Anti-Transglutaminase Antibody ELISA

For the quantitative determination of anti-tissue transglutaminase antibodies in human stool.

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 30-TRAHU-E01, E10
Size: 96 Wells, 10 x 96 wells
Version: 2023-10-10 – ALPCO 6.0

INTENDED USE

The Anti-Transglutaminase Antibody ELISA is intended for the quantitative determination of anti-human tissue transglutaminase (tTG) secretory IgA (sIgA) antibodies in human stool samples. For Research Use Only. Not For Use in Diagnostic Procedures.

INTRODUCTION

Human tissue transglutaminase (htTG) is a 78-kDa calcium-binding enzyme of the protein-glutamine γ-glutamyltransferases family. It cross-links proteins between an ε-amino group of a lysine residue and a γ-carboxamide group of a glutamine residue, creating an inter- or intra-molecular bond that is highly resistant to proteolysis.

PRINCIPLE OF THE ASSAY

This ELISA is used for the quantitative determination of anti-human tissue transglutaminase (anti-htTG) sIgA antibodies in stool. In a first incubation step, the anti-htTG sIgA antibodies in the sample are bound to their antigen (human recombinant transglutaminase), which is immobilized to the surface of the microtiter plates. To remove all unbound foreign substances, a washing step is carried out. In a second incubation step, a peroxidase-labeled anti-sIgA antibody is added. After another washing step, to remove all unbound antibodies, the solid phase is incubated with the substrate, tetramethylbenzidine. An acidic stop solution is then added to stop the reaction. The color converts from blue to yellow. The intensity of the yellow color is directly proportional to the amount of bound anti-htTG antibodies and can be determined photometrically at 450 nm. The sample results are evaluated by comparison with a cut-off value.

MATERIALS SUPPLIED

Product: 30-TRAHU-E01

Component	Quantity	Preparation
Transglutaminase Microplate (96 wells)	12 strips of 8 wells	Ready to use
3 Controls (positive, negative, cut-off)	4 vials each	Lyophilized
Wash Buffer Concentrate	2 x 100 mL	10X
Extraction Buffer Concentrate	1 x 100 mL	2.5X
Conjugate, peroxidase-labeled	1 x 15 mL	Ready to use
TMB Substrate	1 x 15 mL	Ready to use
Stop Solution	1 x 15 mL	Ready to use

Product: 30-TRAHU-E10

Component	Quantity	Preparation
Transglutaminase Microplate (96 wells)	10 Plates: each 12 x 8 wells	Ready to use
3 Controls (positive, negative, cut-off)	40 vials each	Lyophilized
Wash Buffer Concentrate	20 x 100 mL	10X
Extraction Buffer Concentrate	10 x 100 mL	2.5X
Conjugate, peroxidase-labeled	10 x 15 mL	Ready to use
TMB Substrate	10 x 15 mL	Ready to use
Stop Solution	10 x 15 mL	Ready to use

MATERIALS REQUIRED BUT NOT SUPPLIED

- Precision pipettes for dispensing 10 to 1000 µL (with disposable tips)
- Repeating or multi-channel pipette for dispensing up to 1000 µL
- Volumetric containers and pipettes for reagent preparation
- Distilled/Deionized water for reagent preparation
- Microplate washer or wash bottle
- Microplate shaker capable of 550 rpm with an orbit of 2mm
- Microplate reader
- Centrifuge (1,000 x g to 3,000 x g)
- Vortex for sample preparation
- Foil to cover the microplate
- Timer

PRECAUTIONS

1. The human blood products incorporated into this kit have been tested for the presence of HIV (Human Immunodeficiency Virus), HBV (Hepatitis B Virus), and HCV (Hepatitis C Virus). Test methods for these viruses do not guarantee the absence of a virus; therefore, all reagents should be treated as potentially infectious. Handling and disposal should be in accordance with all appropriate national and local regulations for the handling of potentially biohazardous materials.
2. Kit reagents contain sodium azide or Proclin as bactericides. Sodium azide and Proclin are hazardous to health and the environment. Substrates for the enzymatic color reactions may also cause skin and/or respiratory irritation. Any contact with the substances must be avoided. Further safety information can be found in the safety data sheet, which is available from ALPCO upon request.
3. The 10x Wash Buffer concentrate contains surfactants which may cause severe eye irritation in case of eye contact.

   Warning: Causes serious eye irritation
   If in Eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists, get medical advice/attention.
4. The stop solution consists of diluted sulfuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped up immediately with copious quantities of water. Do not breathe vapor and avoid inhalation.
5. Avoid direct contact with skin.
6. This product is not for internal use. Avoid eating, drinking, or smoking when using this product.
7. Do not pipette any reagents by mouth.
8. Reagents from this kit are lot-specific and must not be substituted.
9. Do not use reagents beyond the expiration date.
10. Variations to the test procedure are not recommended and may influence the test results.

STORAGE CONDITIONS

The kit should be stored at 2-8°C. The unopened kit is stable until the expiration date on the box label. The kit can be used up to 4 times within the expiration date stated on the label.

SAMPLE HANDLING

Stool samples are appropriate for use in this assay.

The sample stability is as follows:
Raw stool samples can be stored for 4 weeks at -20°C. Avoid more than 3 freeze-thaw cycles.

Stool extract can be stored for 3 days at 2–8°C or -20°C, or for one day at room temperature (15–30°C). Avoid more than 3 freeze-thaw cycles.

Extraction of the Stool Sample: 1X working extraction buffer is used as a sample extraction buffer. It is recommended to perform the following sample preparation:

1. The raw stool sample must be thawed. For particularly heterogeneous samples it is recommended to perform a mechanical homogenization using an applicator, inoculation loop, or similar device.
2. A 15 mg sample of the stool should be homogenized in 1.5 mL of 1X working extraction buffer.
3. If using the EZ Extraction Device, the device is provided in a prefilled format and does not require any additional extraction buffer.
4. Unscrew the tube (yellow part of cap) to open. Insert yellow dipstick into sample at three different locations. The lower part of the dipstick has notches which need to be covered completely with stool after inserting it into the sample. Place dipstick back into the tube only once. When putting the stick back into the tube, excess material will be stripped off leaving 15 mg of sample to be diluted. Screw tightly to close the tube.
5. Vortex the tube well until no stool sample remains in the notches. Important: Please make sure that you have a maximally homogenous suspension after shaking. Especially with more solid samples, soaking the sample in the tube with buffer for approximately 10 minutes improves the result.
6. Allow sample to stand for approximately 10 minutes until sediment has settled. Floating material like shells of grains can be neglected.
7. Carefully unscrew the complete cap of the tube including the blue ring plus the dipstick. Discard cap and dipstick. Make sure that the sediment will not be dispersed again. This will result in a **dilution of 1:100 (Dilution I).**

Dilution of Samples
The suspension from the sample extraction (Dilution I) is further diluted 1:50 with 1X working wash buffer. For example:

20 µL dilution I + 980 µL 1X working wash buffer, mix well = 1:50 (Dilution II). This results in a final dilution of 1:5,000. For analysis, pipet 100 µL of Dilution II per well.

REAGENT PREPARATION

All reagents must be equilibrated to room temperature prior to preparation and subsequent use in the assay. Prepare enough reagents for only the number of strips used. Store unused strips covered at 2–8 °C. The kit can be used up to 4 times within the expiry date stated on the label. To run the assay more than once, ensure that reagents are stored at the conditions stated on the label.

Controls (positive, negative, cut-off) are provided in a lyophilized form and are stable at 2-8˚C until the expiry date on the label. Reconstitution details are given in the product specification sheet. Reconstituted controls (positive, negative, cut-off) are not stable and cannot be stored.

Wash Buffer Concentrate (10X) must be diluted with distilled or deionized water 1:10 before use (100 mL WASHBUF + 900 mL distilled water) and mixed well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals must be dissolved at room temperature or in a water bath at 37 °C. The WASHBUF ***(10X)*** is stable at 2–8 °C until the expiry date stated on the label. 1X working wash buffer (1:10 diluted WASHBUF) can be stored in a closed flask at 2–8°C for 1 month.

Extraction Buffer Concentrate (2.5X) must be diluted with distilled or deionized water 1:2.5 before use (100 mL Extraction Buffer + 150 mL distilled water) and mixed well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals must be dissolved at 37°C in a water bath. The Extraction Buffer is stable at 2–8°C until the expiry date stated on the label. 1X working extraction buffer (1:2.5 diluted) can be stored in a closed flask at 2–8°C for 4 months.

All other test reagents are ready to use. Test reagents are stable until the expiry date (see label of test package) when stored at 2–8°C.

ASSAY PROCEDURE

All reagents and microplate strips (while sealed in foil pouch) should be equilibrated to room temperature prior to use. Gently mix all reagents before use. Controls and samples should be run in duplicate. Unused well strips should be stored with the desiccant bag in the closed aluminum packaging at 2–8°C. Strips are stable until the expiration date stated on the label.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact ALPCO.

1. Bring all reagents and samples to room temperature (15–30°C) and mix well.
2. Before use, wash the pre-coated microtiter plate wells 5 times with 250 µL 1X working wash buffer. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
3. Add 100 µL of positive, negative, and cut-off control and diluted samples into the respective wells. See *Reagent Preparation* for control reconstitution instructions. A suggested plate layout is included at the end of this protocol.
4. Cover plate tightly and incubate for 1 hour at room temperature (15–30°C) on a horizontal shaker*.
5. Discard the contents of each well. Wash each well 5 times with 250 µL of 1X working wash buffer. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
6. Add 100 µL CONJ (conjugate) into each well.
7. Cover plate tightly and incubate for 1 hour at room temperature (15–30°C) on a horizontal shaker*.
8. Discard the contents of each well. Wash each well 5 times with 250 µL of 1X working wash buffer. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
9. Add 100 µL of SUB (substrate) into each well.
10. Incubate for 10–20 minutes at room temperature (15–30°C) in the dark**.
11. Add 100 µL of STOP (stop solution) into each well, mix thoroughly.
12. Determine absorption immediately with an ELISA reader at 450 nm against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the

extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at 405 nm against 620nm as a reference.

* It is recommended to set the plate shaker to 550 rpm with an orbit of 2mm.

** The intensity of the color change is temperature sensitive. It is recommended to observe the color change and to stop the reaction upon good differentiation.

CALCULATION OF RESULTS

Samples with an optical density higher than the average optical density of the cut-off control are positive.

Cut-off = $OD_{cut\text{-}off\ control}$ = 100U/L

Examples
OD_{sample} = 0.685
$OD_{cut\text{-}off\ control}$ = 0.234 = 100 U/L

Concentration of sample = (0.685 x 100 U/L)/0.234 = 292.7 U/L

Attention: Example calculation is only valid for a sample dilution factor of 1:5000.

Limitations

The lower limit of the measurement range is the LOB. See Performance Characteristics.

Samples with concentrations lower than the measurement range cannot be clearly quantified.

QUALITY CONTROL

Control samples should be analyzed with each run. Results generated from the analysis of control samples should be evaluated for acceptability using appropriate statistical methods. The results for the samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity

Limit of Blank, LoB = 33.01 U/L

Accuracy- Precision

Repeatability (Intra-assay)

The repeatability was assessed with 2 stool samples under constant parameters (same operators, measurement systems, days, and kit lots).

	Sample 1	Sample 2
Mean	159.6 U/L	83.6 U/L
CV (%)	5.8	8.9
n	40	40

Reproducibility (Inter-assay)

The reproducibility was assessed with 2 stool samples under varying parameters (different operators, measurement systems, days, and kit lots).

	Sample 1	Sample 2
Mean	125.7 U/L	150.9 U/L
CV (%)	12.1	10.5
n	18	18

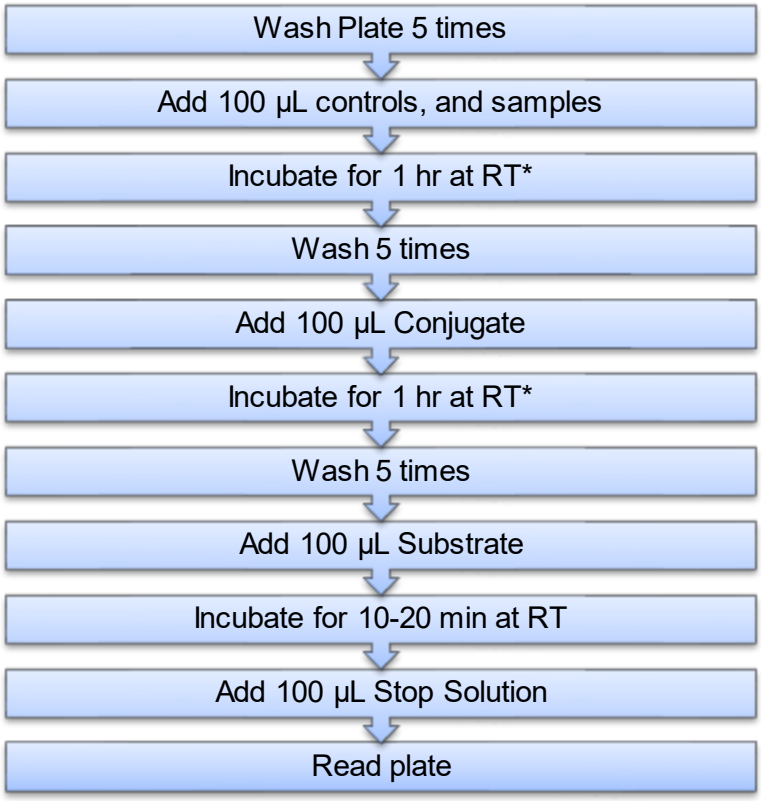
TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore, it is not recommended to assemble wells of different microtiter plates for analysis, even if they are of the same batch.
- Control samples should be analyzed with each run.
- Reagents should not be used beyond the expiration date stated on kit label.
- Substrate solution should remain colorless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.

GENERAL NOTES ON THE ASSAY AND ASSAY PROCEDURE

- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. ALPCO can therefore not be held responsible for any damage resulting from incorrect use.

SHORT ASSAY PROTOCOL



Total Time = 2 hours 20 minutes
***Recommended plate shaker settings are 550 rpm with an orbit of 2mm.**

SUGGESTED PLATE LAYOUT

Below is a suggested plate layout for running standards, controls, and up to 45 samples in duplicate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Ctrl 1	Ctrl 1	6	6	14	14	22	22	30	30	38	38
B	Ctrl 2	Ctrl 2	7	7	15	15	23	23	31	31	39	39
C	Ctrl 3	Ctrl 3	8	8	16	16	24	24	32	32	40	40
D	1	1	9	9	17	17	25	25	33	33	41	41
E	2	2	10	10	18	18	26	26	34	34	42	42
F	3	3	11	11	19	19	27	27	35	35	43	43
G	4	4	12	12	20	20	28	28	36	36	44	44
H	5	5	13	13	21	21	29	29	37	37	45	45

Ctrl = Control
Numbered wells = Samples