Directions for use

Microbial Transglutaminase (MTG) ELISA

ELISA for the detection of
Microbial Transglutaminase (MTG)

Art.-No. E021

For Research & Development Only

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1. Introduction and background

Microbial transglutaminase catalyzes acyl transfer reactions between the γ-carboxyamide group of a protein-bound glutamine residue and primary amines. In simple terms, microbial transglutaminase acts as a biological glue that covalently links proteins. Among others microbial transglutaminase is used in the food industry for the production of dairy products, meat, sausages and bakery products.

The Microbial Transglutaminase (MTG) ELISA (Product Nr. E021) is a sandwich enzyme immunoassay developed for the quantitative determination of native MTG. As part of the kit development, the following foods were examined: glued meat (raw), yoghurt, quark, cheese, salami and bread. It can be assumed that the test is also suitable for the analysis of further foods. Detection and determination limits depend on the respective sample matrix, the degree of processing and the extraction process.
Please note that for each type of sample material, the preparation procedure must be validated separately by the user.

All reagents for the implementation of the enzyme immunoassay are contained in the test kit. A test kit is sufficient for 96 determinations (including standards). A microtiter plate photometer is required for evaluation.

Sample preparation:	homogenizing, extracting
Standard material:	Microbial Transglutaminase (0 - 150 ng/mL)
Detection limit:	0.6 ng/mL (ppb) MTG (1 mL ≙ 1 g)
Determination limit:	3.0 ng/mL (ppb) MTG (1 mL ≙ 1 g)

2. Warnings and precautions

The *Sample buffer* and calibrators contain Na-azide as preservative. Na-azide may react with lead and copper plumbing to form explosive metal azides. On disposal, flush with a large amount of water to prevent azide build-up.
The wash buffer contains bromonitrodioxane (Bronidox) and the conjugates methylisothiazolone / bromonitrodioxane as preservative. The substrate contains 3, 3', 5, 5'-tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2).
The *Stop solution*, 0.2 M sulfuric acid (H_2SO_4), is acidic and corrosive.
All the above mentioned reagents may be toxic if ingested.
Follow routine precautions for handling hazardous chemicals. Avoid all body contact, wear gloves and eye protection. If one of the reagents comes into contact with skin, wash thoroughly with water.
Do not pipette reagents by mouth.
Do not use reagents after expiration date.
Do not mix or use components from kits with different lot numbers.

3. Principle of the test

The microtiter plate wells (solid phase) are coated with antibodies specific to microbial transglutaminase.

1st reaction: Microbial transglutaminase present in the sample binds to the immobilised antibodies, forming the antibody-antigen-complex.

2nd reaction: A microbial transglutaminase specific antibody labeled with biotin binds to the antibody-antigen-complex forming an antibody-antigen-[antibody-biotin] sandwich.

3rd reaction: Streptavidin conjugated to horseradish peroxidase (HRP) binds to the biotin-label fo the antibody-antigen-[antibody-biotin] sandwich.

4th reaction: The HRP-labeled complex converts a substrate (TMB) into a blue product which upon addition of the stop solution turns yellow. Samples containing microbial transglutaminase develop the blue colour (which upon addition of the stop solution turns yellow), whereas samples without microbial transglutaminase remain colourless.

4. Contents of the kit

Reagents and materials are sufficient for 96 determinations (including standards).

1x Microtiter plate (*Coated plate*) packed in a resealable foil laminate pouch with a dessicant bag. The plate consists of 12 strips coated with purified microbial transglutaminase antibody. The strips can be broken into 8 individual wells, thus providing maximum flexibility and economy in use of the assay.

1x *Sample buffer*, 100 mL, ready-to-use, orange coloured. Contains Tris-buffered saline (TBS), bovine serum albumin (BSA), Tween and Na-azide.

1x *Wash buffer*, 100 mL, 10x-concentrate, blue coloured. Contains TBS, Tween and bromonitrodioxane (Bronidox).

1x *MTG-standard*.
Stock solution for preparation of MTG-calibrators, ready-to-use.

1x Purified anti-microbial transglutaminase antibody labeled with biotin (*MTG-Ab conjugate*), 12 mL, ready-to-use, green coloured. Buffered solution containing stabilising protein, methylisothiazolone and bromonitrodioxane (Bronidox).

1x *Streptavidin-HRP* conjugate, 12 mL, ready-to-use, green colored. Buffered solution containing stabilising protein, methylisothiazolone and bromonitrodioxane (Bronidox).

1x *Substrate solution*, 14 mL, ready-to-use, colourless. Contains a buffered solution of TMB and hydrogen peroxide. Bottled in a vial impermeable to light.

1x *Stop solution* (0.2 M H_2SO_4), 14 mL, colourless, ready-to-use.
Caution: sulfuric acid is corrosive, wear gloves and eye protection.

1x Directions for use

5. Materials required but not supplied

- Microcentrifuge
- Homogenization device, according to the sample, e.g. stirrer for yoghurt, mixer for meat
- Deionised or distilled water
- Graduated measuring cylinder, 1,000 mL
- Transfer tubes (recommended)
- Pipettes for 10 µL, 100 µL and 1,000 µL
- Automatic dispenser for 100 µL (recommended)
- Microplate washer (optional)
- Microplate photometer fitted with a 450 nm and optionally a 620 nm filter as reference wavelength
- ELISA evaluation program (recommended)

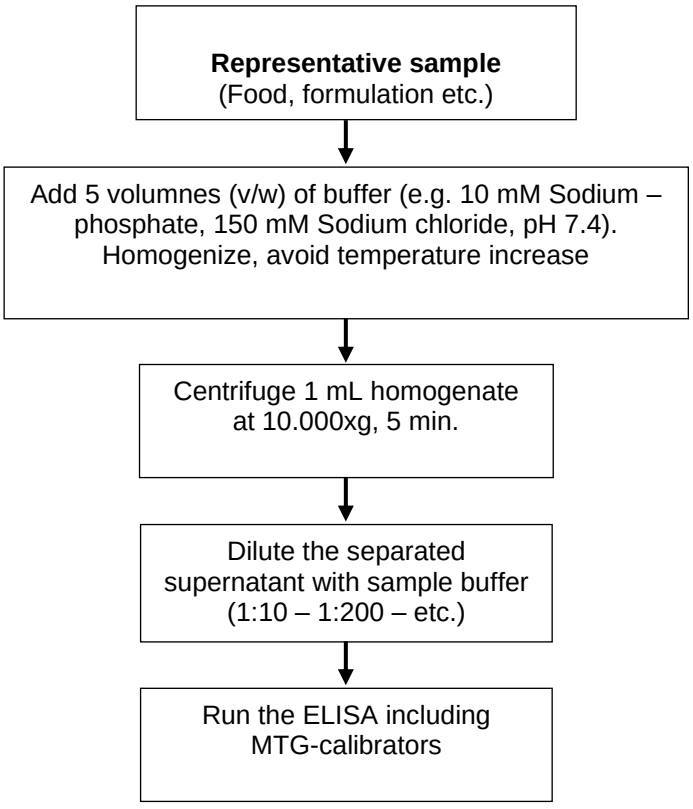
6. Storage of the kit

Store the whole kit at 6 - 8°C. It is important that the *MTG-standard* is not stored below 6°C. The kit 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 resealed to minimize exposure to moisture. *Substrate solution* is sensitive to light, therefore avoid direct exposure to light. After the expiry date (see kit label) no quality guarantee is given.

7. Sample preparation

Prepare samples using normal laboratory techniques. Thorough homogenization of the sample is mandatory. For samples like yoghurt intensive stirring is sufficient, solid samples like sausage and meat have to be homogenized, e.g. using a mixer. Homogenization has to be performed carefully in order to avoid temperature increase.
Due to possible matrix effects from food samples (yoghurt, quark cheese, sausage etc.) dilutions should be done over a broad range. (1:10 – 1:200 – etc.)

The following flowchart describes exemplary the sample preparation procedure for detection of MTG.
Please note that for each type of sample material the preparation procedure must be validated by the user.
The flow chart below is given as guiding principle.



8. Reagent and calibrators preparation

Do not exchange or pool corresponding components from different kits.

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 dessicant bag. Reseal the pouch hermetically and keep it refrigerated for future use.

b. Dilute the wash buffer 10x-concentrate (100 mL, blue) with 900 mL deionised water. Mix thoroughly. The diluted buffer is stable for several weeks if stored refrigerated.

c. Prepare calibrators using normal laboratory techniques and dilute them according to the scheme below. The usage of protein low bind tubes is highly recommended. Take care to avoid any contamination of the sample buffer by calibrator solutions. Calibrators have to be assayed at least in duplicate.

   Preparation of **MTG-calibrators**:

   Add **635 µL** of sample buffer to the vial with **MTG-standard** and preferably spin down shortly on a low speed centrifuge, mix gently afterwards.

   In a second step add **10 µL** of the above prepared MTG-standard dilution to **820 µL** sample buffer and mix gently.
   This dilution is called **MTG-standard (II)** and used for further dilutions as indicated in the table below.

Calibrator No.	Concentration	Volume **(taken from …)**	Sample buffer
1	150 ng/mL	**50** µL **MTG-standard (II)**	**350** µL
2	100 ng/mL	**50** µL **MTG-standard (II)**	**550** µL
3	75 ng/mL	**50** µL **MTG-standard (II)**	**750** µL
4	50 ng/mL	**40** µL **MTG-standard (II)**	**920** µL
5	25 ng/mL	**20** µL **MTG-standard (II)**	**940** µL
6	10 ng/mL	**100** µL **Calibrator No. 2**	**900** µL
7	5 ng/mL	**50** µL **Calibrator No. 2**	**950** µL
8	0 ng/mL (neg. control)	**-**	**500** µL

   Note: This ELISA determines microbial transglutaminase protein only. The deduction of microbial transglutaminase activity in the examined sample is not possible.

9. Assay procedure

Before starting the assay, all components of the kit must have reached room temperature (23 ± 3°C).

To achieve best results, i.e. the maximum ratio between specific and background signal, **careful washing** is essential. It is **crucially 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.

1. Immediately prior to use, wash the solid phase once: fill wells with **350 µL** wash buffer each, soak for about 10 seconds in the wells and remove.

2. Dispense the calibrators, negative control and the diluted samples rapidly into the MTG-antibody-coated microwells; **100 µL** per well. At least duplicate determinations are recommended. Incubate the plate for 30 minutes at room temperature (23 ± 3°C).

3. Wash the wells 4 times with **350 µL** *Wash buffer* each.

4. Rapidly dispense **100 µL** *MTG-Ab conjugate* (ready-to-use, green coloured) per well.
   Incubate the plate for 30 minutes at room temperature (23 ± 3°C).

5. Wash the wells 4 times with **350 µL** *Wash buffer* each.

6. Dispense **100 µL** of *Streptavidin-HRP conjugate* (ready-to-use, green coloured) per well. Incubate the plate for 30 minutes at room temperature (23 ± 3°C).

7. Wash the wells 4 times with **350 µL** *Wash buffer* each.

8. Rapidly dispense **100 µL** *Substrate solution* per well (ready-to-use, black vial); preferably using an automatic dispenser. **Incubate the plate for 30 minutes at room temperature (23 ± 3°C).** As the substrate is photosensitive, avoid intense light exposure (e.g. direct sunlight) during incubation.

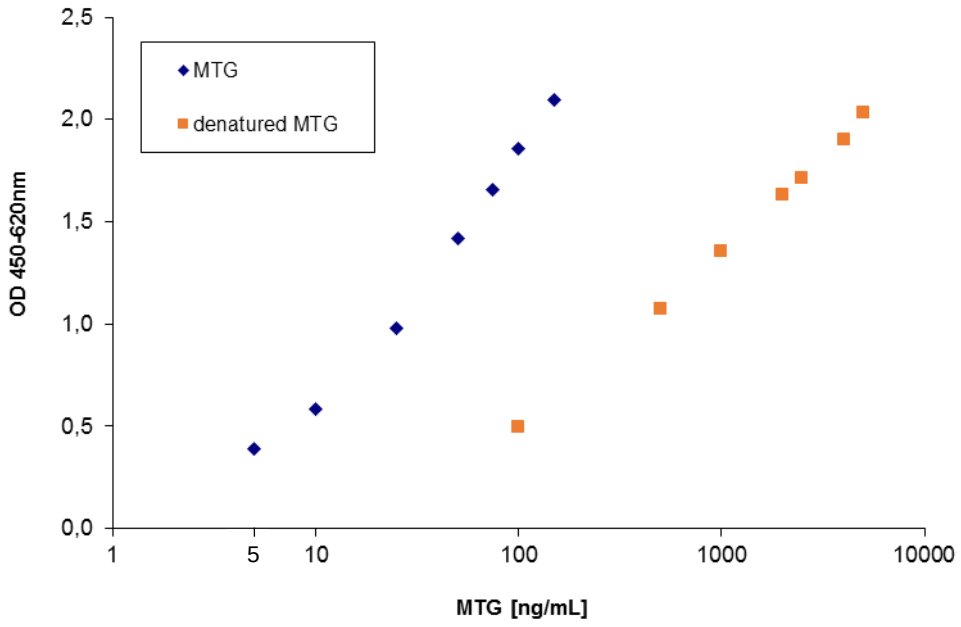
9. Rapidly dispense **100 µL** *Stop solution* per well (14 mL, ready-to-use, colourless); preferably using an automatic dispenser. Use the same sequence as for the *Substrate solution*. Agitate the plate, preferably on an orbital shaker, for about 10 seconds.
   Caution: sulfuric acid, wear gloves and eye protection.

10. Immediately read the absorbance at 450 nm or preferably at 450 nm minus 620 nm.

11. Evaluation and quality control

The data obtained are quantitatively evaluated with the standard curve for MTG respectively, 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 control and actual samples. The curve may be constructed with a conventional evaluation program, using a 4-parameter function. The Spline approximation is also appropriate.

Note:
This ELISA is developed for the detection of microbial Transglutaminase using an MTG-specific polyclonal antibody. However, this antibody not only allows the detection of MTG in its native conformation, but also the detection of denatured MTG. The sensitivity for denatured MTG is about a factor 20 lower than for native MTG.



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 ng/mL MTG.

12. Warranty

This information corresponds to the current state of our knowledge and is intended to provide information about the Microbial Transglutaminase (MTG) ELISA (Art. No. E021) and its possible uses.
Zedira GmbH makes no warranty of any kind, either expressed or implied, except that the test kit has been thoroughly tested to ensure that its properties specified herein are fulfilled. There is no warranty of the merchantability of this product or of the fitness of the product for any purpose.
The performance data presented here were obtained using the procedure indicated. Any modification in the procedure may affect the results in which case Zedira GmbH disclaims all warranties whether expressed, implied or statutory. Moreover, Zedria GmbH accepts no liability for any damage, whether direct, indirect or consequencial, which results from inappropriate use or storage of the product.

Summary flow chart

a. Dilute the centrifuged sample in *sample buffer* (ready-to-use, orange) and mix.

b. Dilute the *wash buffer* 10x-concentrate (100 mL, blue) with water and mix.

c. Arrange the suitable number of coated wells. Wash the wells once with 350 µL *wash buffer* each. Remove *wash buffer* completely.

d. Dispense 100 µL of the calibrators and of the diluted samples into the coated wells of the solid phase.
Duplicate measurements are recommended.

e. Incubate for 30 minutes at room temperature (23 ± 3°C).

f. Wash the wells 4 times with 350 µL wash buffer each.

g. Dispense 100 µL of the *MTG-Ab conjugate* (ready-to-use, green coloured) into the wells.

h. Incubate for 30 minutes at room temperature (23 ± 3°C).

i. Wash the wells 4 times with 350 µL *wash buffer* each.

j. Dispense 100 µL of *Streptavidin-HRP conjugate* (ready-to-use) per well.

k. Incubate for 30 minutes at room temperature (23 ± 3°C).

l. Wash the wells 4 times with 350 µL *wash buffer* each.

m. Dispense 100 µL of the *substrate solution* (ready-to-use, black vial) per well.

n. Incubate for 30 minutes at room temperature (23 ± 3°C).

o. Then, add 100 µL *stop solution* (ready-to-use, colourless) per well and agitate the plate briefly.

p. Immediately measure the absorbance at 450 nm
(or preferably at 450 nm minus 620 nm).

Quantitative evaluation: Determine the standard curve from the absorbance of the calibrators. Using this curve, transform the absorbance of the samples into their respective concentration (ng/mL MTG).

Store the remainder of the reagents refrigerated if they are to be used again.