Anti Tissue Transglutaminase (tTg) IgA GENLISA™ ELISA

REF : KBD914

Ver 2.0

IVD

ELISA for Quantitative Determination of Anti Tissue Transglutaminase (tTg) IgA in human serum or plasma samples.

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

REF KBD914 Σ 96 tests

Krishgen Pudgala LLP Unit Nos#318/319, Shah & Nahar, Off Dr E Moses Road, Worli, Mumbai 400018. India.
Tel: +91-22-49198700 | email: sales@krishgenpudgala.com

Introduction:

Celiac disease (CoD) also known as sprue, non-tropical sprue, gluten-sensitive enteropathy, defined as an autoimmune disorder originating by an aberrant adaptive immune response against gluten-containing grains in susceptible individuals. In celiac subjects the ingestion of gluten leads to an enteropathy with an impairment of the mucosal surface and, consequently, abnormal absorption of nutrients.. The active phase of CoD is accompanied by elevated levels in serum of immunoglobulin A (IgA) autoantibodies against endomysium (IgA-EmA) and tissue transglutaminase (IgA-tTG). Antigliadin antibodies (AGAs) are antibodies of the IgA and IgG classes found in the sera of celiac disease patients. These antibodies mainly target gliadin-derived peptides, which are the main proteins of gluten. The symptoms usually observed are tooth discoloration or loss of enamel, pale sores inside the mouth, irregular menstrual periods, infertility and miscarriage, dermatitis herpetiformis.

Intended Use:

Tissue Transglutaminase (tTg) IgA GENLISATM ELISA is intended for the quantitative determination of IgA class antibodies in human serum or plasma.

Principle:

This kit is developed based on Indirect ELISA analysis system, for quantitative determination of Anti Tissue Transglutaminase IgA in human serum samples. Recombinant Tissue Transglutaminase is coated on the plates. If Anti Tissue Transglutaminase IgA antibody is present in sample, then it will form an immune complex with coated antigen in microwell. Upon addition of Enzyme Conjugate, it will bind to the Anti-Tissue Transglutaminase IgA. After washing microwells in order to remove any non-specific binding, the substrate solution (TMB) is added to microwells and color develops. The optical density (OD value) can be detected with microplate reader and data extrapolated on a graph to obtain the concentration of Anti Tissue Transglutaminase IgA in the samples.

Materials Provided:

1. Tissue Transglutaminase Antigen Microtiter Coated Plate (8 x 12 wells) – 1 no
2. Anti Tissue Transglutaminase IgA Standards (1.2 ml/vial) – 0, 5, 20, 40, 80, 320 AU/mL
3. Positive Control – 1.2 ml
4. Negative Control – 1.2 ml
5. Enzyme Conjugate – 15 ml
6. (10X) Wash Buffer – 50 ml
7. Sample Diluent – 100 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 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.

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:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation. Samples should be aliquoted and must be stored frozen at -20°C to avoid loss of bioactivity. If samples are to be run within 24 hours, they may be stored at 2° to 8 °C. Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

Serum: Use a serum separator tube and allow clotting for 30 minutes, then centrifuge for 10 minutes at 1000 x *g*. Remove serum layer and assay immediately or store serum samples at <-20 °C. Avoid repeated freeze/thaw cycles.

Plasma: Collect blood sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at 1000 x *g* within 30 minutes of collection. Assay immediately or store plasma samples at <-20 °C. Avoid repeated freeze/thaw cycles.

Note: *Grossly hemolyzed samples are not suitable for use in this assay.*

Serum and plasma sample must be pre-diluted to 1:100 with sample diluent.

Reagent Preparation:

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room Temperature before use.
3. To make **(1X) Wash Buffer**; dilute **10 ml of (20X) Wash Buffer in 90 ml of DI water**.

Procedural Notes:

1. For good assay reproducibility and sensitivity, proper washing of the ELISA plate to remove excess/unbound reagents is essential.
2. If the concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect. To overcome Hook Effect samples to be assayed should be sufficiently diluted with our recommended diluent.
3. Avoid assay of Samples containing sodium azide (NaN3), as it could destroy the Enzyme activity of the conjugate resulting in under-estimation of the antibodies.
4. All Standards/Controls and Samples should be assayed at least in duplicates.
5. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
6. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromising the sensitivity of the assay.
7. The plates should be read within 30 minutes after adding the Stop Solution.
8. Make a work list in order to identify the location of Standards/Controls and Samples.

Test Procedure:

1. Bring all reagents to room temperature prior to use.
2. Add **100 ul Standards** to the standard wells.
3. Add **100 ul Positive Control & 100 ul of Negative Control** to the respective wells.
4. Add **100 ul diluted sample** to the sample wells and Mix it well.
5. Seal the plate and then incubate at room temperature for 30 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 **100 ul** of **Enzyme Conjugate** to each well except the blank well.
8. Gently mix, Seal the plate and then Incubate at room temperature for 30 minutes.
9. 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.
10. Add **100 ul** of **TMB Substrate** into each well. Incubate at room temperature for 15 minutes at dark.
11. Stop reaction by adding **100 ul** of **Stop Solution** to each well.
12. Read the microplate with an ELISA reader at 450 nm.

Calculations:

Determine the Mean Absorbance (net of Blank) for each set of duplicate Standards, Controls and Samples. Using graph paper, plot the average value (absorbance 450 nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown Tissue Transglutaminase (tTg) IgA concentrations, find the unknown's 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 Tissue Transglutaminase (tTg) IgA Concentration. If samples were diluted, multiply by the appropriate dilution factor.

Software which is able to generate a cubic spline curve-fit, 4-PL or a polynomial curve (2nd order) is best recommended for automated results.

Note:
It is recommended to repeat the assay at a different dilution factor in the following cases:
- If the sample absorbance value is below the first standard.

If the determination value is higher or lower than normal range, it means there is an abnormal result. The final result should be diagnosed in correlation with the clinical symptoms and other diagnostic methods.

Reference Range:

In a normal range study with serum samples from healthy blood donors the following ranges have been established with the Anti-tTG IgA test:

Anti-Tissue Transglutaminase IgA (AU/ml)	
Cut-Off	20

Limitations:

1. The presence of immune complexes or other immunoglobulin aggregates in the patient sample may cause an increased level of non-specific binding and produce false positives in this assay.
2. A negative htTG IgA result in an untreated patient does not rule out gluten-sensitive eneteropathy, This finding can often be explained by selective IgG deficiency, a relative frequent finding in celiac diseases.

Performance Characteristics of the Kit:

Sensitivity:
88.5%

Limit of Detection:
The lowest concentration of anti-tTG IgA that can be distinguished from the calibrator zero is 0.11 AU/ml with a confidence limit of 95%

Specificity:
This assay has high sensitivity of 100%. There is no significant cross-reactivity or interference seen

Precision:

Sample	1		2	
	SD	%CV	SD	%CV
Intra-assay	5.79	4.6	12.11	6.0
Inter-assay	0.72	7.6	15.92	11.1

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, and 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.

THANK YOU FOR USING KRISHGEN PRODUCT!

Krishgen Pudgala LLP

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

Regulatory Status:

CE Marked	Europe
FDA registered	USA
CDSCO registered	India

SYMBOLS KEY

MTP	**Microtiter Plate (8x12 wells)**
STD	**Anti- Tissue Transglutaminase IgA Standard**
ENZ CONJ	**Enzyme Conjugate**
SAMP DIL	**Sample Diluent**
(20X) WASH BUF	**(20X) Wash Buffer**
SUB TMB	**TMB Substrate**
SOLN STOP	**Stop Solution**
	**Consult Instructions for Use**
REF	**Catalog Number**
	**Expiration Date**
	**Storage Temperature**