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User's Manual

dsDNA Antibody IgA ELISA Kit

REF **DEIA1680**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

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

General Description

Anti-ds-DNA-A ELISA is a solid phase enzyme immunoassay with human recombinant double-stranded DNA (dsDNA) for the quantitative and qualitative detection of IgA antibodies against dsDNA in human serum.

Anti-dsDNA antibodies mainly recognize the phosphate 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.

Principles of Testing

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 intensity 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.

Reagents And Materials Provided

1. Sample Buffer (5×), 1×20ml
2. Wash Buffer (50×), 1×20ml
3. Negative Control, 1×1.5ml
4. Positive Control, 1×1.5ml
5. Cut-off Calibrator, 1×1.5ml
6. Calibrators, 6×1.5ml. 0, 3, 10, 30, 100, 300 U/ml
7. Conjugate IgA, 1×15ml
8. TMB Substrate, 1×15ml
9. Stop Solution, 1×15ml
10. 12×8 well Microtiter plate strips

Materials Required But Not Supplied

Microtiter plate reader 450 nm reading filter and recommended 620 nm reference filter (600-690 nm).

Glass ware (cylinder 100-1000ml), test tubes for dilutions.

Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or adjustable multipipette (100-1000µl).

Microplate washing device (300 µl repeating or multichannel pipette or automated system), adsorbent paper.

Our tests are designed to be used with purified water.

Storage

Store all reagents and the microplate at 2-8°C/35-46°F, in their original containers. Once prepared, reconstituted solutions are stable at 2-8°C/35-46°F for 1 month. 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.

Specimen Collection And Preparation

Sample Collection, Handling and Storage

Use preferentially freshly 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 (<1000 x g). Blood samples should be collected in clean, dry and empty tubes.

After separation, the serum samples should be used during the first 8 hours, respectively stored tightly closed at 2-8°C/35-46°F up to 48 hours, or frozen at -20°C/-4°F for longer periods

Plate Preparation

It is recommended to pipette calibrators, controls and samples as follows:

For Quantitative interpretation

	1	2	3	4...
A	Cal A	Cal A	S1	S1
B	Cal B	Cal B	S2	S2
C	Cal C	Cal C	S3	S3
D	Cal D	Cal D	S4	S4
E	Cal E	Cal E	S5	S5
F	Cal F	Cal F	S6	S6
G	PC	PC	S7	S7
H	NC	NC	S8	S8

For Qualitative interpretation

	1	2	3	4...
A	NC	NC	S6	S6
B	CC	CC	S7	S7
C	PC	PC		
D	S1	S1		
E	S2	S2		
F	S3	S3		
G	S4	S4		
H	S5	S5		

Cal A: calibrator A
Cal B: calibrator B
Cal C: calibrator C
Cal D: calibrator D
Cal E: calibrator E
Cal F: calibrator F
NC: negative control
CC: cut-off calibrator
PC: positive control
S1: sample 1
S2: sample 2
S3: sample 3

Reagent Preparation

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).

To avoid mistakes it is suggested to mark the cap of the different calibrators.

Samples:

Dilute serum samples 1:101 with sample buffer (1×)

e.g. 1000 µl sample buffer (1×) + 10 µl serum. Mix well!

Washing:

Prepare 20 ml of diluted wash buffer (1×) 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/35-46°F).

Assay Procedure

1. Ensure preparations from step Reagent Preparation above have been carried out prior to pipetting.
2. Use the following steps in accordance with quantitative/qualitative interpretation results desired:
3. Pipette into the designated wells as described in chapter 7.2 above, 100 µl of either:

   a. Calibrators (CAL.A to CAL.F) for QUANTITATIVE or

   b. Cut-off Calibrator (CC) for QUALITATIVE interp. and 100 µl of each of the following: Negative control (NC) and Positive control (PC), and diluted serum sample (S1, S2...)
4. Incubate for 30 minutes at 20-32°C/68-89.6°F.
5. Wash 3× with 300 µl washing buffer (diluted 1:50).
6. Pipette 100 µl conjugate into each well.
7. Incubate for 30 minutes at 20-32°C/68-89.6°F.
8. Wash 3× with 300 µl washing buffer (diluted 1:50).
9. Pipette 100 µl TMB substrate into each well.
10. Incubate for 30 minutes at 20-32°C/68-89.6°F, protected from intense light.
11. Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.
12. Incubate 5 minutes minimum.
13. Agitate plate carefully for 5 seconds.
14. Read absorbance at 450 nm (recommended 450/620 nm) within 30 minutes.

Calculation

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 U/ml (x-axis). For best results we recommend log/lin coordinates and 4-Parameter Fit. From the OD of each sample, read the corresponding antibody concentrations expressed in U/ml.

Normal Range	Equivocal Range	Positive Results
< 12 U/ml	12 - 18 U/ml	>18 U/ml

For qualitative interpretation read the optical density of the cut-off calibrator and the samples. Compare sample´s OD with the OD of the cut-off calibrator. For qualitative interpretation we recommend to consider sera within a range of 20% around the cut-off value as equivocal. All samples with higher ODs are considered positive, samples with lower ODs are considered negative.

Negative: OD sample < 0.8 × OD cut-off

Equivocal: 0.8 x OD cut-off ≤ OD sample ≤ 1.2 × OD cut-off

Positive: OD sample > 1.2 × OD cut-off

Typical Standard Curve

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

Detection Range

0-300 U/ml

Sensitivity

1.0 U/ml