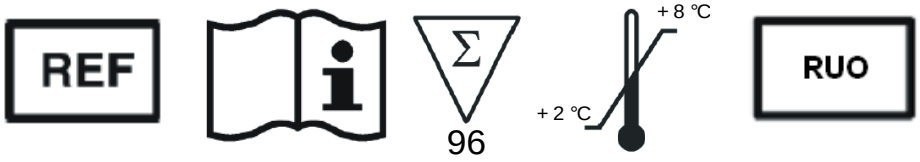
Manual

Anti-human tissue transglutaminase sIgA / IgA

ELISA

For the determination of anti-human tissue transglutaminase sIgA / IgA in stool

Valid from 16.06.2021



IC6400

ImmuChrom GmbH
Lise-Meitner-Str. 13
D 64646 Heppenheim
Tel.: ++49 6252 910084
Fax: ++ 49 6252 910070
info@immuchrom.de

Table of contents Page

1. Intended use

The *ImmuChrom* ELISA Kit is intended for the quantitative determination of anti-human tissue transglutaminase sIgA / IgA in stool. For *research* use only.

2. Introduction

Celiac disease is a chronic illness of the small intestinal mucous membrane. The reason is an intolerance against gluten, which is found in many cereals. This food intolerance against cereal proteins leads to a production of auto-antibodies against tissue transglutaminase, which is the major antigen for endomysium antibodies.

The intake of gluten-containing food leads to an inflammation of the small intestinal mucous membrane, which results in a reduced absorption of nutrients. The symptoms of the disease are reduction of weight, diarrhea, vomitus, anorexia and tiredness. The growth of children is impaired. The characteristic of the symptoms might be different. The only therapeutic treatment is a gluten-free diet. A non-treated celiac disease increases the risk of non-Hodgkin-lymphoma and colon cancer. Celiac disease is associated with type 1 diabetes mellitus in five to ten percent of the patients. Women are more often affected than men. The outcome of the disease is pronounced during infancy and in ages between 30 and 40 years old.

Applications

- Food intolerance
- Monitoring of an elimination diet

The ImmuChrom complete anti-human tissue transglutaminase sIgA / IgA kit allows an easy, rapid and precise quantitative determination of the antibodies in biological samples. The kit includes all reagents ready to use for preparation of the samples.

3. Warnings and precautions

All reagents of this kit are strictly intended for *research* use only.

Do not interchange kit components from different lots.

The stop solution (STOP) contains acid and has to be handled carefully. It is corrosive and causes burns. It should be handled with gloves, eye protection and appropriate protective clothing in a hood. Any spill should be wiped up immediately with copious quantities of water. Do not breath vapor and avoid inhalation. In case of an accident or indisposition contact a physician immediately.

The substrate TMB (tetramethyl benzidine) is toxic by ingestion and contact with the skin. Any spill should be wiped out immediately with copious quantities of water.

Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.

Do not pipette by mouth.

Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.

The reagents of the test kit contain bactericides to protect against bacterial growth. Avoid the contact with the skin or mucous membrane.

Reagents should not be used beyond the expiration date shown on kit label.

Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera. During handling of all kit reagents, controls and serum samples observe the existing legal regulations.

4. Material delivered in the test package

Article no.	Component	Description	Amount
IC6400mtp	MTP	Microtiter plate coated	12 x 8 wells
IC6300wp	WASHBUF	Anti-gliadin / anti transglutaminase ELISA wash buffer conc. 10-fold	100 ml
IC6300pb	SAMPLEBUF	Sample buffer	500 ml
IC6400sp	STABBUF	Stabilization buffer	7 ml
IC6400st	STD	Standards (1 ml) (25; 50; 100; 200 mU/g)	5 vials
IC6400ko	CTRL	Controls (2 levels, 1 ml)	1 vial each
IC6400kg	CONJ	Conjugate, peroxidase-labeled antibody	15 ml
IC6000su	SUB	TMB substrate (tetramethylbenzidine)	15 ml
IC6300sp	STOPP	Stop solution	10 ml

5. Additional special equipment

- Centrifuge, 3000xg
- Plastic vials
- Stool sample extraction vials
- Various pipettes
- Multichannel- or multipipette
- Foil to cover the microtiter plate
- Bidest. water
- ELISA reader with filter 450 nm (reference filter 620)
- Microtiter plate shaker
- Vortex mixer

6. Reagent preparation

Microtiter plate (MTP). Take the needed number of stripes and assemble them on the holder. Please take care that the plate has reached room temperature before usage. Stripes which are not needed yet must be stored at 2-8°C. Please do not dispose of the holder until all stripes are used.

Wash buffer (WASHBUF). Dilute the wash buffer concentrate 1:10 with bidest. water (1 part buffer + 9 parts bidest. water). The dilution is stable for 14 days at 2-8°C.

Important: When storing the wash buffer concentrate at 2-8°C crystallization may occur. Before dilution, all crystals must be dissolved.

It is recommended to dilute only the amount of buffer which is used to process the given samples.

All other test reagents are stable at 2-8 °C up to the date of expiry stated on the label, unless otherwise specified.

7. Specimen

Stool samples

The antibodies are extracted by the sample buffer out of the stool sample.

Extraction in Stool extraction vials

In a stool sample extraction vial mix 15 mg stool with 0.75 ml SAMPLEBUF, then vortex it until the mixture is homogenous. Transfer the resulting slurry to a plastic vial and centrifuge it for 10 min at 3000 xg.

The supernatant is directly pipetted into the microtiter plate wells with no further dilution (please, refer to step 2 of “Sample preparation” for details).

8. Procedure

Principle of the method

The anti-tissue transglutaminase-ELISA test determines anti-tissue transglutaminase sIgA / IgA antibodies according to the “sandwich”-principle. Anti-tissue transglutaminase antibodies in sample, standard and controls bind to tissue transglutaminase, which is coated to the microtiter plate. After a washing step a peroxidase labeled detection antibody is added. A second washing step is followed by the addition of the substrate which is converted to a colored product by the peroxidase. The reaction is terminated by the addition of an acidic stop solution. The optical densities are measured at 450 nm (against the reference wavelength 620 nm) in a microtiter plate reader. The anti-tissue transglutaminase concentration can be calculated from the standard curve.

Sample preparation

All reagents and samples should be warmed up (20-30 °C) and should be mixed well before use.

The position of standards, controls and samples should be noted down in advance on a protocol sheet.

1. Washing step

Pick out the pre-assembled microtiter plate with the needed number of stripes and wash them 5x with 250 µl diluted WASHBUF. Remove residual buffer by tapping the plate on absorbent paper after the washing step.

2. Samples incubation

Pipette **50 µl STABBUF** into all sample wells – not into the wells of the standards and controls.

Pipette **100 µl STD**, **CTRL** or **50 µl** of the supernatant of the **samples** in double values into the microtiter plate.

Cover the stripes with a cover film and incubate the microtiter plate by shaking for **60 min** (20-30 °C).

3. Washing step

Discard the content of the microwells and wash 5x with 250 µl diluted WASHBUF. Remove residual buffer by tapping the plate on absorbent paper after the last washing step.

4. Conjugate incubation

Pipette **100 µl CONJ** in each microwell.

Cover the stripes with a cover film and incubate the microtiter plate by shaking for **60 min** (20-30 °C).

5. Washing step

Discard the content of the microwells and wash 5x with 250 µl diluted WASHBUF. Remove residual buffer by tapping the plate on absorbent paper after the last washing step.

6. Substrate incubation

Pipette **100 µl SUB** in each microwell.

Incubate for **10-15 min** in the dark (20-30 °C).

7. Stopping reaction

Pipette **50 µl STOPP** in each microwell. Mix well.

8. Reading

Read the absorbance at 450 nm. If the microtiter plate reader allows to use a reference wavelength use 620 as reference wavelength.

Reading should be done within 5 min after stopping reaction.

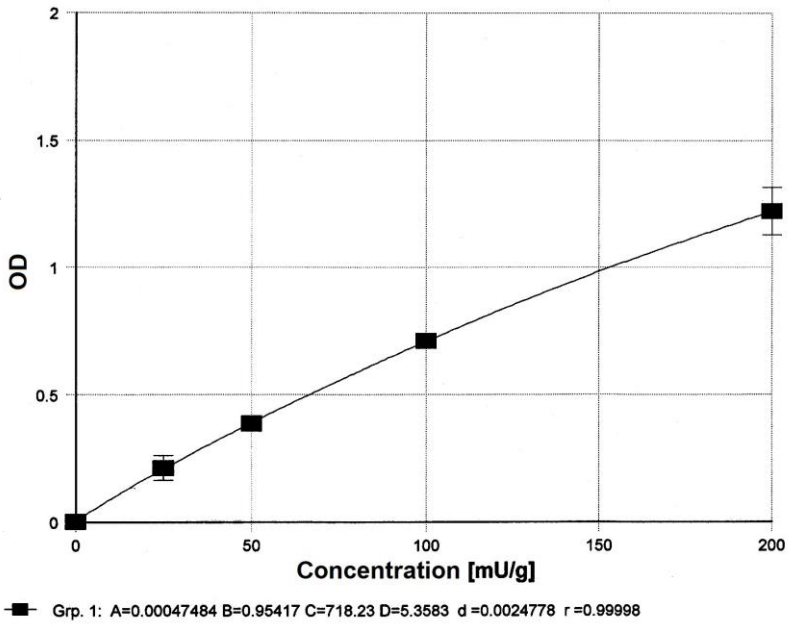
9. Calculation of analytical results

For calculating the results, a “point to point” curve is recommended.

Stool samples

The anti-transglutaminase concentration is read from the standard curve.

Standard curve



The curve given above is only for demonstration. It must not be used for calculation of your samples.

10. Internal quality control

Reference values

Stool: < 100 mU/g

We recommend that each laboratory should develop their own normal range. The values mentioned above are only for orientation and can deviate from other published data.

11. Validation data

Precision and reproducibility

Intra-Assay CV:	7.7 % (7.2 mU/g)	[n = 10]
	2.9 % (105.1 mU/g)	[n = 10]
	2.5 % (206.5 mU/g)	[n = 10]
Inter-Assay CV:	9.1 % (12.4 mU/g)	[n = 10]
	6.5 % (79.0 mU/g)	[n = 10]
	7.6 % (171.7 mU/g)	[n = 10]

Linearity

The linearity of the test ranges from 25 to 200 mU/g stool.

Detection limit

Stool 2.1 mU/g

For the determination, the zero-standard was measured 20 times. The 2-fold standard deviation was added to the mean value of the optical density. The respective concentration was read from the standard curve.

Recovery

Sample	Endogen [mU/g]	Added	Expected [mU/g]	Measured [mU/g]	Recovery [%]
1	9.8	33.3 100.0 150.0	43.1 109.8 159.8	39.5 100.7 141.1	91.6 91.7 88.3
2	12.1	33.3 100.0 150.0	45.4 112.1 162.1	46.5 90.9 134.9	102.4 81.1 83.2

12. Limitations of the method

Stool samples with anti-transglutaminase antibody concentrations above the standard curve should be diluted with sample buffer (SAMPLEBUF) and measured again.

13. Disposal

The substrate (SUB) must be disposed as non-halogenated solvent. The stop solution (STOPP) can be neutralized with NaOH and if the pH value is neutral, it can be disposed as salt solution (**important:** this reaction produces heat and should be handled carefully).

Please refer to the appropriate national guidelines.

distributed in the US/Canada by:
EAGLE BIOSCIENCES, INC.
20A NW Blvd, Suite 112 Nashua, NH 03063
Phone: 617-419-2019 FAX: 617-419-1110
www.EagleBio.com • info@eaglebio.com

EAGLE
BIOSCIENCES