Version 19-04/24

User's Manual

Anti-Human-Tissue-Transglutaminase sIgA ELISA Kit

REF **DEIA009J**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

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

General Description

Human tissue transglutaminase (abbreviated as htTG or TG2) is a 78-kDa, calcium-dependent enzyme of the protein-glutamine γ-glutamyltransferases family. It crosslinks proteins between an ε-amino group of a lysine residue and a γ-carboxamide group of glutamine residue, creating an inter- or intramolecular bond that is highly resistant to proteolysis.

Principles of Testing

This ELISA is designed for the quantitative determination of anti-htTG sIgA antibodies in faeces. In a first incubation step, the anti-htTG sIgA antibodies in the sample are bound to their antigen (human recombinant transglutaminase), which is immobilised 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 peroxidaselabelled 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 colour converts from blue to yellow. The intensity of the yellow colour is directly proportional to the amount of bound antibodies and can be determined photometrically at 450 nm. The results are evaluated by comparison with a cut-off value.

Reagents And Materials Provided

1. PLATE: Microtiter plate, pre-coated 12×8 wells
2. WASHBUF: Wash buffer concentrate, 10×, 2×100 ml
3. CONJ: Conjugate, peroxidase-labelled, ready-to-use 1×15ml
4. CTRL NEG: Control negative, lyophilised (see specification for range) 4×1 vial
5. CTRL POS: Control positive, lyophilised (see specification for range) 4×1 vial
6. CTRL CUT OFF: Cut off control, lyophilised (see specification for concentration) 4×1 vial
7. SUB Substrate (tetramethylbenzidine), ready-to-use 1×15 ml
8. STOP Stop solution, ready-to-use 1×15 ml
9. Extract Extraction buffer concentrate 2.5×, 1×100 ml

Materials Required But Not Supplied

1. Ultrapure water*
2. Horizontal microtiter plate shaker
3. Calibrated precision pipettors and 10–1000 µl single-use tips

4. Foil to cover the microtiter plate
5. Multi-channel pipets or repeater pipets
6. Centrifuge
7. Vortex
8. Standard single-use laboratory glass or plastic vials, cups, etc.
9. Microtiter plate reader

* CD recommends the use of ultrapure water (water type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles >0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25°C (≥18.2 MΩ cm).

Storage

2-8°C/-20°C

Specimen Collection And Preparation

Sample storage

Raw stool

Raw stool samples can be stored for 4 weeks at -20°C. Avoid more than 3 freezethaw-cycles.

Stool suspensions

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 three freeze-thaw cycles.

Extraction of the stool samples

Extraction buffer (1:2.5 diluted Extract) is used as a sample extraction buffer.

We recommend the following sample preparation:

Stool Sample Application System (SAS) (Cat. No.: DEIA009JS)

Stool sample tube – Instructions for use

Please note that the dilution factor of the final stool suspension depends on the amount of stool sample used and the volume of the buffer.

SAS with 1.5 ml extraction buffer:

Applied amount of stool: 15 mg

Buffer Volume: 1.5 ml

Dilution Factor: 1:100

Please follow the instructions for the preparation of stool samples using the SAS as follows:

a) The raw stool sample has to be thawed. For particularly heterogeneous samples we recommend a mechanical homogenisation using an applicator, inoculation loop or similar device.

b) Fill the empty stool sample tube with 1.5ml extraction buffer (1:2.5 diluted Extract) before using it with the sample. Important: Allow the extraction buffer to reach room temperature.

c) Unscrew the tube (yellow part of cap) to open. Insert the yellow dipstick into the sample. 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. 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.

d) 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 ~ 10 minutes improves the result.

e) Allow sample to stand for ~10 minutes until sediment has settled. Floating material like shells of grains can be neglected.

f) 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. **Dilution I: 1:100**

Dilution of samples

The suspension from the sample extraction (dilution I) is further diluted 1:50 with wash buffer.

For example: 20 µl dilution I + 980 µl wash buffer, mix well = 1:50 (dilution II) This results in a final dilution of 1:5000.

For analysis, pipet 100 µl of dilution II per well.

Reagent Preparation

1. To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. **Prepare only the appropriate amount necessary for each run.** The kit can be used up to 4 times within the expiry date stated on the label.
2. **Preparation of the wash buffer:** The **wash buffer concentrate (WASHBUF)** has to be diluted with ultrapure water **1:10** before use (100 ml WASHBUF + 900 ml ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37°C. The WASHBUF is stable at **2–8°C** until the expiry date stated on the label. **Wash buffer** (1:10 diluted WASHBUF) can be stored in a closed flask at **2–8°C** for 1 month.
3. **Preparation of the extraction buffer:** The **extraction buffer concentrate Extract** has to be diluted with ultrapure water **1:2.5** before use (100ml Extract + 150ml ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at 37°C in a water bath. The Extract is stable at **2–8°C** until the expiry date stated on the label. Extraction buffer (1:2.5 diluted Extract) can be stored in a closed flask at **2–8°C for 4 months**
4. The **lyophilised controls** (CTRL NEG, CTRL POS and CTRL CUT OFF) are stable at **2–8°C** until the expiry date stated on the label. **Reconstitution** details are given in the specification data sheet. Controls (reconstituted CTRL NEG, CTRL POS and CTRL CUT OFF) are **not stable and cannot be stored.**
5. All other test reagents are ready-to-use. Test reagents are stable until the expiry date (see label) when stored at **2–8°C**.

Assay Procedure

Bring all reagents and samples to room temperature (15–30°C) and mix well.

Mark the positions of controls/samples on a protocol sheet.

Take as many microtiter strips as needed from the kit. Store unused strips together with the desiccant bag in the closed aluminium packaging at 2–8°C. Strips are stable until expiry 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.

We recommend to carry out the tests in duplicate.

1. Before use, wash the wells 5 times with 250 µl wash buffer. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
2. Add 100 µl controls/diluted samples into the respective well.
3. Cover the strips and incubate for 1 hour at room temperature (15–30°C) on a horizontal shaker*.
4. Discard the content of each well and wash 5 times with 250 µl wash buffer. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
5. Add 100 µl conjugate (CONJ) into each well.
6. Cover the strips and incubate for 1 hour at room temperature (15–30°C) on a horizontal shaker*.
7. Discard the content of each well and wash 5 times with 250 µl wash buffer. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
8. Add 100 µl substrate (SUB) into each well.
9. Incubate for 10–20 min** at room temperature (15–30°C) in the dark.
10. Add 100 µl stop solution (STOP) into each well and mix well.
11. 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 620 nm as a reference.

* We recommend shaking the strips at 550 rpm with an orbit of 2 mm.

** The intensity of the colour change is temperature sensitive. We recommend observing the colour change and stopping the reaction upon good differentiation.

Quality Control

CD recommends the use of external controls for internal quality control, if possible.

Control samples should be analysed 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.

Interpretation Of Results

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

Cut off = $OD_{cut\ off\ control}$ = 100 U/l

Example

OD_{sample} = 0.685

$OD_{cut\ off\ control}$ = 0.234 = 100 U/l

Concentration sample = (0.685 * 100 U/l)/0.234= 292.7 U/l

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

Precision

Repeatability (Intra-Assay); n = 40

The repeatability was assessed with 2 stool samples under constant parameters (same operator, instrument, day and kit lot).

Reproducibility (Inter-Assay); n = 18

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

Detection Range

We recommend each laboratory to establish its own reference range

Detection Limit

The lower limit of the measurement range can be calculated as: LoB × sample dilution factor to be used Samples with concentrations lower than the measurement range cannot be clearly quantified.

The following value has been estimated based on the concentration of the calibrator without considering possibly used sample dilution factors.

Limit of blank, LoB: 33.01 U/l

Precautions

1. All reagents in the kit package are for research use only.
2. Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
3. Kit reagents contain sodium azide or ProClin as bactericides. Sodium azide or ProClin are hazardous to health and the environment. Substrates for enzymatic colour 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.
4. The 10× Wash buffer concentrate (WASHBUF) 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.
5. The stop solution consists of diluted sulphuric 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 breath vapour

and avoid inhalation.

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