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User's Manual

Human DNA (Anti-dsDNA Antibody) ELISA Kit

REF **IVDIA1002-FA**

Σ **96T**

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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

The Human DNA (Anti-dsDNA Antibody) ELISA Kit is a quantitative immunoassay for the determination of IgG antibodies against double-stranded desoxyribonucleic acid (dsDNA) in human serum. The kit is for research use only and is not intended for diagnostic use.

General Description

Systemic autoimmune diseases such as SIE 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 SIE diagnostics and are included in the diagnostic criteria of the American College of Rheumatology for SLE.

Principles of Testing

The ELISA (Enzyme Linked Immunosobent Assay) is an immunoassay for the determination of specific antibodies. The strips of the microtiter plate are coated with test-specific antigens. If antibodies are present in the patient's sample, they bind to the antigens. A secondary antibody conjugated with the enzyme peroxidase detects the generated immune complex. A colorless substrate is converted into the colored product. The signal intensity of the reaction product is proportional to the antibody activity in the sample. After stopping the signal intensity of the reaction product is measured photometrically.

Reagents And Materials Provided

1. **Calibrators(0-4):** 5 x 1 ml, white cap, ready-to-use, contains ProClin 950;
   Colored dilutions of human serum; The antibody activities are indicated on the quality control certificate.
2. **Microtiter plate(A):** 12 breakable microtiter strips (ready-to-use), 8 wells per strip, each well coated with purified dsDNA.
3. **Wash buffer (10x)(B):** 1 x 100 ml, white cap, contains ProClin 950.
4. **Sample diluent(C):** 1 x 100 ml, black cap, ready-to-use, contains sodium azide.
5. **Conjugate IgG(D):** 1 x 15 ml, red cap, ready-to-use, contains ProClin 950;
   Colored solution of polyclonal anti-human IgG antibody conjugated to horseradish peroxidase.
6. **Substrate(E):** 1 x 15 ml, blue cap, ready-to-use, 3,3',5,5'-Tetramethylbenzidine.
7. **Stop solution(F):** 1 x 15 ml, yellow cap, ready-to-use, 0.25 M sulfuric acid.

8. **Positive Control(P):** 1 x 1 ml, red cap, ready-to-use, contains ProClin 950, human serum (diluted); The antibody activities are indicated on the quality control certificate.
9. **Negative Control(N):** 1 x 1 ml, green cap, ready-to-use, contains ProClin 950, human serum (diluted); The antibody activities are indicated on the quality control certificate.

Materials Required But Not Supplied

1. Common laboratory equipment
2. Precision pipettes (5-1000 µL), multi-channel pipettes (100-1000 µL) and disposable pipette tips
3. Graduated cylinders (100 -1000 mL)
4. Sample tubes for the preparation of dilutions
5. Vortex mixer or other rotators
6. Microtiter plate washer or wash comb
7. Microtiter plate reader with optical filters for 450 nm and 620 nm or 690 nm
8. Adsorbent paper or paper towel

Storage

Upon receipt, all test components must be stored at 2°C to 8°C, preferably in the original kit box. If stored property in their original containers, all components are stable until their expiry date. All components are stable for at least 2 months after opening when stored properly at 2°C to 8°C.

Specimen Collection And Preparation

1. Sample Material

The use of freshly collected serum from blood taken by venipuncture is recommended. The use of icteric, lipemic, hemolytic or bacterially contaminated samples should be avoided. Insoluble substances must be removed from the sample by centrifugation. Samples must not be thermally inactivated.

2. Sample Dilution

The samples must be diluted 1:101 (e. g. 10 µL + 1000 µL) with sample diluent and mixed thoroughly. Building of foam should be avoided.

3. Sample Storage

Samples may be kept at 2°C to 8°C up to three days. Long-term storage requires -20°C. Repeated freezing and thawing should be avoided. For multiple use, samples should be aliquoted and kept at -20°C.

Plate Preparation

The following pipetting scheme is recommended:

	1	2	3	4
A	CAL 0	Sample 2		
B	CAL 1	Sample 3		
C	CAL 2	Sample 4		
D	CAL 3	Sample 5		
E	CAL 4	…		
F	N	…		
G	P	…		
H	Sample 1	…		

Reagent Preparation

All components including the microtiter plate must be brought to room temperature (RT: 18°C to 25°C) before use for at least 30 min. All liquid components must be mixed gently to ensure homogeneity.

1. **Microtiter Plate:** The microtiter plate is sealed in an aluminium bag. Unused test strips should always be stored refrigerated and protected from moisture with the desiccant in the property sealed aluminum bag. Carefully resealed, the test strips can be used for 8 weeks after opening.
2. **Calibrators:** The calibrators are ready-to-use and must not be diluted any further. Calibrators must be used in each test run.
3. **Controls:** The positive and the negative controls are ready-to-use and must not be diluted any further. Controls must be used in each test run. Laboratories can also validate their own control samples and use them alternatively.
4. **Sample Diluent:** The sample diluent is ready-to-use.
5. **Wash Buffer:** The wash buffer is concentrated and must be diluted 1:10 with distilled water before use (e.g. 100 ml+ 900 ml). A sufficient amount of washing solution must be prepared. The diluted washing solution can be stored at 2°C to 8°C up to 30 days.
6. **Conjugate:** The conjugate is ready-to-use and stable up to 8 weeks after opening when stored at 2°C to 8°C.
7. **Substrate:** The substrate is ready-to-use. Exposure of the substrate solution to strong light should be avoided.
8. **Stop Solution:** The stop solution is ready-to-use.

Assay Procedure

General Information

This product is for research use only. The instructions for use must be carefully read before use. They are valid only for the present product with the given composition and must be strictly followed to ensure reliable test results. Deviations can lead to erroneous test results. Components must not be exchanged by test reagents of different lots or of other manufacturers.

Contamination of reagents must be avoided by use of aseptic techniques when removing aliquots from the vials. After use, reagent vials must be tightly closed with their corresponding caps.

Cross-contamination of samples or reagents can lead to inconsistent test results and must be avoided by use

of consistent pipetting techniques.

Exposure of reagents to strong light must be avoided throughout the entire test procedure and storage.

Insufficient washing will result in poor precision and elevated measurement signals. After each washing step any residual fluid has to be removed completely.

The indicated incubation times and temperatures must be adhered to and significant time shifts during pipetting samples and reagents must be avoided. The microtiter plate should be shortly shaken after addition of reagents.

Protocol

1. **Addition of calibrators, controls and diluted samples:** Add 100 µL ready-to-use calibrators, controls and diluted samples per well.
2. **Incubation:** Cover the plate and incubate for 60 min. at RT.
3. **Wash cycle:** Aspirate the solution and wash 3 times with 300 µL washing solution with at least 5 seconds soaking time each; dry by tapping the microtiter plate on a paper towel to remove any residual droplets.
4. **Addition of conjugate:** Add 100 µL ready-to-use conjugate to each well.
5. **Incubation:** Cover the plate and incubate for 30 min. at RT.
6. **Wash cycle:** Aspirate the solution and wash 3 times with 300 µL washing solution with at least 5 seconds soaking time each; dry by tapping the microtiter plate on a paper towel to remove any residual droplets.
7. **Addition of substrate:** Add 100 µL ready-to-use substrate to each well.
8. **Incubation:** Cover the plate and incubate for 15 min. in the dark at RT.
9. **Addition of Stop Solution:** Add 100 µL ready-to-use stop solution to each well.
10. **Analysis:** Read optical density (OD) at 450 nm versus 620 or 690 nm within 30 min. after stopping the reaction.

Automation: Automated processing of the immunoassays must be performed analogous to manual use and validated by the user.

Quality Control

Test runs are only valid if the following criteria of validity are fulfilled:

- OD CAL 0 < CAL 1 < CAL 2 < CAL 3 < CAL 4

- OD CAL 4 > 1.2

- The negative control must be evaluated negative.

- The positive control must be evaluated positive and present an antibody activity within the validity range indicated on the quality control certificate.

If these criteria are not met, the test is not valid and must be repeated.

In case of an invalid test run, the expiry dates and storage conditions, incubation times and temperatures, and precise calibration of all instruments used should be verified. If no reason for an invalid test run could be identified, please contact the supplier or manufacturer of the product.

Interpretation Of Results

A positive test result indicates the presence of specific antibodies. A negative result indicates the absence of specific antibodies, but does not exclude the possibility of an autoimmune reaction. In case of a borderline test result, a reliable evaluation is not possible.

Typical Standard Curve

For generation of a standard curve, the optical signals (optical density, OD) of the calibrators are plotted against their antibody activities and correlated by a 4-parameter logistic (4 PL) fit. Antibody activities of unknown samples can be derived directly from their optical signals by use of the generated standard curve.

Reference Values

The immunoassay is calibrated using the international reference preparation WO/80 as a reference. Quantitative results are expressed in IU/ml.

The reference ranges are indicated below:

Antibody activity < 30 IU/mL - negative

Antibody activity 30 – 35 IU/mL - borderline

Antibody activity > 35 IU/mL - positive

As a result of different seroprevalences in individual regions, each laboratory should verify the reference ranges by own analysis and adapt, if necessary.

Performance Characteristics

Sensitivity and specificity were assessed by the analysis of 61 serum samples from patients with SLE and 102 serum samples from unselected blood donors using the commercially available anti-dsDNA ELISA of a European manufacturer as a reference.

Sensitivity 94.6 %

Specificity > 99 %

Precision

The precision of test results was assessed by the determination of the intra- and interassay variation by the analysis of multiple samples with different antibody activities.

	Intraassay Precision		Interassay Precision	
	IU/mL	CV (%)	IU/mL	CV (%)
Sample 1	151	3.5	139	10.3
Sample 2	56	6.1	61	6.2
Sample 3	29	4.4	32	7.7

Detection Range

Reliable accuracy, trueness, precision, linearity and recovery of test results have been observed within the measurement range of the assay from the LoQ to the upper calibrator in comprehensive studies. Samples with test results above the upper calibrator should be reported as > max. Samples with test results below the LoQ should be reported as < min. If test results above the upper calibrator are observed, the samples may be tested at a higher dilution. The resulting antibody activity must be multiplied with the additional dilution factor.

Precautions

1. The product is designed exclusively for research use by qualified, authorized and trained personnel. All test components and human samples should be handled with care as potentially hazardous. Good laboratory practices (GLP) and all relevant regulations should be adhered to.
2. In case the product is damaged or product information including labelling is wrong or incorrect, please contact the manufacturer or supplier.
3. This product contains preparations of human and / or animal origin. Any material derived from human body fluids or organs used for the preparation of components were tested and found negative for HBsAg (Hepatitis B-Virus-surface Antigen) and antiHIV as well as anti-HCV antibodies. However, all components and all patient samples should be handled as potentially hazardous in accordance with national laws and appropriate guidelines on biological safety.
4. As the product contains potentially hazardous materials, the following precautions should be followed: Do not smoke, eat or drink while handling kit material or samples. Avoid direct contact to kit material or samples by wearing protective gloves laboratory coat and safety glasses. Never pipette material by mouth. Wipe up spills promptly and wash the affected surface thoroughly with a decontaminant. Wash hands thoroughly after use.
5. Some of the reagents contain ProClin (< 1.0 %) as a preservative, may cause skin sensitization and must not be swallowed or allowed to come into contact with skin or mucosa.
6. Some of the reagents contain sodium azide (< 0.1 %) as a reservative and must not be swallowed or allowed to come into contact with skin or mucosa. The possible formation of heavy metal azides in the drainage has to be prevented by sufficient rinsing with water.
7. The information in the safety data sheet on possible hazards, first aid measures, measures in the event of the unintentional release of large quantities, handling and storage, personal protective equipment, information on disposal as well as information on toxicology must be observed.
8. Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the member state in which the user and/or the patient is established.
9. For decontamination and disposal the recommendations of the CDC as well as the relevant local and national environmental guidelines and regulations should be adhered to. Samples, potentially contaminated materials and infectious waste must be decontaminated, e.g. by autoclaving for 20 min. at 121°C.

Limitations

Autoimmune patients suffering from rheumatoid arthritis, Sjögren's syndrome or autoimmune hepatitis may exhibit positive dsDNA autoantibodies levels.

The interpretation of test results must always be considered in combination with the clinical picture of the patient. The diagnosis should not be based on the results of a sole diagnostic method. All clinical and laboratory findings should be evaluated to state a diagnosis. For confirmation, further investigations should

be carried out.

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