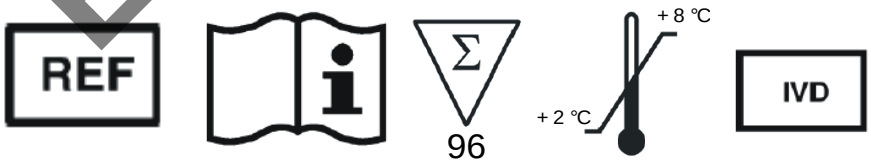
Manual

Secretory IgA

ELISA

For the determination of sIgA in stool

Valid from 28.12.2022



IC6100

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1. Intended use

This ELISA kit works according to the "sandwich principle" and determines the secretory immunoglobulin A (sIgA) in the stool. Only for in vitro diagnostics by trained personnel in laboratories.

2. Introduction

The sIgA consists of two immunoglobulin A molecules, which are connected to each other via a J protein (joining) and a secretory component. The secretory component is synthesized by the epithelial cells of the mucous membranes of the gastrointestinal, respiratory and urogenital tracts, as well as in the salivary, lacrimal and mammary glands. The plasma cells in the subendothelial space of the mucous membranes secrete a complex of two IgA molecules linked via the J protein. This complex then binds to the secretory component that sits on the surface of the epithelial cell. After binding, the sIgA is transported through the epithelial cell and excreted at the mucosal surface by exocytosis.

The determination of the secretory IgA (sIgA) provides an initial overview of the functional status of the gut-associated immune system (GALT). Here, the secretion capacity and the degree of stimulation of the plasma cells in the submucosa of the intestine are recorded.

Taking non-steroidal anti-inflammatory drugs (e.g. aspirin, ibuprofen, diclofenac) or COX-2 inhibitors (e.g. celecoxib) can lead to enteropathies, which result in an increase in inflammatory parameters in the stool (10). Before carrying out the determination, the corresponding medication should therefore not be taken for a period of 14 days, so as not to influence the measurement of the degree of intestinal inflammation.

Indications

- Allergic disease
- Increased liability for infections
- Inflammatory processes in the gut
- Autoimmune disease

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. Standards and controls contain sIgA from human colostrum. The starting reagent was examined for all parameters required by the FDA. 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 wear 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
IC6100mtp	MTP	Mikrotiter plate coated	12 x 8 wells
IC6100wp	WASHBUF	ELISA wash buffer conc. 10 fold	100 ml
IC6100st	STD	Standards (1.0 ml) The concentrations are given in the specification	5 vials
IC6100ko	CTRL	Controls (2 levels, 1.0 ml) The concentrations are given in the specification	1 vial each
IC6100kg	CONJ	Conjugate, peroxidase-labeled antibody	15 ml
IC6100su	SUB	TMB substrate (tetramethylbenzidine)	15 ml
IC6100sp	STOPP	Stop solution	7 ml

5. Additional special equipment

- Centrifuge, 3000xg
- Plastic reaction 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 20-30 °C 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

sIgA is extracted by the extraction buffer out of the stool sample in a ratio 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** diluted wash buffer (WASHBUF), then vortex it until the mixture is homogenous. Transfer the resulting slurry to a plastic vial and centrifuge it for 10 min at 3000 x g.

Dilute the supernatant **1:250** with diluted wash buffer (e.g. 4 µl supernatant + 996 µl diluted wash buffer)

8. Procedure

Principle of the method

The sIgA-ELISA test determines human secretory IgA according to the “sandwich”-principle. sIgA in sample, standard and controls binds to antibodies, which are 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 read at 450 nm (against the reference wavelength 620 nm) in a microtiter plate reader. The sIgA concentration can be calculated from the standard curve.

Sample preparation

All reagents and samples should be at 20-30 °C and mixed well before use.

The position of standards, controls and samples are noted on a protocol sheet.

1. Washing step

Take out the pre-assembled microtiter plate with the needed number of stripes and wash them 1x with 250 µl diluted WASHBUF per cavity. Remove residual buffer by tapping the plate on absorbent paper after the washing step.

2. Incubation samples

Pipette **100 µl STD**, **CTRL** and diluted **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 per cavity. Remove residual buffer by tapping the plate on absorbent paper after the last washing step.

4. Incubation conjugate

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. Incubation substrate

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

Incubate by shaking for **10-15 min** in the dark (20-30 °C, 400 rpm; 2 mm orbit diameter).

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 nm as reference wavelength.

Reading should be done within 5 min after stopping reaction.

9. Calculation of analytical results

For calculating the results, we recommend to use the 4-parameter-Marquardt algorithm.

Stool samples

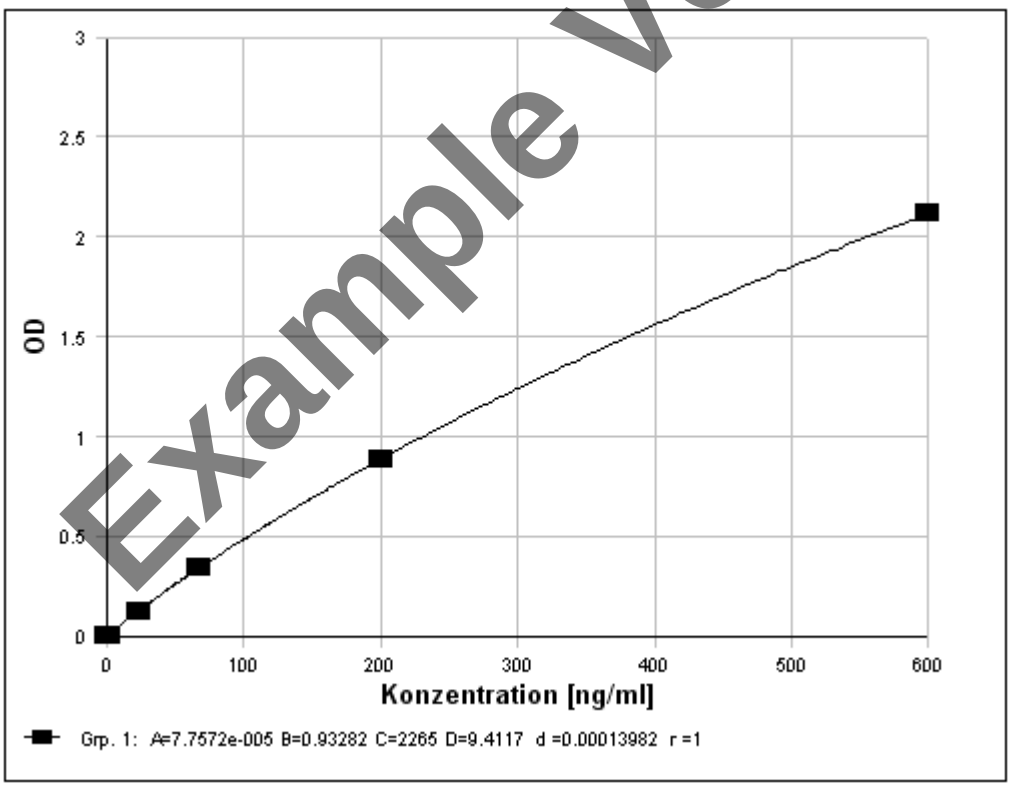
The obtained sIgA concentration is multiplied with **12.5**

Dilution 1: 15 mg in 0.75 ml corresponds to a factor **50** (assumption: 1 g stool = 1 ml)

Dilution 2: Factor **250** (4 µl supernatant + 996 µl diluted wash buffer)

Calculation: Conc. Patient [µg/ml] = obtained conc. [ng/ml] x 50 x 250 / 1000

Standard curve



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

10. Internal quality control

Reference values

Stool: 510 - 2040 µg/ml

Ref: M. Martin (Hrsg.). Gastroenterologische Aspekte in der Naturheilkunde
ISBN 3-930620-29-4; S.31

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 s-IgA is between a sample concentration of 277.5 – 7500 µg/ml.

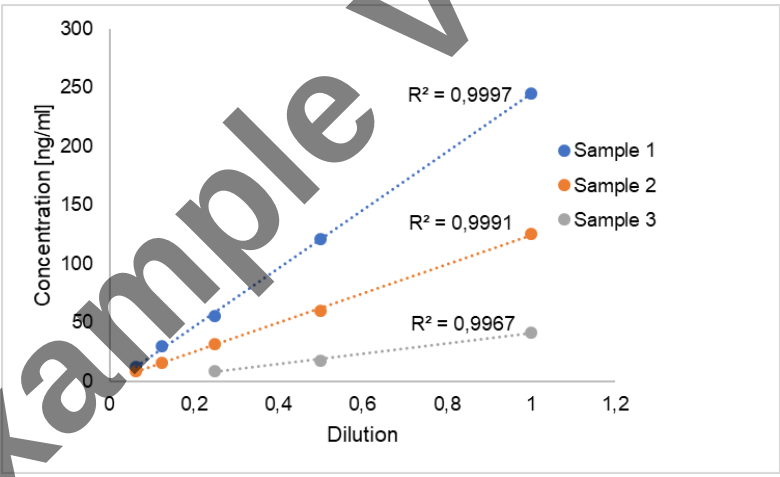
Precision and reproducibility

Intra-assay CV:	5.4 % (224.4 ng/ml)	[n = 10]
	4.5 % (111.9 ng/ml)	[n = 10]
	6.3 % (33.4 ng/ml)	[n = 10]
Inter-assay CV:	6.0 % (227.4 ng/ml)	[n = 10]
	5.0 % (108.4 ng/ml)	[n = 10]
	8.2 % (31.8 ng/ml)	[n = 10]

Linearity

The dilution of the samples was done with diluted wash buffer.

Sample	Dilution factor	Expected [ng/ml]	Measured [ng/ml]	Recovery [%]
1	-- 1:2 1:4 1:8 1:16	-- 123 61.3 30.6 15.3	245 121 55.6 29.4 12.3	 98.4 90.8 96.0 80.3
2	-- 1:2 1:4 1:8 1:16	-- 62.3 31.1 15.6 7.8	125 59.4 31.2 15.5 8.5	 95.4 100 99.6 109
3	-- 1:2 1:4	-- 20.6 10.3	41.2 17.7 8.5	 85.9 82.5



Detection limit

3.0 ng/ml

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.

Limit of quantification

7.5 ng/ml

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	Endogenous [ng/ml]	Added [ng/ml]	Expected [ng/ml]	Measured [ng/ml]	Recovery [%]
1	31.5	22.2 66.6 200	53.7 98.1 232	56.9 106 279	106 108 120
2	112	22.2 66.6 200	135 179 312	128 181 336	94.7 101 108
3	249	22.2 66.6 200	271 316 449	279 353 484	103 112 108

Cross reactivity

Cross reactivity to other plasma proteins could not be detected in stool samples.

12. Limitations of the method

Stool samples with sIgA concentrations above the standard curve should be diluted with diluted wash buffer (WASHBUF) and measured again.

In the case of severe diarrhea, reduced levels can be measured despite an intact state of the gut-associated immune system.

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

1. Dzidiz et al 2017, Aberrant IgA responses to the gut microbiota during infancy precede asthma and allergy development. J. Allergy Clin. Immunol. 1017-1025

2. Edman et al 2018, Increased Cathepsin S activity associated with decreased protease inhibitory capacity contributes to altered tear proteins in Sjögren's Syndrome patients. Sci Rep 11044.

3. Siddiqui et al 2017. Update on clinical and research application of fecal biomarkers for gastrointestinal diseases. *World J Gastrointest Pharmacol Ther* 2017 February 6; 8(1): 39-46

4. M. Martin (Hrsg) 2000. Gastroenterologische Aspekte in der Naturheilkunde. ISBN 3-930620-29-4 S.28ff

Referenzbereich: < 0,27 mg/g (2)

6. Velikova et al, 2020. Lactobacilli Supplemented with Larch Arabinogalactan and Colostrum Stimulates an Immune Response towards Peripheral NK Activation and Gut Tolerance. Nutrients, DOI 10.3390/nu12061706

7. Mijatovic et al. 2018. Biomedical approach in autism spectrum disorders—the importance of assessing inflammation. AIMS Molecular Science, 5(3): 173–182 (Aussage: 68% der Patienten mit Autismus zeigten abnormale sIgA Werte, Allgemein gesteigerte Aktivität des GALT)

8. Beckmann, Rüffer (2000). Mikroökologie des Darmes. ISBN 3-87706-521-X D. S. 261-262

9. Martin Hrsg. (2013) Das Standardlabor in der naturheilkundlichen Praxis S. 412, 432f. ISBN 978-3-437-56303-4

10. Tibble JA, et al. 1999. High prevalence of NSAID enteropathy as shown by a simple faecal test. Gut. 45(3): 362-6