dsDNA GENLISA® ELISA

REF : KBD201

Ver 2.4

IVD

Enzyme Immunoassay for the Quantitative and Semi-Quantitative Determination of dsDNA IgG 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**

For *In-vitro Diagnostic Use only. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of KRISHGEN Pudgala LLP is strictly prohibited.*

REF KBD201 **Σ 96 tests**

Krishgen Pudgala LLP Unit No.1/2, Om Sainath Commercial Complex, Off Makoli-Anjur Phata Road. Village Dapode, Bhiwandi 421302.

Introduction:

SLE is a systemic autoimmune disease characterized by the production of autoantibodies and a broad spectrum of clinical manifestations. The presence of antibodies to double stranded DNA (dsDNA) has been a criterion for SLE. The practical approach has been to use an assay that detects both high and low avidity anti-dsDNA antibodies as a primary screen such as ELISA. The detection of antibodies against single-stranded DNA (ssDNA) remains as disadvantage of ELISA . Antibodies against ssDNA only recognize ssDNA. They are observed not only in patients with SLE but also in other connective tissue diseases, such as systemic sclerosis and myositis. Thus, using highly pure dsDNA as well as digestion of ssDNA using nuclease S1 are critical strategies in establishing well-qualified ELISA.
The prevalence of anti-dsDNA has been reported as 20-80% of SLE depending on the detection methods. Due to the high frequency, sensitivity (57%), and specificity (70-98%), the presence of these autoantibodies could be an important serological marker for the diagnosis of SLE. Furthermore, once the diagnosis of SLE is made, periodic
measurements are considered essential to monitor the clinical course of the patient because an increase in anti-dsDNA antibody titer can predict a flare. Moreover, the presence of anti-dsDNA antibodies has been associated with a more severe disease pattern characterized by renal involvement, and a titer increase may predict a disease relapse. Anti-dsDNA antibodies have different subclasses. Most pathogenic antibodies are affiliated with the IgG class in SLE patients.
The anti-dsDNA IgG ELISA kit is designed for the quantitative and semi-quantitative detection of IgG antibodies to dsDNA that could be applicable as an aid to diagnosis of SLE patients accompany to other clinical manifestations.

Intended Use:

The dsDNA GENLISA® ELISA is intended for the quantitative and semi-quantitative detection of IgG antibodies to double-stranded DNA (dsDNA) in human serum or plasma samples.

Principle:

The dsDNA GENLISA® ELISA is an indirect enzyme linked immnunosorbent assay for quantitative and semi-quantitative determination of IgG antibody present in the human serum and plasma. The Microtiter wells are pre-coated with specific dsDNA antigens. Standards, Samples and Controls are pipetted into microwells and dsDNA IgG present in sample binds to the antigen coated on the wells. Enzyme Conjugate antibody is pipetted and incubated to form an immune complex. After washing microwells in order to remove any non-specific binding, the substrate solution is added to microwells and color develops proportionally to the amount of dsDNA IgG present in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. dsDNA Antigen Microtiter Coated Plate (96 wells) - 1 no
2. dsDNA IgG Standard (0, 100, 400, 800 IU/ml) – 1 ml/vials
3. Sample Diluent– 2 x 50 ml
4. Low Control Serum – 1 ml
5. High Control Serum – 1 ml
6. Enzyme Conjugate – 12 ml
7. (10X) Wash Buffer – 50 ml
8. TMB Substrate - 12 ml
9. Stop Solution - 12 ml
10. 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 pipette to measure volumes ranging from 10 ul, 100 ul to 1000 ul.
3. Deionized (DI) water.
4. Distilled Water.

5. Graph paper or software for data analysis.
6. Timer.
7. Absorbent Paper.

Handling/Storage:

1. Kit should be stored at 2-8°C upon receipt and when it is not in use.
2. Keep un-used wells in their sealed bag with desiccants.
3. Do not use expired date reagents.
4. Do not freeze.
5. Protect from light and moisture.

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, re-centrifuge.

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, re-centrifuge.

For Serum & Plasma - Samples have to be **diluted 1:101 (v/v)**, e.g. **10 ul sample + 1000 ul (1X) Sample Diluent** prior to assay.

Reagent Preparation:

1. Allow all components to reach RT (Room Temperature) prior to use in the assay.
2. Wash Buffer (1X) Dilution: To make Wash Buffer (1X), add 50 ml of Wash Buffer (10X) to 450 ml of DI water. This is the working solution.

Test Procedure:

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

2. Add **100 ul Standard, Control Serum and diluted Sample** in appropriate wells.

3. Seal the plate and Incubate at room temperature (22-28ºC) for 60 minutes.

4. 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.

5. Add **100 ul** of **Enzyme Conjugate** to each well.

6. Seal the plate and Incubate at room temperature (22-28ºC) for 30 minutes.

7. 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.

8. Add **100 ul** of **TMB Substrate** into each well.

9. Incubate at room temperature for 15 minutes in dark.

10. Add **100 ul of Stop Solution**. Read result with an ELISA reader at 450 nm within 30 minutes of stopping the reaction.

Validity of the Test

The assay is considered valid if:
1. In the quantitative assay the OD (450 nm) of standards 0 and 800 IU/ml less than 0.1 and higher than 1.2, respectively, are acceptable. In semi-quantitative assay, the OD (450 nm) of standard 2 (100 IU/ml) higher than 0.1 is valid.
2. The OD (450 nm) of blank is acceptable whenever it become less than 0.1.

Result Calculation

Semi-Quantitative

1. Calculate mean absorbance value of standards and samples at 450 nm. (Use 630 nm filters as reference filter if it's available).
2. Subtract blank ODs from samples and standard ODs.
3. Calculate a ratio of the absorbance value of the control or patient sample over the absorbance value of standard 2 (100 IU/ml) according to the following.

Formula:
Index= absorbance value of the control or patient
sample/ absorbance value of standard 2 (100 IU/ml)

Interpret results as follows:

Index <1.0	**Negative**
Index ≥1.0	**Positive**

Quantitative

Determine the mean absorbance for each set of duplicate or triplicate standards and samples. Using semi-log graph paper or computer programs, plot the optical densities of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit straight line through the standard points. To determine the unknown concentrations, find the unknowns mean absorbance value on the y-axis and draw a horizontal line to the standard curve.

At the point of intersection, draw a vertical line to the x-axis and read the Carbamazepine concentration. If samples were diluted, multiply by the appropriate dilution factor.

Computer based curve-fitting software; 4-PL or cubic spline or polynomial regression (2nd order) may be preferred.

Expected Values

It is important for each laboratory to establish the normal range limits. The following reference range from healthy persons should be considered as a guideline only:

<100 IU/ml	Negative
≥100 IU/ml	**Positive**

Performance Characteristics:

Comparative Study

The Diagnostics anti-dsDNA IgG kit was evaluated using the serum samples confirmed by two commercially anti-dsDNA IgG ELISA assays. The positive serum specimens were collected from rheumatologic patients attended to one of referral clinical centers. The results are summarized below.

Comparative Study (n= 451)		Competitor (anti-dsDNA IgG ELISA kit)		
		+	-	Total
anti-dsDNA IgG ELISA Kit	+	136	5	141
	-	12	298	310
	Total	148	303	451

The comparative study on the samples indicates the Sensitivity: 91.9%, Specificity: 98.35%, and Accuracy: 96.2% for the anti-dsDNA IgG kit.

Minimum Detection Limit
The LoB and LoD of anti-dsDNA by this assay are estimated to be 7.6 IU/ml and 30 IU/ml, respectively.

Test Precision
Intra, as well as Inter-assay precision, carried out by 3 different sera, and the results are shown in tables 1 and 2:

Intra-assay

No.	No. of tests	Mean (IU/ml)	SD (IU/ml)	CV%
1	20	53.49	5.03	9.40
2	20	379.57	7.23	1.90
3	20	613.61	15.51	2.53

Inter-assay

No.	No. of tests	Mean (IU/ml)	SD (IU/ml)	CV%
1	20	62.94	6.48	10.30
2	20	321.82	24.50	7.60
3	20	640.65	35.74	5.60

*Each test was run in duplicate.

Recovery
The certain amounts of anti-dsDNA IgG were added into 3 different sera with known concentrations of anti-dsDNA IgG and then their recovery were determined. The results shown below:

No.	anti-dsDNA IgG level (IU/ml)	anti-dsDNA IgG added (IU/ml)	Exp. (IU/ml)	Obs. (IU/ml)	Rec. (%)
1	39.25	128.21	83.73	80.73	96.4
2	301.82	705.68	503.75	601.70	119.4
3	678.46	106.48	392.47	424.35	108.1

Exp.: Expected, Obs.: Observed,
Rec.: Recovery

Linearity
To verify test linearity, three different serum samples with known anti-dsDNA IgG concentration were diluted sequentially by Sample Diluent. Then the sera were tested by the ELISA. The results of serum recovery were determined considering dilution factor.

No	Anti-dsDNA IgG (IU/ml) undiluted specimen	1:2	1:4	1:8
1	708.38	108.0	117.5	115.5
2	755.9	112.5	116.9	115.4
3	577.79	100.5	94.9	93.9

Cross Reactivity

No cross reactivity was seen with sera of scleroderma (n=20) and rheumatoid arthritis (n=12) patients in the anti-dsDNA IgG ELISA kit.

References

Isenberg D. Anti-dsDNA antibodies: still a useful criterion for patients with systemic lupus erythematosus? Lupus. 2004;13(11):881-5.

Petri M, Orbai AM, Alarcón GS, et al. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum. 2012;64(8):2677-86.

Zigon P, Lakota K, Cucnik S, et al. Comparison and evaluation of different methodologies and tests for detection of anti-dsDNA antibodies on 889 Slovenian patients' and blood donors' sera. Croat Med J. 2011;52(6):694-702.

Pisetsky DS, Lipsky PE. New insights into the role of antinuclear antibodies in systemic lupus erythematosus. Nat Rev Rheumatol. 2020;16(10):565-579.

Bragazzi NL, Watad A, Damiani G, Adawi M, Amital H, Shoenfeld Y. Role of anti-DNA auto-antibodies as biomarkers of response to treatment in systemic lupus erythematosus patients: hypes and hopes. Insights and implications from a comprehensive review of the literature. Expert Rev Mol Diagn. 2019;19(11):969-978.

Wichainun R, Kasitanon N, Wangkaew S, et al. Sensitivity and specificity of ANA and anti-dsDNA in the diagnosis of systemic lupus erythematosus: a comparison using control sera obtained from healthy individuals and patients with multiple medical problems. Asian Pac J Allergy Immunol. 2013;31(4):292-8.

Conti F, Ceccarelli F, Perricone C, et al. Systemic Lupus Erythematosus with and without Anti-dsDNA Antibodies: Analysis from a Large Monocentric Cohort. Mediators Inflamm. 2015;2015:328078.

Cozzani E, Drosera M, Gasparini G, Parodi A. Serology of Lupus Erythematosus: Correlation between Immunopathological Features and Clinical Aspects. Autoimmune Dis. 2014;2014:321359.

Rekvig OP. The dsDNA, Anti-dsDNA Antibody, and Lupus Nephritis: What We Agree on, What Must Be Done, and What the Best Strategy Forward Could Be. Front Immunol. 2019;10:1104.

Ho A, Magder LS, Barr SG, Petri M. Decreases in anti-double-stranded DNA levels are associated with concurrent flares in patients with systemic lupus erythematosus. Arthritis Rheum. 2001;44(10):2342-9.

Villalta D, Bizzaro N, Bassi N, et al. Anti-dsDNA antibody isotypes in systemic lupus erythematosus: IgA in addition to IgG anti-dsDNA help to identify glomerulonephritis and active disease. PLoS One. 2013;12;8(8):e71458.

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.

LIMITED WARRANTY

Krishgen Pudgala LLP does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the product; against defects in products or components not manufactured by Krishgen Pudgala LLP, or against damages resulting from such non-Krishgen Pudgala LLP made products or components. Krishgen Pudgala LLP passes on to customer the warranty it received (if any) from the maker thereof of such non-Krishgen made products or components. This warranty also does not apply to product to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Pudgala LLP.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Pudgala LLP shall be to repair or replace the defective product in the manner and for the period provided above. Krishgen Pudgala LLP shall not have any other obligation with respect to the products or any part thereof, whether based on contract, tort, strict

liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall Krishgen Pudgala LLP be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of Krishgen Pudgala LLP with respect to the product. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

Krishgen Pudgala LLP, 2026.

THANK YOU FOR USING A KRISHGEN PRODUCT!

Krishgen Pudgala LLP

EC REP

Unit No.1/2, Om Sainath Commercial Complex,
Off Mankoli-Anjur Phata Road. Village Dapode, Bhiwandi 421302.
KinesisDx, Lyoner Strasse 14, Frankfurt, Germany

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 Standard, Control Serum and Diluted Sample** in appropriate wells.
3	Seal the plate and Incubate at room temperature (22-28ºC) for 60 minutes.
4	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.
5	Add **100 ul** of **Enzyme Conjugate** to each well.
6	Seal the plate and Incubate at room temperature (22-28ºC) for 30 minutes.
7	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.
8	Add **100 ul of TMB Substrate** into each well.
9	Incubate at room temperature for 15 minutes in dark.
10	Add **100 ul of Stop Solution**. Read result with an ELISA reader at 450 nm within 15 minutes stopping the reaction.

SYMBOLS KEY

MTP	Microtiter Plate (96 wells)
STD	Standard
SAMP DIL	Sample Diluent
Low CNTRL SERUM	Low Control Serum
HIGH CNTRL SERUM	High Control Serum
ENZY CONJ	Enzyme Conjugate
10X WASH BUF	(10X) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature