ZediXclusive

E I A

for the determination of tissue Transglutaminase

Directions for use

Art.-no. E018

Zedira GmbH Roesslerstrasse 83 D-64293 Darmstadt
Tel.: + 49 6151 66628-0 Fax: + 49 6151 66628-19 contact@zedira.com

For Research & Development only

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Contents

1. Introduction and background

Tissue transglutaminase (tTG, TG2) displays diverse functions in various physiological processes. In addition to these physiological functions, there is strong evidence for the implication of tTG in a number of pathological conditions, including celiac disease, fibrosis, neurodegeneration and cancer. This test may be used to detect TG2 in order to explore the expression and function of tTG during (patho)physiological conditions.

2. Warnings and precautions

The test kit is intended for *in vitro* use only. It is applicable only for research and development, not for diagnostic purposes.
Follow routine precautions for handling hazardous chemicals. Avoid all body contact, wear gloves and eye protection.

a. There are no available test methods that can absolutely assure that Hepatitis B and C viruses, HIV-1/2, or other infectious agents are present in biological samples. All human products, including patient samples, should be considered potentially infectious. Handling and disposal of this material should comply with the rules defined by appropriate local biohazard safety guidelines.

b. Avoid contact with 5% H_2SO_4. It may cause skin irritation and burns. If one of the reagents comes into contact with skin, wash thoroughly with water.

c. Do not use reagents after expiration date.

d. Do not mix or use components from kits with different lot numbers.

e. Replace caps on reagents immediately. Do not swap caps.

f. Do not pipette reagents by mouth.

3. Principle of the test

This test is based on two-site sandwich enzyme immunoassay principle. Tested specimen is placed into the microwells coated by the antibody to tissue transglutaminase (tTG). Antigen from the specimen bind coated antibody on the microwell surface. Unbound material is removed by washing procedure. Secondary antibodies directed towards tTG, and labelled with HRP, are then added into the microwells. After subsequent washing procedure, the remaining enzymatic activity bound to the microwell surface is detected and quantified by addition of chromogen-substrate mixture, stop solution and photometry at 450 nm. Optical density in the microwell is directly related to the quantity of antigen in the specimen.

4. Specificity

The specificity of E018 was determined with human transglutaminases: TG1 (Zedira product no. T009), TG3 (T012), TG4 (T042), TG5 (T023), TG6 (T021), TG7 (T011) and FXIII (T027).
Result: The EIA E018 is specific for TG2. It does not detect other human transglutaminases.

Specificity was further determinded with TG2 from different species: human (T002, T022, T034, T051), guinea pig (T006, T039), rat (T038), mouse (T040) and dog (T072).
Result: The EIA E018 is specific for human TG2. It does not detect TG2 from other species tested.

5. Contents of the kit

a. Microtiterplate, coated with tissue transglutaminase antibody and packed in a foil laminate pouch with a desiccant bag. The plate consists of 12 strips, each of which can be broken into 8 individual wells, thus providing maximum flexibility and economy in use of the assay. Adhesive tape to reseal unused wells are included.

b. Sample buffer, 50 mL, ready-to-use.

c. Wash buffer, 22 mL, 26x-concentrate.

d. Calibrator, >80 µg lyophilised tissue transglutaminse as a standard.

e. Conjugate anti-tTG antibody-HRP, 11 mL, ready-to-use.

f. Substrate solution, 11 mL, ready-to-use.
Contained in a vial impermeable to light.

g. Stop solution (5% H_2SO_4), 11 mL, ready-to-use.
Caution: sulfuric acid is corrosive.

h. Directions for use

6. Materials required but not supplied

a. Deionised or distilled water

b. Graduated cylinder, 500 mL

c. Low protein binding tubes, clean and dry (recommended)

d. Pipettes for 10 and 100 µL (1- and 8-channel pipettes recommended)

e. Microplate washer (optional)

f. Microplate photometer fitted with a 450 nm filter

g. Dry thermostat for 37°C

h. ELISA evaluation program (recommended)

7. Storage of the kit

Store the whole kit at 2 - 8°C. It is stable up to the expiry date stated on the label of the box. Do not use kit beyond its expiry date. After opening the pouch keep unused microtiter wells tightly sealed by adhesive tape (included) to minimize exposure to moisture.

8. Reagent preparation

Do not exchange or pool corresponding components from different kits, due to possibly different shipping or storage conditions.

a. Before opening the pouch of the solid phase, it must have reached room temperature. Remove the supernumerary microwells from the frame and immediately put them back into the pouch, together with the desiccant bag. Reseal the pouch hermetically and keep it refrigerated for future use.

b. Dilute 20 mL of wash buffer 26x-concentrate with 500 mL deionised water in a graduated cylinder. Mix thoroughly. The diluted washing solution is stable for 5 days at 18-25°C or 2 weeks at 2-8°C.

c. Reconstituting the standard: To make 100 µg/mL calibrator solution (Calibrator stock solution) reconstitute the lyophilized recombinant tissue transglutaminase **by adding 675 µL of deionized water** and mix gently until complete dissolution **followed by 1:2 dilution with sample buffer** (e.g. 250 µL calibrator + 250 µL sample buffer = stock).

Prepare calibrators using normal laboratory techniques and dilute them according the scheme below. Mix thoroughly. It is recommended to spin down shortly the tubes containing calibrators on a low speed centrifuge. Take care to avoid any contamination of the sample buffer by calibrator solutions. Calibrators are normally assayed in duplicate.

Scheme for calibrator preparation:

Calibrator No.	Concentration	Volume (taken from previous sample)	Sample buffer
1	10 µg/mL	60 µL (**stock**)	540 µL
2	7.5 µg/mL	360 µL (1)	120 µL
3	5 µg/mL	240 µL (2)	120 µL
4	1 µg/mL	100 µL (3)	400 µL
5	0.5 µg/mL	220 µL (4)	220 µL
6	0.2 µg/mL	160 µL (5)	240 µL
7	0.1 µg/mL	125 µL (6)	125 µL
8	0 (Blank)	--	250 µL

Note: Tissue transglutaminase preparation contains buffer salts and additives required for solubilizing the protein.

9. Specimen collection and storage

This kit is intended for use with biological samples. Grossly hemolytic, lipemic, or turbid samples should be avoided. Specimens may be stored short-term at 2-8°C before testing. For a longer storage, the specimens should be frozen at -20°C or lower. Repeated freezing/ thawing should be avoided.

10. Assay procedure

Before starting the assay, all components of the kit (including the desired number of unsealed microstrips) must have reached room temperature (23 ± 3°C). Put the desired number of microstrips into the frame. All reagents should be mixed by gentle inversion or vortexing prior to use. Do not allow foam formation. To achieve best results, careful washing is essential. It is important to remove the wash solution completely. For that purpose, tap the plate firmly on several layers of absorbent tissue. Automated washers must be verified according to results obtained by manual washing.

a. If suggested analyte concentration in the sample exceeds the highest calibrator, dilute this sample accordingly, using sample buffer.

b. Dispense the calibrators (reconstituted and diluted tissue transglutaminase, see 7. c) and the diluted samples rapidly into the microwells, 100 µL per well. Duplicate measurements are recommended.

   Incubate the plate for 30 minutes at 37 °C.

c. Wash the wells 3 times with 200 µL wash buffer.

d. Dispense 100 µL of Conjugate solution (ready-to-use) per well.

   Incubate the plate for 30 minutes at 37 °C.

e. Wash the wells 3 times with 200 µL wash buffer.

f. Rapidly dispense 100 µL of substrate solution (ready-to-use) per well.

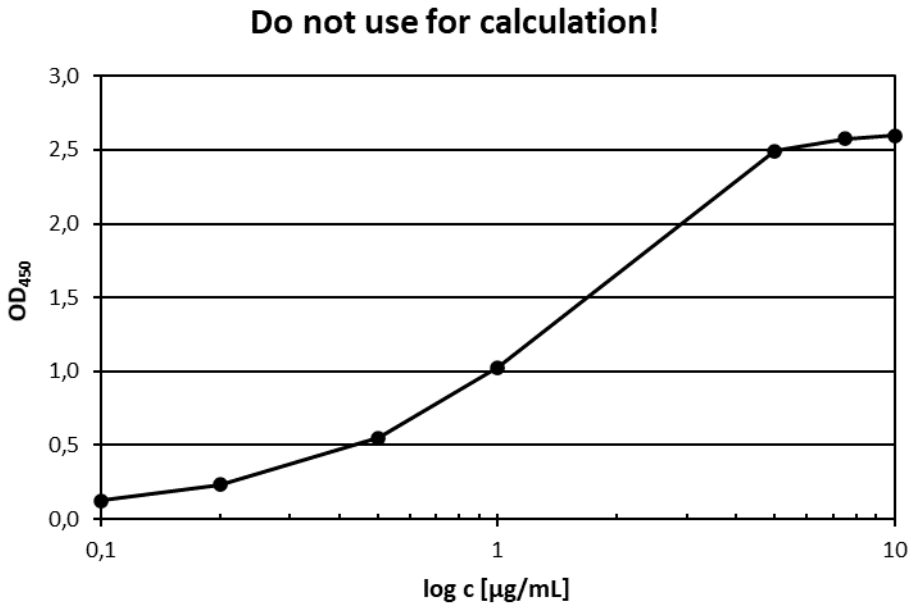
g. Incubate the plate for 15 min at 20-25°C. As the substrate is photosensitive, avoid intense light exposure (e.g. direct sunlight).

h. Rapidly dispense 100 µL stop solution (ready-to-use) per well. Use the same sequence as for the substrate. Agitate the plate, preferably on an orbital shaker, for about 10 seconds.

i. Immediately read the absorbance at 450 nm.

11. Evaluation

Quantitative evaluation: The data obtained are quantitatively evaluated with the standard curve, as shown below. However, the depicted curve can only serve as a model. It can not substitute the measurement of the calibrators, together with the controls and actual samples. Use 4-parameter or Spline functions of conventional ELISA evaluation programs to construct curve.



If no computer supported evaluation is possible, the standard curve may be drawn by hand. It allows transformation of the absorbance value of a sample into its concentration, i.e. into µg tTG/mL.