

Anti-Tissue Transglutaminase (tTg) IgA GENLISA® ELISA

REF : KBD914

Ver 1.4

IVD

Enzyme Immunoassay for the Quantitative Determination of
Anti-Tissue Transglutaminase (tTg) IgA 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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 **96 tests**

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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 GENLISA® 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 (96 wells) – 1 no
2. Anti-Tissue Transglutaminase IgA Standards (0, 5, 20, 40, 80, 320 AU/ml) – 1.2 ml/vial
3. Positive Control – 1.2 ml
4. Negative Control – 1.2 ml
5. Enzyme Conjugate – 15 ml
6. (20X) Wash Buffer – 2 x 25 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.
To make **(1X) Wash Buffer**; dilute **25 ml of (20X) Wash Buffer in 475 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.

Calculation of Results:

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.

Validity of test:

The test is validity if following conditions are met.

Negative Control < 20.0 AU/ml

Positive Control > 20.0 AU/ml

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 of the Assay:

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 tTG IgA result in an untreated patient does not rule out gluten-sensitive enteropathy, 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.



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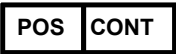
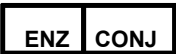
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**KinesisDx, Lyoner Strasse 14, Frankfurt, Germany****Regulatory Status:**

CE Marked	Europe
FDA registered	USA
CDSCO registered	India

SYMBOLS KEY

	Microtiter Plate (96 wells)
	Standards
	Positive Control
	Negative Control
	Enzyme Conjugate
	Sample Diluent
	(20X) Wash Buffer
	TMB Substrate
	Stop Solution
	Consult Instructions for Use
	Catalog Number
	Expiration Date
	Storage Temperature