, or drink when manipulating the kit.

Do not pipette by mouth.

All human source material used in kit reagents (e.g., controls, standards) has been tested by approved methods and found negative for HBsAg, Hepatitis C, and HIV 1. However, no test can guarantee the absence of viral agents in such material completely. Handle kit controls, standards, and samples as if capable of transmitting infectious diseases and according to national requirements.

### 5.2 General directions for use

Do not mix or substitute reagents or microplates from different lot numbers. This may lead to variations in the results.

Allow all components to reach room temperature (20-26°C/64-78.8°F) before use, mix well, and follow the recommended incubation scheme for an optimum performance of the test.

Never expose components to higher temperature than 37°C/98.6°F.

Always pipette substrate solution with brand new tips only. Protect this reagent from light. Never pipette conjugate with tips used previously with other reagents.

## 6. Sample Collection, Handling, and Storage

It is preferable to use newly collected serum samples. Blood withdrawal must follow national requirements.

Do not use icteric, lipemic, hemolyzed, or bacterially contaminated samples. Sera with particles should be cleared by low speed centrifugation (<1,000 x g). Blood samples should be collected in clean, dry, and empty tubes. After separation, the serum samples should be used immediately, stored tightly closed at 2-8°C/35-46°F for up to three days, or frozen at -20°C/-4°F for longer periods.

## 7. Assay Procedure

### 7.1 Preparations prior to pipetting

Dilute concentrated reagents:

Dilute the concentrated sample buffer 1:5 with deionized water (e.g., 20 ml plus 80 ml). Dilute the concentrated wash buffer 1:50 with deionized water (e.g., 20 ml plus 980 ml).

#### **Samples:**

Dilute serum samples 1:101 with sample buffer (1X) (e.g., 1,000 µl sample buffer (1X) + 10 µl serum). Mix well!

**Washing:**

Prepare 20 ml of diluted wash buffer (1X) per 8 wells or 200 ml for 96 wells (e.g., 4 ml concentrate + 196 ml deionized water).

**Automated washing:**

Consider excess volumes required for setting up the instrument and dead volume of robot pipette.

**Manual washing:**

Discard liquid from wells by inverting the plate. Knock the microwell frame with wells downside vigorously on clean absorbent paper. Pipette 300 µl of diluted wash buffer into each well, wait for 20 seconds. Repeat the whole procedure twice again.

**Microplates:**

Calculate the number of wells required for the test. Remove unused wells from the frame, replace and store in the provided plastic bag, together with desiccant, seal tightly (2-8°C/35-46°F).

**7.2 Work flow**

For pipetting scheme see Annex A.

- Pipette 100 µl of each diluted serum sample into the designated microwells.
- Pipette 100 µl of the calibrators OR the cut-off control and negative and positive controls into the designated wells.
- Incubate for 30 minutes at room temperature (20-26°C/64-78.8°F).
- Wash 3X with 300 µl of wash buffer (diluted 1:50).
- Pipette 100 µl of conjugate into each well.
- Incubate for 15 minutes at room temperature (20-26°C/64-78.8°F).
- Wash 3X with 300 µl of wash buffer (diluted 1:50).
- Pipette 100 µl of TMB substrate into each well.
- Incubate for 15 minutes at room temperature (20-26°C/64-78.8°F), in the dark.
- Pipette 100 µl of stop solution into each well, using the same order as when the substrate was pipetted.
- Incubate for a minimum of 5 minutes.
- Agitate plate carefully for 5 seconds.
- Read absorbance at 450 nm (optionally 450/620 nm) within 30 minutes.

**8. Quantitative and Qualitative Interpretation**

For **quantitative interpretation** establish the standard curve by plotting the **optical density (OD) of each calibrator (y-axis)** with respect to the corresponding concentration values in **IU/ml (x-axis)**. Log/lin coordinates and 4-Parameter Fit are recommended for best results. From the OD of each sample, read the corresponding antibody concentrations expressed in **IU/ml**.

<b>Normal Range</b>	<b>Positive Results</b>
$\leq 15$ IU/ml	$> 15$ IU/ml

### Example of a standard curve

It is recommended to pipette calibrators in parallel for each run.

Calibrators IgG	OD 450/620 nm	CV% (Variation)
0 IU/ml	0.036	2.9
3 IU/ml	0.176	2.3
10 IU/ml	0.314	2.9
30 IU/ml	0.618	2.9
100 IU/ml	1.312	0.1
300 IU/ml	2.076	0.7

### Example of calculation

Sample	Replicate (OD)	Mean (OD)	Result (IU/ml)
S 01	0.799/0.744	0.772	40.3
S 02	1.404/1.393	1.399	119.5

For lot specific data, see enclosed quality control leaflet. Medical laboratories might perform an in-house Quality Control by using own controls and/or internal pooled sera, as foreseen by EU regulations.

**This kit is for research use only. It is not for use in diagnostic procedures.**

Each laboratory should establish its own normal range based upon its own techniques, controls, equipment, and sample population according to its established procedures.

For **qualitative interpretation** read the optical density of the cut-off control and the samples. Compare the sample's OD with the OD of the cut-off control. All samples with higher ODs are considered positive.

**Negative:**  $OD_{\text{sample}} < OD_{\text{cut-off}}$

**Positive:**  $OD_{\text{sample}} > OD_{\text{cut-off}}$

## 9. Technical Data

<b>Sample material:</b>	serum
<b>Sample volume:</b>	10 µl of sample diluted 1:101 with 1X sample buffer
<b>Total incubation time:</b>	60 minutes at 20-26°C/64-78.8°F
<b>Calibration range:</b>	0-300 IU/ml
<b>Analytical sensitivity:</b>	1.0 IU/ml
<b>Storage:</b>	at 2-8°C/35-46°F use original vials, only
<b>Number of determinations:</b>	96 tests

## 10. Performance Data

### 10.1 Analytical sensitivity

The analytical sensitivity of this kit has been found to be 1.0 IU/ml.

### 10.2 Specificity and sensitivity

The microplate is coated with **recombinant human dsDNA**. No crossreactivities to other autoantigens have been found. Antibodies targeting dsDNA show a diagnostic sensitivity of 85% for SLE thus allowing the differentiation from other inflammatory rheumatic diseases. Combining all three subclasses results in a diagnostic sensitivity of this assay of 90%.

### 10.3 Linearity

Chosen sera have been tested with this kit and found to dilute linearly. However, due to the heterogeneous nature of human autoantibodies there might be samples that do not follow this rule.

Sample No.	Dilution Factor	measured concentration (IU/ml)	expected concentration (IU/ml)	Recovery (%)
1	1 / 100	42.9	43.2	99.3
	1 / 200	20.4	21.6	99.4
	1 / 400	9.3	10.8	86.1
	1 / 800	4.9	5.4	90.7
2	1 / 100	179.4	176.0	101.9
	1 / 200	86.4	88.0	98.2
	1 / 400	41.8	44.0	95.0
	1 / 800	19.8	22.0	90.0

### 10.4 Precision

To determine the precision of the assay, the variability (intra- and inter-assay) was assessed by examining its reproducibility on three serum samples selected to represent a range that spans the standard curve.

Intra-assay		
Sample No.	Mean (IU/ml)	CV (%)
1	> 300.0	2.1
2	138.0	2.4
3	26.4	4.7

Inter-assay		
Sample No.	Mean (IU/ml)	CV (%)
1	463.3	2.6
2	171.6	2.3
3	58.2	4.6

## 10.5 Calibration

This assay is calibrated against WHO/80. The results are expressed in IU/ml.

## 11. Literature

1. **Tan EM, Cohen AS, Fries JF, et al. (1982).**  
*Revised criteria for the classification of systemic lupus erythematosus.*  
Arthritis Rheumatism 25: 1271-1277.
  
2. **Witte T, Hartung K, Matthias T, Sachse C, Fricke M, Deicher H, Kalden JR, Lakomek HJ, Peter HH, Schmidt RE (1998).**  
*Association of IgA anti-dsDNA antibodies with vasculitis and disease activity in system lupus erythematosus.*  
Rheumatol Int 18: 63-69.
  
3. **Witte T, Hartung K, Matthias T, Sachse C, Fricke M, Deicher H, Kalden JR, Lakomek HJ, Peter HH, Schmidt RE, SLE study group (1998).**  
*IgM anti-dsDNA antibodies in systemic lupus erythematosus: negative association with nephritis.*  
Rheumatol Int 18: 85-91.

## ANNEX A: Pipetting scheme

It is suggested to pipette the calibrators, controls, and samples as follows: for **quantitative interpretation** use the calibrators to establish a standard curve, for **qualitative interpretation** use the cut-off control.

	for <b>quantitative interpretation</b> use calibrators to establish a standard curve						for <b>qualitative interpretation</b> use cut-off control					
	1	2	3	4	5	6	7	8	9	10	11	12
<b>A</b>	CalA	CalE	S1				NC	S2				
<b>B</b>	CalA	CalE	S1				NC	S2				
<b>C</b>	CalB	CalF	S2				CC	S3				
<b>D</b>	CalB	CalF	S2				CC	S3				
<b>E</b>	CalC	PC	S3				PC	...				
<b>F</b>	CalC	PC	S3				PC	...				
<b>G</b>	CalD	NC	...				S1	...				
<b>H</b>	CalD	NC	...				S1	...				

CalA: calibrator A, CalB: calibrator B, CalC: calibrator C, CalD: calibrator D, CalE: calibrator E, CalF: calibrator F  
 PC: positive control      NC: negative control      CC: cut-off control  
 S1: sample 1              S2: sample 2              S3: sample 3