

Tissue Transglutaminase (tTg) IgG GENLISA® ELISA

REF : KBD915

Ver 1.3

IVD

Enzyme Immunoassay for the Quantitative Determination of
Tissue Transglutaminase (tTg) 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 KBD915

 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 is a chronic autoimmune disorder characterised by inflammation of the intestinal mucosa and villous atrophy, triggered by intolerance to gluten, a protein found in wheat, rye and barley. Clinical manifestations include diarrhoea, gastrointestinal disturbances, anaemia, fatigue and, in some cases, extraintestinal or asymptomatic presentations. Untreated disease is associated with an increased risk of lymphoma, gastrointestinal malignancies and other autoimmune conditions such as diabetes mellitus and thyroid disorders.

Diagnosis is based on a combination of clinical findings, serological testing and, where required, small intestinal biopsy. Serological assays detecting antibodies against tissue transglutaminase (tTG) play a central role. While tTG IgA is the primary screening marker, tTG IgG is particularly useful in patients with IgA deficiency or when IgA-based testing is inconclusive. Clinical improvement following a gluten-free diet further supports the diagnosis.

Intended Use:

Tissue Transglutaminase (tTg) IgG GENLISA® ELISA is intended for the quantitative determination of IgG class antibodies in human serum and plasma.

Principle:

Tissue Transglutaminase (tTg) IgG GENLISA® ELISA is an indirect enzyme linked immunosorbent assay for quantitative determination of IgG antibody present in the human serum and plasma. Antigens are pre-coated onto microwells. Standards, Samples and Controls are pipetted into microwells and Tissue Transglutaminase (tTg) IgG Antibody present in sample binds to the antigen coated on the wells. Enzyme conjugate 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 Tissue Transglutaminase (tTg) IgG present in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Tissue Transglutaminase Antigen Microtiter Coated Plate (96 wells) – 1 no
2. Tissue Transglutaminase IgG 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.

Sample Dilution:

To make 1:100 Dilution, dilute 10 ul Sample + 990 ul Sample Diluent.

Reagent Preparation:

1. Bring all reagents to Room Temperature before use.
2. To make **(1X) Wash Buffer**; dilute **25 ml of (20X) Wash Buffer** in **475 ml of DI water**.

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) IgG 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) IgG 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.

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 htTG IgG result in an untreated patient does not rule out gluten-sensitive enteropathy, The patient may be IgA positive or have no antibody to tTG.

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.

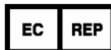
This Limited Warranty states the entire obligation of Krishgen Pudgala LLP with respect to the product. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

Krishgen Pudgala LLP, 2026.

THANK YOU FOR USING A KRISHGEN PRODUCT!**Krishgen Pudgala LLP**

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	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.

SYMBOLS KEY

MTP	Microtiter Plate (96 wells)
STD	Anti- Tissue Transglutaminase IgG Standard
NC	Negative Control
PC	Positive Control
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