Instructions for Use

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dsDNA-Ab IgA ELISA

Cat.-No. : RE70201

Size : 96
(Break apart)

Storage : 2 - 8 °C

Enzyme immunoassay for the quantitative detection of IgA antibodies against dsDNA in human serum.

- For in-vitro diagnostic use only -

IBL IMMUNO-BIOLOGICAL LABORATORIES
FLUGHAFENSTRASSE 52A, D-22335 HAMBURG PHONE: + 49- (0) 40-53 28 91-0, FAX: -11
E-MAIL: IBL@IBL-HAMBURG.COM INTERNET: HTTP://WWW.IBL-HAMBURG.COM

Instruction manual

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1. Intended Use

dsDNA-Ab IgA ELISA is a solid phase enzyme immunoassay with human recombinant double-stranded DNA (dsDNA) for the quantitative detection of antibodies against dsDNA in human serum.
Anti-dsDNA antibodies mainly recognize the phosphat units of the DNA, thus these autoantibodies also bind single stranded DNA (ssDNA). To ensure correct quantitation of anti-dsDNA antibodies the used antigen has been proven to be free of contamination with ssDNA.
The assay is a tool in the differential diagnosis of systemic lupus erythematosus (SLE).

2. Clinical Application and Principle of the Assay

Antibodies binding to 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 the 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 diagnosis and the clinical monitoring of SLE. Consequently it has been established as 1 of the 11 ACR-criteria for the diagnosis 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 develop IgA class anti-dsDNA antibodies, additionally. There have been suggestions that the presence of these IgA class anti-dsDNA antibodies may define a certain subset of SLE patients. Indeed studies demonstrated the association of this subclass with certain parameters of the 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. While 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. Patient‘s antibodies, if present in the specimen, 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 patient sample.

3. Kit Contents

To be reconstituted:

5x Sample Buffer 1 vial, 20 ml - 5x concentrated (capped white: yellow solution)
Containing: Tris, NaCl, BSA, sodium azide (preservative)

50x Wash Buffer 1 vial, 20 ml - 50x concentrated (capped white: green solution)
Containing: Tris, NaCl, Tween, sodium azide (preservative)

Ready to use:

Negative Control 1 vial, 1.5 ml (capped green: yellow solution)
Containing: Human serum (diluted), sodium azide (preservative)

Positive Control 1 vial, 1.5 ml (capped red: yellow solution)
Containing: Human serum (diluted), sodium azide (preservative)

Calibrators 6 vials, 1.5 ml each 0, 3, 10, 30, 100, 300 U/ml
(color increasing with concentration : yellow solutions)
Containing: Human serum (diluted), sodium azide (preservative)

Conjugate 1 vial,15 ml IgA (capped red: red solution)
Containing: Anti-human immunoglobulins conjugated to horseradish peroxidase

TMB Substrate 1 vial, 15 ml (capped black)
Containing: Stabilized TMB/H_2O_2

Stop Solution 1 vial, 15 ml (capped white: colorless solution)
Containing: 1M Hydrochloric Acid

Microtiterplate 12x8 well strips with breakaway microwells
Coating see paragraph 1

Material required but not provided:

Microtiter plate reader 450 nm reading filter and optional 620 nm reference filter (600-690 nm). Glass ware, test tubes for dilutions. Distilled water. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or multipipette. Microplate washing device (multichannel pipette or automated system), adsorbent paper.

4. Storage and Shelf Life

Store all reagents and the microplate at 2-8°C, in their original containers. Once prepared, reconstituted solutions are stable for 1 month at 4°C, at least. ***Reagents and the microplate shall be used within the expiry date indicated on each component, only. Avoid intense exposure of TMB solution 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 IN VITRO DIAGNOSTIC USE only. Thus, only staff trained and specially advised in methods of in vitro diagnostics shall perform the kit. 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. Reagents may be irritating to eyes and skin thus 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 for some reagents of this kit (controls, standards e.g.) 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. Thus handle kit controls, standards and patient samples as if capable of transmitting infectious diseases.

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) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test. Always pipet substrate solution with clean tips only. Protect this reagent from light. Never pipette conjugate with tips used with other reagents prior.

6. Sample Collection, Handling and Storage

Use preferentially freshly collected serum samples. Do not use icteric, lipemic, hemolysed or bacterially contaminated samples. Sera with particles should be cleared by low speed centrifugation. Blood samples should be collected in dry tubes. After separation, the serum samples should be used immediately, respectively stored at 2-8°C up to three days, or frozen at -20°C for longer periods.

7. Assay Procedure

7.1 Preparations prior to pipetting

Dilute concentrated reagents:

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

Samples

Dilute serum samples 1:101 with sample buffer (1x)
e.g. 1000 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 plus 196 ml distilled 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 adsorbent 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).

7.2 Work flow

Pipette 100 µl of each patient‘s diluted serum into the designated microwells.
Pipette 100 µl calibrators OR cut-off control and negative and positive controls into the designated wells.
Incubate for 30 minutes at room temperature (20-26°C).
Wash 3x with 300 µl washing buffer (diluted 1:50).
Pipette 100 µl conjugate into each well.
Incubate for 15 minutes at room temperature (20-26°C).
Wash 3x with 300 µl washing buffer (diluted 1:50).
Pipette 100 µl TMB substrate into each well.
Incubate for 15 minutes at room temperature (20-26°C), in the dark.
Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.
Incubate 5 minutes minimum.
Agitate plate carefully for 5 sec.
Read absorbance at 450 nm (optionally 450/620 nm) within 30 minutes.

8. Quantitative Interpretation

Establish the standard curve by plotting the **optical density (O.D.) of each calibrator (y-axis)** with respect to the corresponding concentration values in **U/ml (x-axis)**. For best results we recommend log/lin coordinats and 4-Parameter Fit. From the O.D. of each sample, read the corresponding antibody concentrations expressed in **U/ml.**

Normal Range	**Positive Results**
< 15 U/ml	**> 15 U/ml**

Example of a standard curve

We recommend pipetting calibrators in parallel for each run.

Calibrators IgA	***O.D. 450/620 nm***	***CV %***
0 U/ml	0.038	2.7
3 U/ml	0.148	1.9
10 U/ml	0.323	1.1
30 U/ml	0.596	2.9
100 U/ml	1.232	1.1
300 U/ml	2.139	0.2

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. ***Do not use this example for interpreting patients results!***

9. Technical Data

Sample material: serum

Sample volume: 10 µl of sample diluted 1:101 with 1x sample buffer

Total incubation time: 60 minutes at room temperature (20-26°C)

Calibration range: 0-300 U/ml

Analytical sensitivity: 1.0 U/ml

Storage: at 2-8°C use original vials, only

Number of determinations: 96 tests

10. Performance Data

10.1 Analytical sensitivity

The analytical sensitivity of this kit has been found at 1.0 U/ml.

10.2 Specificity

The microplate is coated with **recombinant human dsDNA**.

No crossreactivities to other autoantigens have been found.

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.

10.4 Calibration

Due to the lack of international reference calibration this assay is calibrated in arbitrary units (U/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 systemic lupus erythematosus.
   Rheumatol Int 18: 63-69.

3. **Witte T, Hartung K, Sachse C, Matthias T, 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.

Pipetting Scheme

		Calibrators (A-F)	Controls	Samples
Pipette	Calibrators (A-F)	**100 µl each**		
Pipette	Controls		**100 µl each**	
Pipette	Prediluted samples (1:101)			**100 µl each**
Incubate		***30 min at room temperature (20-26°C)***		
Decant		***Wash 3x with 300 µl of wash buffer (1x)***		
Pipette	Conjugate	**100 µl**	**100 µl**	**100 µl**
Incubate		***15 min at room temperature (20-26°C)***		
Decant		***Wash 3x with 300 µl of wash buffer (1x)***		
Pipette	Substrate	**100 µl**	**100 µl**	**100 µl**
Incubate		***15 min at room temperature (20-26°C), in the dark.***		
Pipette	Stop Solution	**100 µl**	**100 µl**	**100 µl**
Incubate		***5 min at room temperature (20-26°C)***		

Agitate plate for 5 seconds and read OD at λ450 nm (optionally λ450/620 nm) within 30 minutes. Resulting color is stable for 30 minutes, at least.