AccuDiag™
Anti Tissue Transglutaminase IgA
ELISA Kit

Cat# 3173-17

IVD See external Label 96 Tests

Test	Anti Tissue Transglutaminase IgA
Method	Indirect -Solid Phase ELISA
Principle	Sandwich Complex
Detection Range	0.11 AU/mL -20 AU/mL
Sample	10 µL serum or plasma
Total Time	~ 75 min
Shelf Life	12-14 Months from the manufacturing date
Specificity	100%
Sensitivity	88.5%

INTENDED USE

The Anti Tissue Transglutaminase IgA ELISA Test is an Indirect-solid phase enzyme immunometric assay for the quantitative detection of IgA antibodies to transglutaminase in serum or plasma. The Anti Tissue Transglutaminase IgA ELISA is for use only by a laboratory. As an aid in identifying celiac disease and dermatitis herpetiformis, the Anti Tissue Transglutaminase IgA ELISA test is for in-vitro diagnostic use only.

SUMMARY AND EXPLANATION

Celiac disease is characterized by chronic inflammation of the intestinal mucosa and flattening of the epithelium or positive (villous atrophy). The origin of the celiac disease is the intolerance to gluten, the protein of wheat, rye and barley. The main symptoms are diarrhea, gastrointestinal problems, anemia, fatigue, psychiatric problems and other diverse side effects. In some cases patients may be asymptomatic (1).

Clinical and mucosal recovery after institution of a gluten free diet is objective evidence that the enteropathy is gluten induced. [2].
Diagnosis of celiac disease is confirmed by abnormal findings on the small bowel biopsy and later verified by the clinical response to a gluten-free diet, i.e. the avoidance of wheat, barley, rye, oats and triticale.
Left untreated patients suffering from celiac disease have an increased risk of lymphoma or gastrointestinal neoplasm. Furthermore, even if clinically silent, longstanding untreated celiac disease predisposes for other autoimmune diseases, like Diabetes mellitus, rheumatoid diseases, autoimmune hepatitis or thyroiditis.

The European Society for Pediatric Gastroenterology and Nutrition (ESPGAN) draw the guidelines for the celiac disease diagnosis, including: a) an initial positive gut biopsy, b) 6 months on a gluten-free diet, c) a negative second gut biopsy, d) a gluten challenge for 6 months e) a positive third gut biopsy.

Recently, the development of serum tests for three different antibodies of the IgA isotype made it possible to modify these guidelines for celiac disease. These revised ESPGAN criteria include: a) a single positive gut biopsy and b) the demonstration of at least two of the three IgA class antibodies against gliadin, endomysium or transglutaminase.

The enzyme tissue Transglutaminase (tTG) has been reported to be the main, if not sole, target for endomysial antibodies. These antibodies fall once a gluten-free diet has begun, thus facilitating monitoring of dietary compli-ance.Anti-tTG IgG are a highly sensitive marker for celiac diesease with 95-100 %, and have a specificity of 90 to 97 % [3, 4, 5, 6].

Since some years the human tTG antigen has been produced by recombinant technology. This new antigen allows certain advantages compared with the traditional guinea pig liver antigen.

There is a percentage of patients that don't produce any IgA and probably they represent the single largest contributor to a false negative serological result in biopsy confirmed celiac patients. To correctly evaluate this group of patients, many laboratories perform IgG determinations of several samples tested for the presence of antibodies against celiac disease. Most IgA deficient celiac patients are found to be positive for IgG class reticulin and gliadin.

Several studies on the use of IgG against t-TG to find patients not producing IgA demonstrated that test sensitivity was increased from 91.5% for IgA antibody alone to 98.5% when both IgA and IgG results were considered.

TEST PRINCIPLE

The principle of the Anti-Tissue Transglutamiase IgA ELISA test is a three-incubation process whereby the first incubation (30 minutes) involves the binding of serum/plasma antibodies to the transglutaminase that has been coated on the microplates. Next, non-reactive serum is washed from the microplates.

At the second incubation stage (30 minutes), anti-human IgA horseradish peroxidase conjugate will bind to IgA class antibodies that are bound to the immobilized antigens. After incubation, any excess unbound enzyme conjugate will be washed away.

At this point, a chromogen solution (tetramethylbenzidine or TMB) is added. Following a 15 minute incubation, the chromogen develops into a blue color. After the addition of a stop solution, the color turns to yellow. The color intensity can be gauged proportionally to the amount of IgA antibodies in the sample.

MATERIALS AND COMPONENTS

Materials provided with the test kits
1. **Anti-Tissue Transglutaminase IgA Calibrators (6 vials, 1.2mL each)**
   Phosphate buffer 0.1M, NaN3 < 0.1%, human serum.
   CAL0
   CAL1
   CAL2
   CAL3
   CAL4
   CAL5
2. **Controls (2 vials, 1.2 mL each)**
   Phosphate buffer 0.1M, NaN3 <0.1%, human serum.
   Negative Control
   Positive Control
3. **Sample Diluent (1 vial, 100 mL)**
   Phosphate buffer 0.1 M, NaN3 <0.1%
4. **Conjugate (1 vial, 15 mL)**
   Anti h-IgA conjugated with peroxidase, BSA 0.1%,

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Proclin <0.0015%

5. **Coated Microplate**
   (1 breakable microplate coated with human recombinant tissue transglutaminase)
6. **TMB Substrate (1 vial, 15 mL)**
   3,3',5,5' – tetramethylbenzidine 0.26 g/L, hydrogen peroxide 0.05%.
7. **Stop Solution (1 vial, 15 mL)**
   Sulphuric acid 0.15M
8. **10X Conc. Wash Solution (1 vial, 50 mL)**
   Phosphate buffer 0.2M, proclin <0.0015%

Materials required but not provided

1. Distilled water.

Auxiliary materials and instrumentation

1. Automatic dispenser.
2. Microplates reader (450 nm)

ASSAY PROCEDURE

Preparation of the Calibrator (C0...C5)
Since no international reference preparation for Anti-tissue Transglutamise antibodies is available, the assay system is calibrated in relative arbitrary units. The Calibrators are ready to use and have the following concentration:

	C_0	C_1	C_2	C_3	C_4	C_5
AU/mL	0	5	20	40	80	320

Once opened, the Calibrators are stable 6 months at 2-8ºC.

Preparation of the Sample
Either human serum or plasma samples can be used for the test. **All serum and plasma samples have to be diluted 1:100 with sample diluent**; for example 10 µL of sample may be diluted with 990 µL sample diluent. Test samples should be clear. Contamination by lipemia is best avoided, but does not interfere with this assay. Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months. Avoid repetitive freezing and thawing of samples. This may result in variable loss of autoantibody activity. Testing of heat-inactivated sample is not recommended.

Preparation of the Wash Solution
Dilute the contents of each vial of the buffered wash solution concentrate (10x) with distilled water to a final volume of 500 mL prior to use. For smaller volumes respect the 1:10 dilution ratio. The diluted wash solution is stable for 30 days at 2-8°C.In the concentrated wash solution is possible to observe the presence of crystals; in this case mix at room temperature until complete dissolution of crystals; for greater accuracy dilute the whole content of the bottle of concentrated wash solution to 500 mL, taking care also to transfer completely the crystals, then mix until crystals are completely dissolved.

PROCEDURE

1. **Allow all reagents to reach room temperature (22-28°C) for at least 30 minutes.**
2. Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C**.**
3. To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
4. As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the calibration curve (C_0-C_5), two for each Control, two for each sample, one for Blank.

Reagent	Calibrator	Sample/Control	Blank
Calibrator C_0-C_5	100 µL		
Controls		100 µL	
Diluted Sample		100 µL	
Incubate 30 minutes at room temperature (22-28ºC). Remove the contents from each well, the wells 3 times with 300 µL of diluted wash solution			
Conjugate	100 µL	100 µL	
Incubate 30 minutes at room temperature (22-28ºC). Remove the contents from each well, the wells 3 times with 300 µL of diluted wash solution			
TMB Substrate	100 µL	100 µL	100 µL
Incubate 15 minutes in the dark at room temperature (22-28 ºC)			
Stop Solution	100 µL	100 µL	100 µL
Shake the microplate gently. Read the absorbance (E) at 450 nm against Blank within 5 minutes.			

RESULTS

For the test Anti Tissue Transglutaminase IgA a 4-Parameter-Fit with Lin-Log coordinates for optical density and concentration is the data reduction method of choice. Smoothed-Spline Approximation and log-log coordinates are also suitable. However we recommend using a Lin-Log curve.

First calculate the averaged optical densities for each calibrator well. Use Lin-Log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

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

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

Please pay attention to the fact that the determination of a range of expected values for a "normal" population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore each laboratory should consider the range given by the Manufacturer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.

Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually. It is recommended that

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each laboratory establishes its own normal and pathological ranges of serum Anti-tTG antibodies.

QUALITY CONTROL

1. The Positive and Negative Control should be run with every batch of samples to ensure that all reagents and procedures perform properly.
2. Because Positive and Negative Control are prediluted, they do not control for procedural methods associated with dilution of specimens.
3. Additional suitable control sera may be prepared by aliquoting pooled human serum specimens and storing at < - 20ºC.
4. In order for the test results to be considered valid, all of the criteria listed below must be met. If any of these are not met, the test should be considered invalid and the assay repeated:
   ➤ The absorbance of the prediluted h-tTG IgA Positive must be greater than the absorbance of the prediluted Negative Control.
   ➤ The negative and Positive Control are intended to monitor for substantial reagent failure and they will not ensure precision at this assay cut-off.
   ➤ This test is only valid if the optical density at 450 nm for Positive Control and Negative Control as well as for the Calibrator C0-C5 complies with the respective range indicated on the Quality Control Certificate enclosed to each test kit: If any of these criteria is not met, the results are invalid and the test should be repeated.

PERFORMANCE CHARACTERISTICS

Precision and reproducibility
Precision and reproducibility are evaluated by eight reply of two positive samples by two different runs with two different lots.

Dispensing and washing operations were performed manually by an operator.
The results in terms of Calibrator deviation and coefficient of variation were below:

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

Specificity
Comparison test against a commercial reference kit, performed on 40 sera (23 of them positive sera and 17 negative sera) showed 100.0% specificity.

Sensitivity
Comparison test against a commercial reference kit, performed on 40 sera (23 of them positive sera and 17 negative sera) showed a 88.5% sensitivity.

Detection Limit
The lowest concentration of anti-tTG IgA that can be distinguished from Calibrator zero is 0.11 AU/mL with a confidence limit of 95%.

WASTE MANAGEMENT
Reagents must be disposed off in accordance with local regulations.

LIMITATIONS OF PROCEDURE

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 h-tTG IgA result in an untreated patient does not rule out gluten-sensitive enteropathy. This finding can often be explained by selective IgA deficiency, a relatively frequent finding in celiac disease.

PRECAUTION

1. This kit is intended for in vitro use by professional persons only. Not for internal or external use in Humans or Animals.
2. Use appropriate personal protective equipment while working with the reagents provided.
3. Follow Good Laboratory Practice (GLP) for handling blood products.
4. All human source material used in the preparation of the reagents has been tested and found negative for antibody to HIV 1 & 2, HbsAg, and HCV. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, Calibrators and Controls should be handled in the same manner as potentially infectious material.
5. Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy and the bovine protein has been obtained from countries not infected by BSE, but these materials should be handled as potentially infectious.
6. Some reagents contain small amounts of Sodium Azide (NaN3) or Proclin 300R as preservatives. Avoid the contact with skin or mucosa.
7. Sodium Azide may be toxic if ingested or absorbed through the skin or eyes; moreover it may react with lead or copper plumbing to form potentially explosive metal azides. If you use a sink to remove the reagents, allow scroll through large amounts of water to prevent azide build-up.
8. The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
9. The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
10. Avoid the exposure of reagent TMB/H2O2 to directed sunlight, metals or oxidants. Do not freeze the solution.
11. Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
12. All reagents should be stored refrigerated at 2-8 ºC in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated.
13. Allow all kit components and specimens to reach room temperature (22-28 ºC) and mix well prior to use.
14. Do not interchange kit components from different lots. The expiry date printed on box and vials labels must be observed. Do not use any kit component beyond their expiry date.
15. **WARNING: the conjugate reagent is designed to ensure maximum dose sensitivity and may be contaminated by external agents if not used properly:** therefore, it is recommended to use disposable consumables (tips, bottles, trays, etc.) For divided doses, take the exact amount of conjugate needed and do not re-introduce any waste product into the original bottle. In addition, **for doses dispensed with the aid of automatic and semi-automatic devices,** before using the conjugate, it is advisable to clean the fluid handling system, ensuring that the procedures of washing, deproteinization and decontamination are effective in avoiding contamination of the conjugate; this procedure is highly recommend when the kit is processed using analyzers which are not equipped with disposable tips.
   For this purpose, Diagnostic Automation, Inc. supplies a separate decontamination reagent for cleaning needles
16. If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.
17. The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background.
18. It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order

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of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate.

19. Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
20. Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
21. Maximum precision is required for reconstitution and dispensation of the reagents.

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