BIOGENIX
INC. PVT. LTD.

Anti-double Stranded DNA (ds-DNA) Antibody Enzyme Immunoassay Test Kit
（ELISA）

INTENDED USE

This kit is a qualitative detection of human serum/plasma of Anti-double Stranded DNA antibody. The kit is suitable for clinical screening and diagnosis. Detection of ds-DNA antibodies is very important for the diagnosis and treatment of lupus erythematosus (SLE). Patients with other connective tissue diseases can also get positive result for anti ds-DNA, and the disease is generally SLE overlap syndrome. Therefore, anti ds-DNA antibody is one of the criteria for the diagnosis of lupus erythematosus.

PRINCIPLE OF THE TEST

This kit uses indirect ELISA principle to detect ds-DNA antibody. Purified ds-DNA antigen is pre-coated on the microplate, the ds-DNA antibody in sample will combine with ds-DNA antigen first, then combine with enzyme-labeled second antibody to form antigen-antibody-anti-antibody complex, and shows blue color in the microplate. This kit is used for the specific detection of ds-DNA antibody in human serum/plasma.

COMPONENTS

Materials provided with the kit:

	96T		48T	
Coated Microtiter Plate	1 bag	12*8	1 bag	12*4
Conjugate	1 vial	6.5 mL	1 vial	3.5 mL
Sample Diluent	1 vial	11 mL	1 vial	6 mL
Wash Buffer Concentrate	1 vial	20 mL	1 vial	10 mL
Substrate A	1 vial	7 mL	1 vial	3.5 mL
Substrate B	1 vial	7 mL	1 vial	3.5 mL
Stop Solution	1 vial	6 mL	1 vial	3 mL
Cut-off Control	1 vial	1 mL	1 vial	1 mL
Positive Control	1 vial	1 mL	1 vial	1 mL
Closure Plate Membrane	3 sheet		3 sheet	

Note: different batches of reagent kit, and different component can not be exchanged for use. Once open, stable for 3 months at 2-8°C.

SPECIMEN COLLECTION AND PREPARATION

1. **Specimen Collection:** No special patient's preparation required. Collect the specimen in accordance with the normal laboratory practice. Either fresh serum/plasma specimens can be used with this assay. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum must be separated from the clot as early as possible to avoid haemolysis of the RBC. Care should be taken to ensure that the serum/plasma specimens are clear and not contaminated by microorganisms.

2. ***Highly lipaemic, icteric, or hemolytic specimens should not be used*** as they can give false results in the assay. ***Do not heat inactivate specimens***. This can cause deterioration of the target analyte. Samples with visible microbial contamination should never be used.

3. ds-DNA ELISA is intended ONLY for testing of individual serum/plasma samples. Do not use the assay for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.

4. Transportation and Storage: Store specimens at 2-8°C. Specimens not required for assaying within 3 days should be stored frozen (-20°C or lower). Multiple freeze-thaw cycles should be avoided. For shipment, samples should be packaged and labeled in accordance with the existing local and international regulations for transportation of clinical samples and ethological agents.

TEST PROCEDURE

1. All reagents should be allowed to reach room temperature for 15 minutes before use.

2. Dilute the wash buffer at the rate of 1:40 dilution with distilled water before use.

3. Add 2 drops (100μL) of Sample Dilute in the corresponding hole, Add 5μL Sample in the corresponding hole (Do not add in the blank hole). Add 1 drop (50μL) of positive control and cut-off control to the positive control hole and cut-off control hole. The sample should be corresponding to the number of micro plate, each plate should be provided with cut-off control 2 holes, positive control 1 hole and blank control 1 hole. **Note: Use a separate disposal pipette tip for each specimen, Cut-off and Positive Control as to avoid cross-contamination.**

4. Shake gently to mix for 30 s. Incubate at 37 ℃ for 20 minutes with the sealing plate membrane sealing the plate.

5. At the end of the incubation, remove and discard the plate cover. Take out, add wash buffer to each well for 20 seconds. Repeat 5 times. After the final washing cycle, turn the plate over onto blotting paper or clean towel, and tap it to remove any remainders.

6. Respectively adding Conjugate 50μL (Do not add in the blank hole)

7. Incubate at 37 ℃ for 20 minutes with the sealing plate membrane sealing the plate. Repeat the wash step for 5 times as in step 5.

8.Add Substrate A 50μL and Substrate B 1 drop (50μL) (Do not add in the blank hole). Incubate at 37 ℃ for 10 minutes with the sealing plate membrane sealing the plate.

9. Add 50μL Stop Solution to each well (Do not add in the blank hole). Mix gently by shaking, read the absorbance within 10 minutes after stopping the reaction. Calibrate the plate reader with the Blank well and read the absorbance at 450nm. If a dual filter instrument is used, set the reference wavelength at

630nm. Set no blank holes is allowed if use dual wavelength to detect. Calculate the Cut-off value and evaluate the results.

INTERPRETATION OF RESULTS

Colorimetry: Read O.D at 450nm with a microplate reader.

Cut off= The average O.D. value of cut-off control.

Positive Results: Sample O.D ≥ Cut-off O.D.

Specimens giving an absorbance equal to or greater than the average O.D. value of cut-off control are considered initially reactive, which indicates that ds-DNA has probably been detected using ds-DNA ELISA. All initially reactive specimens should be retested in duplicates using ds-DNA ELISA before the final assay results interpretation. Repeatedly reactive specimens can be considered positive for ds-DNA with ds-DNA ELISA.

Negative Results: Sample O.D < Cut-off O.D.

Specimens giving absorbance less than the average O.D. value of cut-off control are negative for this assay, which indicates that no ds-DNA antibody has been detected with ds-DNA ELISA, therefore the blood unit do not contain ds-DNA antibody.

Follow-up, confirmation and supplementary testing of any positive specimen with other analytical system (e.g. PCR) is required. Clinical diagnosis should not be established based on a single test result. It should integrate clinical and other laboratory data and findings.

LIMITATIONS OF PROCEDURE

1. Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.
2. The reagent is a qualitative reagent, and can not be used as a quantitative reagent.
3. This reagent is only used for the detection of human serum/plasma samples.

PRECAUTIONS

1. Do not exchange reagents from different lots or use reagents from other commercially available kits. The components of the kit are precisely matched for optimal performance of the tests.
2. Specimens with Hyperlipidemia and icterus show no effects on the detection results. But specimen with severe hemolysis will affect the color of the result, other test methods are suggested to use.
3. Allow the reagents and specimens to reach room temperature before use. Shake reagent gently before use.
4. Concentrated washing liquid will produce crystal at room temperature, should be diluted completely before use.
5. Never reuse microplate sealing membrane.

STORAGE & VALIDITY

1. Store at 2-8℃.DO NOT FREEZE.
2. Once open, stable for 3 months at 2-8°C. Other liquid components have the same validity period with the reagent box.

REFERENCES

Chinese Pharmacopoeia
China Biological Products Procedures

	Keep in Dark Place		Keep Dry
	Do Not Reuse		Temperature Limitation
	Consult Instruction for Use		In Vitro Diagnostic Medical Product
	Batch Code		Contains Sufficient for <n> Tests
	Manufacturer		Date of Manufacture
	This side up		Fragile

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