

Instructions for Use



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Secretory IgA ELISA

For the determination of sIgA in stool and saliva.

REF 30132345

 96

For illustrative purposes only.

To perform the assay the instructions for use provided with the kit have to be used

Distributed by: **IBL International GmbH**
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Always there for you



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1. Intended use

The *ImmuChrom* ELISA Kit is intended for the quantitative determination of sIgA in stool and saliva. For in vitro diagnostic use by trained personnel in laboratories only.

2. Introduction

Secretory IgA is comprised of two immunoglobulin A molecules, which are joined by a J-protein and a secretory component. The secretory component is synthesized by epithelial cells of the mucous membrane of gastrointestinal, respiratory and urogenitaltract. It is also produced by the saliva, tear and mammary glands. The plasma cells in the subendothelial area of mucous membranes are releasing a complex of two IgA-molecules, which are joined over the J-protein. This complex is binding to a secretory component, located at the surface of the epithel cell. After binding, the sIgA is transported across the cell and excreted by exocytosis.

The determination of secretory IgA (sIgA) allows a first overview of the functionality of the gastrointestinal associated immune system (GALT). At this the secretory power and the degree of stimulation of the plasma cells of the intestinal submucosa is determined.

Indications

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

The *ImmuChrom* complete sIgA kit allows an easy, rapid and precise quantitative determination of secretory IgA 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 in vitro diagnostic use only.

This assay was produced and put on the market according to the IVD guidelines of 98/79/EC.

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 out immediately with copious quantities of water. Do not breath vapor and avoid inhalation. In case of an accident or indisposition contact immediately a physician.

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 testkit 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.	Compont	Description	Amount
IC6100mtp	MTP	Mikrotiterplate coated	12 x 8 wells
IC6100wp	WASHBUF	ELISA waschbuffer conc. 10 fold	100 ml
IC6100st	STD	Standard (1 ml) (0; 22,2; 66,6; 200; 600)	5 vials
IC6100ko	CTRL	Control 1 and 2 (1 ml)	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

- Laboratory balance
- Centrifuge, 2000xg
- Stool sample extraction vials
- Various pipettes
- Foil to cover the microtiterplate
- Multichannel or multipipette
- ELISA reader with filter 450 nm (reference filter 620 or 690 nm)
- Microtiterplate shaker
- Vortex mixer

6. Reagent preparation

Microtiterplate (MTP). Take the needed strips out of the bag and mount them on the holder. Please take care that the package has reached room temperature before opening the bag. Stripes which are not needed yet could be stored at 2-8°C. Please dispose the holder when all stripes are used.

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

Important: When storing the washbuffer concentrate at 2-8°C crystalization could 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.

7. Specimen

Stool samples

slgA is extracted by the sample dilution buffer out of the stool sample.

Extraction in glass or plastic vials

100 mg stool are mixed with 5 ml washbuffer on a vortex mixer until the mixture is homogenous.

1 ml of the mixture is transferred into an "Eppendorf" reaction vial and centrifuged for 10 min at 2000xg.

Dilute the supernatant 1:250 with washbuffer (4 µl + 996 µl washbuffer)

100 µl of the dilution are used in the test per well.

Extraction in stick vials

Alternatively stick vials can be used for extraction.

We recommend to use 20 mg stool per ml extractionbuffer (washbuffer). In case of using a 15 mg stick vial 0.75 ml of washbuffer should be filled in the vials.

When the top of the stick is submersed in the buffer it can be left over night at 2-8 °C to improve solution.

The suspension is mixed on a vortex mixer and centrifuged for 10 min at 2000xg.

Dilute the supernatant 1:250 with washbuffer (4 µl + 996 µl washbuffer)

100 µl of the dilution are used in the test per well.

Saliva samples

To get a good comparability between different patient samples, we recommend to take the samples at the same time. The patient should not eat or drink 30 min before taking the sample. The sample should be stored or sent on ice.

The sample is centrifuged for 10 min at 2000xg. The centrifugation will create a sediment, a liquid phase and a foamy supernatant.

Dilute the liquid phase 1:10000 with WASHBUF

Dilution A: 10 µl + 990 µl WASHBUF

Dilution B: 10 µl + 990 µl WASHBUF

100 µl of the dilution B are used in the test per well.

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 microtiterplate. 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 microtiterplate reader. The sIgA concentration can be calculated from the standard curve.

Sample preparation

All reagents and samples should have room temperature (18-26°C) and mixed well before use.

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

1. Washing step

Take out the neededstrips of the microtiter plate and wash 1x with 250 µl diluted WASHBUF. Remove residual buffer by tapping the plate on absorbent paper after the washing step.

2. Incubation samples

Pipette **100µl STD, CTRL** and **samples** in double values in the microtiterplate.

The stripes are covered and incubated by shaking for **60 min** at room temperature (18-26 °C).

The reaction starts on pipetting to the antibody coated microwell. Pipetting should be as quickly as possible. When processing many samples at once the samples should be pipetted to a separate microtiterplate (150 µl) and transferred simultaneously using a multichannel pipette.

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. Incubation conjugate

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

The stripes are covered and incubated by shaking for **60 min** at room temperature (18-26 °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. Incubation substrate

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

Incubate for **10-15 min** at room temperature (18-26 °C) in the dark.

7. Stopping reaction

Pipette **50 µl STOPP** in each microwell, mix well.

8. Reading

Read the absorbance at 450 nm. If the microtiterplate reader allows to use a reference wavelength use 620 or 690 nm as reference wavelength.

Reading should be done within 5 min after stopping reaction.

In case that the highest standard exceeds the range of the reader the reading should be done at 405 nm against 620 nm (690 nm).

9. Calculation of analytical results

For calculating the results we recommend to use the 4-parameter algorithm. Is this algorithm not available a "point to point" or a "spline" function can be used.

Stool samples

The obtained sIgA concentration is multiplied with **12,5**

Dilution 1: 100 mg in 5 ml corresponds to a factor **50** (assumption: 1 g stool = 1 ml)

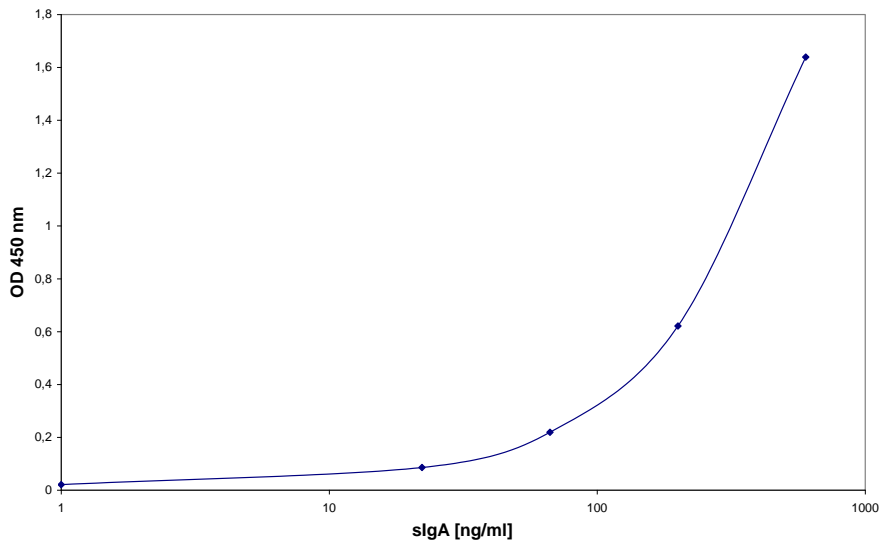
Dilution 2: Factor **250**

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

Saliva samples

The obtained sIgA concentration [ng/ml] is multiplied with **10** to get calculated concentration in µg/ml.

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: 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

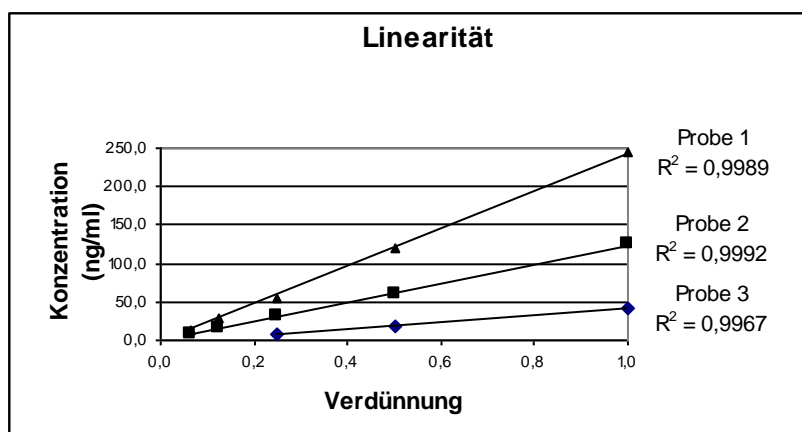
Precision and reproducibility

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 WASHBUF.

Sample	Dilution faktor	Expected [ng/ml]	Measured [ng/ml]	Recovery [%]
1	--	--	245	
	1:2	122,5	120,5	98,4
	1:4	61,3	55,6	90,8
	1:8	30,6	29,4	96,0
	1:16	15,3	12,3	80,3
2	--	--	124,5	
	1:2	62,3	59,4	95,4
	1:4	31,1	31,2	100,2
	1:8	15,6	15,5	99,6
	1:16	7,8	8,5	109,2
3	--	--	41,2	
	1:2	20,6	17,7	85,9
	1:4	10,3	8,5	82,5



Detection limit

3.1 ng/ml

For the determination of the detection limit 20 replicates of the standard 0 were measured. After addition of the twofold standard deviation to the mean value the concentration was read from the standard curve.

Recovery

Sample	Endogen [ng/ml]	Added	Expected [ng/ml]	Measured [ng/ml]	Recovery [%]
1	31,5	22,2	53,7	56,9	106,0
		66,6	98,1	105,8	107,8
		200	231,5	278,6	120,3
2	112,4	22,2	134,6	127,5	94,7
		66,6	179,0	180,9	101,1
		200	312,4	335,7	107,5
3	248,9	22,2	271,1	279,4	103,1
		66,6	315,5	352,7	111,8
		200	448,9	484,1	107,8

Cross reactivity

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

12. Limitations of the method

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

In case of strong diarrhea it is possible that even patients with an intact gut associated immune system show lowered values.

13. Disposal

The substrate (SUB) must be disposed as non-halogenated solvent. The stop solution (STOPP) could be neutralized with NaOH and if the pH value is neutral it can be disposed as salt solution. (**Important:** Reaction will produce heat, be careful)

Please refer to the appropriate national guidelines.

14. Literature references

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

Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.-Cat.: / Αριθμός-Κατ.:
	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλισμένο
	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di valutazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazemar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbicante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!
<p>Symbols of the kit components see MATERIALS SUPPLIED.</p> <p>Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.</p> <p>Voir MATERIEL FOURNI pour les symbôles des composants du kit.</p> <p>Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.</p> <p>Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.</p> <p>Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.</p> <p>Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.</p>	

COMPLAINTS: Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

WARRANTY: The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

LIMITATION OF LIABILITY: IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER'S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.

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