






dsDNA IgG GENLISA™ ELISA

REF : KBD201

Ver 1.1

IVD

Enzyme Immunoassay for the Qualitative Determination of dsDNA antibody in human serum and plasma.

IVD	For In-vitro Diagnostic Use	REF	Catalog Number
	Store At	LOT	Batch Code
	Manufactured By		Biological Risk
	Expiry Date		Consult Operating Instructions

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REF KBD201

 96 tests

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Introduction:

ds-DNA is used for the semi-quantitative determination of autoantibodies to nuclear and cytoplasmic antigens in human serum and plasma.

Connective tissue diseases (CTD) are a group of autoimmune disorders which are characterized by presence of antinuclear antibodies (ANA) in the blood of patients. ANA are a specific class of autoantibodies that have the capability of binding and destroying certain structures within the nucleus of the cells. Although lower amounts of these antibodies can be seen in the normal population as well, a spurt in titers is seen in patients of CTD. These antibodies are involved in the disease pathogenesis and also constitute the basis for diagnosis and treatment of CTD. ANA have been categorized in two groups:

- Auto-antibodies to DNA (these include antibodies against single and double-stranded DNA (ssDNA, dsDNA)) and histone;
- Autoantibodies to extractable nuclear antigens (ENA).

Systemic lupus erythematosus (SLE) is a severe disease that involves dysregulation of the immune system, excessive production of pathogenic autoantibodies (and their upregulation in serum), and multiple immune-system-mediated injuries. Significant levels of anti-dsDNA and anti-ssDNA antibodies are considered to be confirmatory in diagnosis of SLE. The symptoms usually observed are muscle pain, swelling, stiffness.

Intended Use:

The dsDNA GENLISA™ ELISA is intended for the qualitative determination of ds-DNA IgG antibody in human serum and plasma.

Principle:

dsDNA GENLISA™ ELISA is an indirect enzyme linked immunosorbent assay which is designed to qualitatively detect ds-DNA antibody present in the human serum and plasma. Purified ds-DNA antigen is pre-coated onto microwells. Samples and Controls are pipetted into microwells and ds-DNA antibody present in test sample binds to the antigen coated on the wells. And then enzyme labeled antibody conjugate is pipetted and incubated to form an immune complex. After washing microwells in order to remove any non-specific binding, the substrate solution (A and B) is added to microwells and color develops proportionally to the amount of ds-DNA antibody present in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Microtiter Coated Plate (8x12 wells) - 1 no
2. Cut-Off Control - 1 ml
3. Positive Control - 1 ml
4. Enzyme Conjugate - 6.5 ml
5. (40X) Wash Buffer - 20 ml
6. Sample Diluent - 11 ml
7. TMB Substrate - 12 ml
8. Stop Solution - 12 ml
9. Instruction Manual.

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Graph paper or software for data analysis
6. Timer
7. Absorbent Paper

Handling/Storage:

1. Store main kit components at recommended storage temperature indicated on the component label.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.
3. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

**Specimen Collection and Handling:**

Serum- Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.

Plasma- Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 15-min at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, recentrifuge.

Reagent Preparation:

1. Wash Buffer (1X) Dilution: To make Wash Buffer (1X), add 2.5ml of Wash Buffer (40X) to 97.5ml of DI water. This is the working solution.
2. Allow all components to reach RT (Room Temperature) prior to use in the assay.

Test Procedure:

1. All reagents should be allowed to reach room temperature before use.
2. Add **100 ul Sample Diluent** to the sample wells.
3. Add **5 ul Sample** to the respective sample wells. Mix gently.
4. Dispense **50 ul Cut-Off Control** and **50 ul Positive Control** to the control wells respectively.
5. Shake gently for 30 seconds to mix well. Incubate at 37°C for 20 minutes.
6. Aspirate and wash plate 5 times with **(1X) Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
7. Add **50 ul of Enzyme Conjugate** to each well except the blank well.
8. Incubate at 37°C for 20 minutes.
9. Repeat the Aspiration / Wash Step.
10. Add **100 ul of TMB Substrate** into each well except blank well.
11. Incubate at 37°C for 10 minutes.
12. Add **100 ul of Stop Solution**. Read result with an ELISA reader at 450 nm within 15 minutes of stopping the reaction.

Interpretation of Results:

Determine the Mean Absorbance (net of Blank) for each set of duplicate Controls and Samples. Results are interpreted qualitatively by calculating a cut-off value for each sample on the basis of the cut-off determined. Read Absorbance at 450nm with an ELISA reader.

Cut-Off value (CO) = OD_{mean} of Cut-Off Control

Positive Results: OD value \geq CO

Specimens giving an absorbance equal to or greater than the CO are considered initially reactive, which indicates that dsDNA Antibody has probably been detected using the ELISA. All initially reactive specimens should be retested in duplicates using dsDNA ELISA before the final assay results interpretation. Repeatedly reactive specimens may be considered positive for dsDNA IgG with dsDNA antibody ELISA.

Negative Results: OD value $<$ CO

Specimens giving absorbance less than the CO are negative for the assay, which indicates that no Anti-dsDNA has been detected with the dsDNA IgG ELISA.

Cut Off Value	OD_{mean} of Cut-Off Control
Positive	\geq CO
Negative	$<$ CO

Criteria of Validation:

The results are considered to be valid, if

Blank	O.D. $<$ 0.250
Cut-Off Control (CO)	OD between 0.3 - 1.50
Positive Control	OD $>$ Cut-Off

OD = Optical Density / Absorbance at 450nm

Reference Values:

It is recommended that each laboratory establishes its own normal and pathological reference ranges, as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

Limitations of Method:

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

Performance Characteristics:**Sensitivity:**

Limit of Detection: When detecting anti-dsDNA limits, laboratory quality control positive samples diluted till 1:8 with the ds-DN IgG ELISA kit should be in the positive.

Specificity:

The recombinant antigen used in the kit is specific for ds-DNA.

Precision:

Intra-Assay: CV% \leq 15%.

Inter-Assay: CV% \leq 20%

Safety Precautions:

- **This kit is For In-vitro Diagnostic Use only.** Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
 - Do not smoke, eat or drink while handling kit material
 - Always use protective gloves
 - Never pipette material by mouth
 - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.



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Regulatory Status:

CE Marked	Europe
FDA registered	USA
CDSCO registered	India

SCHEMATIC ASSAY PROCEDURE

1	All reagents should be allowed to reach room temperature before use.
2	Add 100 ul Sample Diluent to the sample wells .
3	Add 5 ul Sample to the respective sample wells . Mix gently.
4	Dispense 50 ul Cut-Off Control and 50 ul Positive Control to the cut-off and positive wells respectively.
5	Shake gently for 30 seconds to mix well. Incubate at 37°C for 20 minutes .
6	Aspirate and wash plate 5 times with (1X) Wash Buffer and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
7	Add 50 ul of Enzyme Conjugate to each well except the blank well.
8	Seal the plate and incubate at 37°C for 20 minutes .
9	Repeat the Aspiration / Wash Step.
10	Add 100 ul of TMB Substrate into each well except blank well.
11	Incubate at 37°C for 10 minutes .
12	Add 100 ul of Stop Solution . Read result with an ELISA reader at 450 nm within 15 minutes of stopping the reaction.

SYMBOLS KEY

	Microtiter Plate (8x12 wells)
	Controls
	Enzyme Conjugate
	TMB Substrate
	Stop Solution
	Consult Instructions for Use
	Catalog Number
	Expiration Date
	Storage Temperature