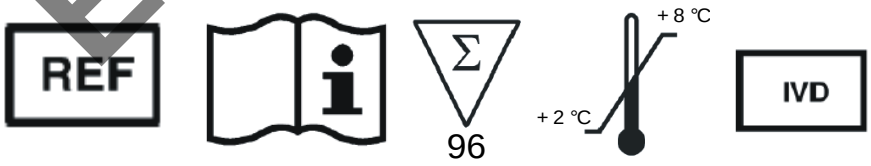
Manual

Anti-human tissue transglutaminase sIgA / IgA

ELISA

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

Valid from 22.11.2022



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1. Intended use

The *ImmuChrom* ELISA Kit is intended for the quantitative determination of anti-human tissue transglutaminase sIgA / IgA in stool. For *in vitro* diagnostic use by trained personnel in laboratories only.

2. Introduction

In the case of gluten intolerance, the body reacts hypersensitively to the gluten proteins (gliadin and glutenin) that are present in grain. 80-90% of patients have atypical or no symptoms and often do not know anything about their disease. In people with celiac disease, the intake of gluten leads to inflammation of the intestinal mucosa. The intestinal villi recede. Since the diseased intestinal mucosa can no longer absorb enough nutrients, a vitamin and mineral deficiency can occur. Hereditary factors (95% of patients have the HLA-DQ2 and HLA-DQ8 genes; up to 5% have the HLA-DR4 gene) play an important role in celiac disease, but the immune system and environmental factors also influence its development. Women get sick than men. According to the German Celiac Society, more recent screening tests show that the frequency of the disease is now between 1:100 and 1:200.

Antibodies against transglutaminase and/or gliadin can be detected in patients with gluten enteropathy. These antibodies can be detected regardless of whether the disease is manifest or silent or latent. In contrast to manifest gluten enteropathy, silent or latent forms do not show any atrophy of the villi of the small intestine, but pronounced intestinal or extraintestinal symptoms can still be present.

The detection of anti-transglutaminase sIgA / IgA in the stool is suitable for screening and therapy monitoring. Elevated titers are not evidence of celiac disease (2). Celiac disease is detected by serum tests and a histological finding of villous atrophy.

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. General notes, warnings and precautions

This assay was produced and put on the market according to the IVD guidelines of 98/79/EC. All reagents of this kit are strictly intended for in vitro diagnostic use only.

Individual components from different batches and test kits should not be interchanged. The expiry dates stated on the relevant packaging must be observed.

The test kit reagents contain preservatives to protect against bacterial growth. Therefore contact with the skin and/or mucous membranes should be avoided.

The test kit contains components of human origin. The starting reagents were tested for antibodies against HIV1/2, hepatitis B and anti-HCV using immunoassay methods. All parameters tested were found negative. As a precautionary measure, all test kit reagents should always be treated as potentially infectious material in accordance with health care accident prevention regulations.

The substrate TMB (tetramethylbenzidine) is toxic by ingestion and skin contact. In the event of contact with the skin, the affected area must be washed immediately with plenty of water and soap.

Avoid contact of the stop solution, which consists of acid, with the skin. It causes burns on contact. You should therefore work with protective gloves and goggles. In the event of contact, the burned area must be immediately and thoroughly rinsed with plenty of water. If necessary, a doctor should be consulted.

Adherence to the prescribed protocol for performing the test is essential. ImmuChrom GmbH assumes no liability for any damage caused by unauthorized changes in the test procedure.

The guidelines for carrying out quality control in medical laboratories must be observed. Appropriate controls must be carried along.

The reagents must not be used after the expiration date.

Wear disposable gloves when handling specimens or kit reagents and wash hands thoroughly afterwards. Do not pipette by mouth. Do not eat, drink, smoke, or put on makeup in areas where specimens or kit reagents are being handled.

Patient samples may contain unknown interfering substances. This can lead to false high or false low results.

The final clinical diagnosis should not be based on the results of a single test, but should be considered by a physician only after all clinical and laboratory results have been evaluated.

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) The concentrations are given in the specification	5 vials

IC6400ko	CTRL	Controls (2 levels, 1 ml) The concentrations are given in the specification	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
- Vortex mixer
- Various pipettes
- Multichannel- or multipipette
- Foil to cover the microtiter plate
- Bidest. water
- Microtiter plate shaker
- ELISA reader with filter 450 nm (reference filter 620)

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 in a ration of 1:50 (e.g. 20 mg/ml).

Extraction in Stool extraction vials

For the extraction stick vials could be used.

We recommend to 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 3000xg.

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.

Calibration: Calibration was done in relative units as no higher order reference material is available.

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, 400 rpm; 2 mm orbit).

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, 400 rpm; 2 mm orbit).

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** by shaking in the dark (20-30 °C, 400 rpm; 2 mm orbit).

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.

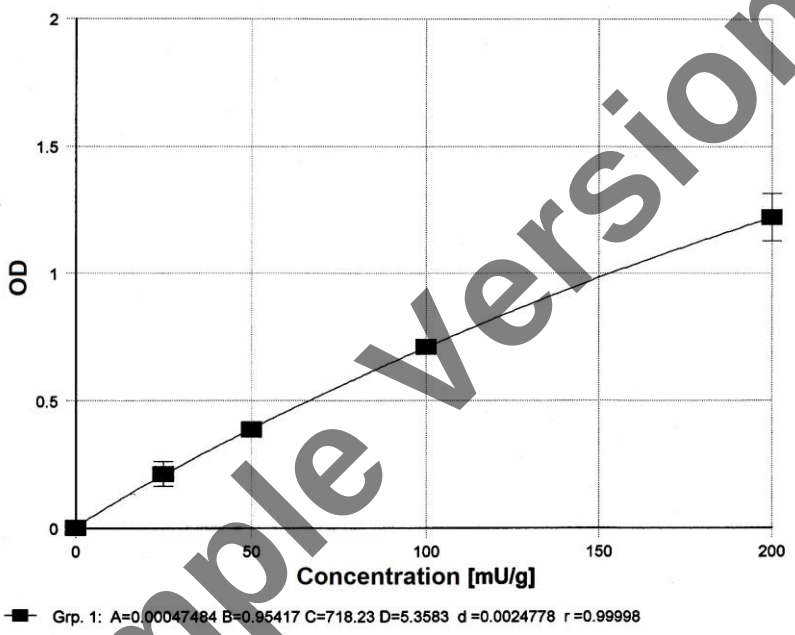
9. Calculation of analytical results

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

(Ref: Martin (Hrsg.), Das Standardlabor in der naturheilkundlichen Praxis, 4. Auflage, 2014 ISBN 978-3-437-56303-4)

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

Measuring range

The measuring range of the anti-transglutaminase sIgA / IgA is between a sample concentration of 25 mU/g and 200 mU/g

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

2.9 mU/g

For the determination, the zero-standard was measured 20 times. The 3-fold standard deviation was added to the mean value of the optical density. The respective concentration was read from the standard curve.

Limit of quantification

7.9 mU/g

For the determination, the zero-standard was measured 20 times. The 10-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

Cross reactivity

Cross reactivities could not be determined in an autoantibody test

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.

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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.

14. Literature references

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