Manual

IDK® sIgA ELISA

For the in vitro determination of secretory IgA in saliva and stool

For Reference Use Only

Valid from 2022-02-11

REF **K 8870** Σ 96

REF **K 8870.20** Σ 20 x 96

+8°C
+2°C

IVD

CE

Immundiagnostik AG, Stubenwald-Allee 8a, 64625 Bensheim, Germany
Tel.: +49 6251 70190-0 Fax: + 49 6251 70190-363
e.mail: info@immundiagnostik.com www.immundiagnostik.com

Table of Contents

For Reference Use Only

1. INTENDED USE

This Immundiagnostik AG assay is an enzyme immunoassay intended for the quantitative determination of secretory IgA (sIgA) in saliva and stool. For *in vitro* diagnostic use only.

2. INTRODUCTION

Secretory IgA (sIgA) consists of two IgA monomers joined by the J-chain and an additional secretory component. It is secreted in plasma cells located in the *lamina propia* of mucosal membranes. Synthesis of sIgA is independent from the synthesis of serum IgA. This means that lack of serum IgA does not necessarily correlate with a lack of sIgA1. Secretory IgA is the major immunoglobulin in saliva, tears, colostrum, nasal mucous, mother's milk, tracheobronchial and gastrointestinal secretes. It plays a major role in preventing adherence of microorganisms to mucosal sites, in activation of the alternative complement pathway and in activating inflammatory reactions. Newborns are provided with sIgA by mother's milk and are passively immunised against gastrointestinal infections.

Indications

- Proof of an imbalanced immunological barrier on the intestinal mucosa
- Autoimmune disease

3. MATERIAL SUPPLIED

Art. no.	Label	Kit components	Quantity for cat. no.	
			K 8870	K 8870.20
K 8870	PLATE	Microtiter plate, pre-coated	12 x 8 wells	20 x 12 x 8 wells
K 0001.C.100	WASHBUF	Wash buffer concentrate, 10x	2 x 100 ml	-
K 8870	CONJ	Conjugate concentrate, peroxidase-labelled (mouse anti-sIgA)	1 x 200 µl	15 x 200 µl
K 8870	STD	Standards, lyophilised (0; 22.2; 66.6; 200; 600 ng/ml)	2 x 5 vials	25 x 5 vials

For Reference Use Only

Art. no.	Label	Kit components	Quantity for cat. no.	
			K 8870	K 8870.20
K 8870	CTRL1	Control, lyophilised (see specification for range)	2 x 1 vial	25 x 1 vial
K 8870	CTRL2	Control, lyophilised (see specification for range)	2 x 1 vial	25 x 1 vial
K 0002.15	SUB	Substrate (tetramethylbenzidine), ready-to-use	1 x 15 ml	20 x 15 ml
K 0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml	20 x 15 ml
K 6999.C.100	IDK Extract®	Extraction buffer concentrate *IDK Extract*® 2.5x	2 x 100 ml	–

For reorders of single components, use the catalogue number followed by the label as product number.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Calibrated precision pipettors and 10–1000 µl single-use tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Centrifuge, 3000 *g*
- Vortex
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* Immundiagnostik AG recommends the use of ultrapure water (water type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

For Reference Use Only

5. STORAGE AND PREPARATION OF REAGENTS

- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. **Prepare only the appropriate amount necessary for each run.** The kit can be used up to 4 times within the expiry date stated on the label.
- Reagents with a volume less than **100 µl** should be centrifuged before use to avoid loss of volume.
- **Preparation of the wash buffer:** The **wash buffer concentrate (WASHBUF)** has to be diluted with ultrapure water **1:10** before use (100 ml WASHBUF + 900 ml ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37 °C. The **WASHBUF** can be used until the expiry date stated on the label when stored at **2–8 °C**. **Wash buffer** (1:10 diluted WASHBUF) can be stored in a closed flask at **2–8 °C for 1 month**.
- **Preparation of the extraction buffer:** The **extraction buffer concentrate *IDK Extract*®** has to be diluted with ultrapure water **1:2.5** before use (100 ml *IDK Extract*® + 150 ml ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at 37 °C in a water bath. The ***IDK Extract*®** can be used until the expiry date stated on the label when stored at **2–8 °C**. **Extraction buffer** (1:2.5 diluted *IDK Extract*®) can be stored in a closed flask at **2–8 °C for 4 months**.
- The **lyophilised standards (STD)** and **controls (CTRL)** can be used at **2–8 °C** until the expiry date stated on the label. Before use, the STD and CTRL have to be reconstituted with **500 µl of ultrapure water** and mixed by gentle inversion to ensure complete reconstitution. Allow the vial content to dissolve for 10 minutes and then mix thoroughly. **Standards and controls** (reconstituted STD and CTRL) **can be stored at -20 °C for 4 weeks and can be subjected to a maximum of two freeze-thaw cycles**.
- **Preparation of the conjugate:** Before use, the **conjugate concentrate (CONJ)** has to be diluted **1:101** in **wash buffer** (100 µl CONJ + 10 ml wash buffer). The **CONJ** can be used at **2–8 °C** until the expiry date stated on the label. **Conjugate** (1:101 diluted CONJ) **is not stable and cannot be stored.**
- All other test reagents are ready-to-use. Test reagents can be used until the expiry date (see label) when stored at **2–8 °C**.

For Reference Use Only

6. STORAGE AND PREPARATION OF SAMPLES

Sample stability

The **sample stability** is as follows:

Raw stool: 2 days at room temperature (15–30 °C), 2 days at 2–8 °C or 8 weeks at -20 °C

Stool extracts (1:100): 1 day at room temperature (15–30 °C), 7 days at 2–8 °C or 7 days at -20 °C, maximum 2 freeze-thaw cycles

Saliva: 1 day at 2–8 °C, 4 weeks at -20 °C

Saliva

To avoid variation in sIgA content, take saliva samples always at the same time of the day. No food or liquid should be consumed 30 min before sample collection. Collect saliva samples using salivettes and centrifuge at 3000 *g* for 10 min.

For analysis, the **saliva supernatant** is diluted **1:2000 in wash buffer**, e. g.

10 µl saliva supernatant + **990 µl** wash buffer = **dilution I** (1:100)

50 µl dilution I + **950 µl** wash buffer = **dilution II** (1:20)

Final dilution: 1:2000

For analysis, pipet **100 µl** of **dilution II** per well.

Extraction of the stool samples

Extraction buffer (1:2.5 diluted *IDK Extract®)* is used as a **sample extraction buffer**. We recommend the following sample preparation:

Stool Sample Application System (SAS) (Cat. No.: K 6998SAS)

Stool sample tube – Instructions for use

Please note that the dilution factor of the final stool suspension depends on the amount of stool sample used and the volume of the buffer.

SAS with 1.5 ml sample extraction buffer:

Applied amount of stool: 15 mg

Buffer Volume: 1.5 ml

Dilution Factor: 1:100

Please follow the instructions for the preparation of stool samples using the SAS as follows:

For Reference Use Only

a) The raw stool sample has to be thawed. For particularly heterogeneous samples we recommend a mechanical homogenisation using an applicator, inoculation loop or similar device.

b) Fill the **empty stool sample tube** with 1.5 ml **sample extraction buffer** (1:2.5 diluted *IDK Extract®)* before using it with the sample. **Important:** Allow the sample extraction buffer to reach room temperature.

c) Unscrew the tube (yellow part of cap) to open. Insert the yellow dipstick into the sample. The lower part of the dipstick has notches which need to be covered completely with stool after inserting it into the sample. Place dipstick back into the tube. When putting the stick back into the tube, excess material will be stripped off, leaving 15 mg of sample to be diluted. Screw tightly to close the tube.

d) Vortex the tube well until no stool sample remains in the notches. **Important:** Please make sure that you have a maximally homogenous suspension after shaking. Especially with more solid samples, soaking the sample in the tube with sample extraction buffer for ~10 minutes improves the result.

e) Allow sample to stand for ~10 minutes until sediment has settled. Floating material like shells of grains can be neglected.

f) Carefully unscrew the complete cap of the tube including the blue ring plus the dipstick. Discard cap and dipstick. Make sure that the sediment will not be dispersed again.

Dilution I: 1:100

Dilution of stool samples

The supernatant of the sample preparation procedure (dilution I) is further diluted **1:125 in wash buffer**. For example:

40 µl dilution I + **960 µl** wash buffer (mix well) = **dilution II** (1:25)

200 µl dilution II + **800 µl** wash buffer (mix well) = **dilution III** (1:5)

Final dilution: 1:12 500

For analysis, pipet **100 µl of dilution III** per well.

For Reference Use Only

7. ASSAY PROCEDURE

Principle of the test

This ELISA is intended for the quantitative determination of secretory IgA in stool and saliva. In a first incubation step, the sIgA in the samples is bound to polyclonal antibodies (rabbit anti human IgA), which are immobilised to the surface of the microtiter wells. To remove all unbound substances, a washing step is carried out. In a second incubation step, a peroxidase-labelled conjugate (mouse anti-sIgA) is added which recognises specifically the bound secretory IgA. After another washing step, to remove all unbound substances, the solid phase is incubated with the substrate, tetramethylbenzidine (TMB). An acidic stop solution is then added to stop the reaction. The colour converts from blue to yellow. The intensity of the yellow colour is directly proportional to the concentration of secretory IgA. A dose response curve of the absorbance unit (optical density, OD) vs. concentration is generated, using the results obtained from the standards. Secretory IgA, present in the patient samples, is determined directly from this curve.

Test procedure

Bring all **reagents and samples to room temperature** (15–30 °C) and mix well.

Mark the positions of standards/controls/samples on a protocol sheet.

Take as many microtiter strips as needed from kit. Store unused strips together with the desiccant bag in the closed aluminium packaging at 2–8 ° C. Strips are stable until expiry date stated on the label.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact your supplier or Immundiagnostik AG.

We recommend to carry out the tests in duplicate.

1.	Before use, wash the wells **5 times** with **250 µl wash buffer**. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
2.	Add each **100 µl standards/controls/diluted samples** into the respective wells.
3.	Cover the strips and incubate for **1 hour on a horizontal shaker*** at room temperature (15–30 °C).

For Reference Use Only

4.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
5.	Add **100 µl conjugate** (diluted CONJ) in each well.
6.	Cover the strips and incubate for **1 hour on a horizontal shaker*** at room temperature (15–30 °C).
7.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
8.	Add **100 µl substrate** (SUB) in each well.
9.	Incubate for **10–20 minutes**** at room temperature (15–30 °C) **in the dark.**
10.	Add **100 µl stop solution** (STOP) and mix well.
11.	Determine **absorption immediately** with an ELISA reader at **450 nm** against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at **405 nm** against 620 nm as a reference.

* We recommend shaking the strips at 550 rpm with an orbit of 2 mm.

** The intensity of the colour change is temperature sensitive. We recommend observing the colour change and stopping the reaction upon good differentiation.

8. RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend using the "4 parameter algorithm".

1. 4 parameter algorithm

It is recommended to use a linear ordinate for the optical density and a logarithmic abscissa for the concentration. When using a logarithmic abscissa, the zero standard must be specified with a value less than 1 (e. g. 0.001).

2. Point-to-point calculation

We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

For Reference Use Only

3. Spline algorithm

We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

The plausibility of the duplicate values should be examined before the automatic evaluation of the results. If this option is not available with the programme used, the duplicate values should be evaluated manually.

Saliva

The obtained results have to be multiplied by the **dilution factor of 2 000** to get the actual concentrations.

Stool

The obtained results have to be multiplied by the **dilution factor of 12 500** to get the actual concentrations.

In case **another dilution factor** has been used, multiply the obtained result by the dilution factor used.

9. LIMITATIONS

Samples with concentrations above the measurement range (see definition below) can be further diluted and re-assayed. Please consider this higher dilution when calculating the results.

Samples with concentrations lower than the measurement range (see definition below) cannot be clearly quantified.

The upper limit of the measurement range can be calculated as:

highest concentration of the standard curve × *sample dilution factor to be used*

The lower limit of the measurement range can be calculated as:

LoB × *sample dilution factor to be used*

LoB see chapter "Performance Characteristics".

10. QUALITY CONTROL

Immundiagnostik AG recommends the use of external controls for internal quality control, if possible.

Control samples should be analysed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid if within the

For Reference Use Only

same assay one or more values of the quality control sample are outside the acceptable limits.

Reference range

Secretory IgA in saliva (saliva samples collected using salivettes)

Children (n = 37)	18–237 µg/ml (mean 128 µg/ml)*
Age >16 years (n = 33)	102–471 µg/ml

* Hofman LF, Le T (2002) Preliminary pediatric reference range for secretory IgA in saliva using an enzyme immunoassay. Clinical Chemistry 48 (6):A169, Suppl.

Secretory IgA in stool 510–2040 µg/ml (n = 76)*

* Based on Immundiagnostik studies of stool samples of apparently healthy persons

We recommend each laboratory to establish its own reference range.

11. PERFORMANCE CHARACTERISTICS

Accuracy – Precision

Repeatability (Intra-Assay); n = 20

The repeatability was assessed with 2 stool samples under **constant** parameters (same operator, instrument, day and kit lot).

Sample	Mean value [µg/ml]	CV [%]
1	971.0	5.6
2	1 136.0	5.8

Reproducibility (Inter-Assay); n = 12

The reproducibility was assessed with 2 stool samples under **varying** parameters (different operators, instruments, days and kit lots).

Sample	Mean value [µg/ml]	CV [%]
1	1 279.6	8.2
2	1 277.4	7.7

For Reference Use Only

Accuracy – Trueness

The trueness states the closeness of the agreement between the result of a measurement and the true value of the measurand. Therefore, sIgA spikes with known concentrations were added to 2 different stool samples. The results below were obtained without consideration of the sample dilution factor.

Sample [ng/ml]	Spike [ng/ml]	Expected [ng/ml]	Obtained [ng/ml]	Recovery [%]
112.8	150.0	262.8	278.5	106.0
	75.0	187.8	194.9	103.8
	50.0	162.8	165.3	101.8
	25.0	137.8	149.7	108.6
	150.0	262.8	289.7	110.2
	150.0	262.8	295.0	112.2
	50.0	162.8	164.8	101.2
	50.0	162.8	146.8	106.0
109.3	150.0	259.3	271.2	104.6
	75.0	184.3	212.2	115.2
	50.0	159.3	170.9	107.3
	25.0	134.3	135.9	101.2
	150.0	259.3	250.3	96.6
	50.0	159.3	160.2	100.6

Linearity

The linearity states the ability of a method to provide results proportional to the concentration of analyte in the test sample within a given range. This was assessed according to CLSI guideline EP6-A with a serial dilution of 2 different stool samples.

For secretory IgA in stool and saliva, the method has been demonstrated to be linear from 26.8 to 276.8 ng/ml based on the standard curve without considering possibly used sample dilution factors, showing a non-linear behaviour of less than ± 20 % in this interval.

For Reference Use Only

Sample	Dilution	Expected [ng/ml]	Obtained [ng/ml]	Recovery [%]
A	1:12 500	276.0	276.0	100.0
	1:25 000	138.0	107.3	77.7
	1:50 000	69.0	53.8	78.0
	1:187 500	36.8	29.2	79.3
B	1:12 500	214.4	214.4	100.0
	1:25 000	107.2	96.3	89.8
	1:50 000	53.6	57.4	107.0
	1:100 000	26.8	28.8	107.4

Analytical specificity

The specificity of the antibody was tested by measuring the cross-reactivity against a range of compounds with structural similarity to sIgA. There was no cross-reactivity observed.

Substance tested	Concentration added	Concentration obtained [ng/ml]	Conclusion
α1-antitrypsin	90 µg/l	< 2.088	< LoB
Albumin	800 µg/l	< 2.088	< LoB
PMN elastase	40 ng/ml	< 2.088	< LoB
Lysozyme	30 ng/ml	< 2.088	< LoB
Hemoglobin	1 000 µg/ml	< 2.088	< LoB
Hemoglobin-hapto-globin complex	40 mU/l	< 2.088	< LoB
CRP	150 ng/ml	< 2.088	< LoB
Pancreatic amylase	28 333 mU/l	< 2.088	< LoB
Chymotrypsin	1 000 ng/ml	< 2.088	< LoB
Myeloperoxidase	100 ng/ml	< 2.088	< LoB

For Reference Use Only

Analytical sensitivity

The following values have been estimated based on the concentrations of the standards without considering possibly used sample dilution factors.

Limit of blank, LoB	2.088 ng/ml
Limit of detection, LoD	6.947 ng/ml
Limit of quantitation, LoQ	11.965 ng/ml

The evaluation was performed according to the CLSI guideline EP-17-A2. The specified accuracy goal for the LoQ was 20 % CV.

12. PRECAUTIONS

- All reagents in the kit package are for *in vitro* diagnostic use only.
- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- Kit reagents contain sodium azide or ProClin as bactericides. Sodium azide or ProClin are hazardous to health and the environment. Substrates for enzymatic colour reactions may also cause skin and/or respiratory irritation. Any contact with the substances must be avoided. Further safety information can be found in the safety data sheet, which is available from Immundiagnostik AG on request.
- The 10x Wash buffer concentrate (WASHBUF) contains surfactants which may cause severe eye irritation in case of eye contact

  Warning: Causes serious eye irritation. **IF IN EYES:** Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: get medical Advice/attention.
- The stop solution consists of diluted sulphuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped up immediately with copious quantities of water. Do not breath vapour and avoid inhalation.

13. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch.

For Reference Use Only

- Control samples should be analysed with each run.
- Reagents should not be used beyond the expiration date stated on kit label.
- Substrate solution should remain colourless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- The guidelines for medical laboratories should be followed.
- *IDK*® and *IDK Extract*® are trademarks of Immundiagnostik AG.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.
- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.

15. REFERENCES

General literature

1. Brandtzaeg, P., 2010. Update on mucosal immunoglobulin A in gastrointestinal disease. *Current opinion in gastroenterology*, **26**(6), pp.554–63.
2. Corthésy, B., 2012. Autoimmunity Reviews Role of secretory IgA in infection and maintenance of homeostasis. *Autoimmunity Reviews*.

For Reference Use Only

Literature using K8870

3. Kalach, N. et al., 2013. Intestinal permeability and fecal eosinophil-derived neurotoxin are the best diagnosis tools for digestive non-IgE-mediated cow's milk allergy in toddlers. *Clinical chemistry and laboratory medicine : CCLM / FESCC*, **51**(2), pp.351–61.

4. Kaur, R. et al., 2012. Antibody in middle ear fluid of children originates predominantly from sera and nasopharyngeal secretions. *Clinical and vaccine immunology : CVI*, **19**(10), pp.1593–6.

5. Kabeerdoss, J. et al., 2011. Effect of yoghurt containing Bifidobacterium lactis Bb12® on faecal excretion of secretory immunoglobulin A and human beta-defensin 2 in healthy adult volunteers. *Nutrition journal*, **10**(1), p.138.

6. Senol, A. et al., 2011. Effect of probiotics on aspirin-induced gastric mucosal lesions. *The Turkish journal of gastroenterology : the official journal of Turkish Society of Gastroenterology*, **22**(1), pp.18–26.

7. Chalkias, A. et al., 2011. Patients with colorectal cancer are characterized by increased concentration of fecal hb-hp complex, myeloperoxidase, and secretory IgA. *American journal of clinical oncology*, **34**(6), pp.561–6.

8. Mohan, R. et al., 2008. Effects of Bifidobacterium lactis Bb12 supplementation on body weight, fecal pH, acetate, lactate, calprotectin, and IgA in preterm infants. *Pediatric research*, **64**(4), pp.418–22.

9. Hofman, L. & Le, T., 2002. Preliminary pediatric reference range for secretory IgA in saliva using an enzyme immunoassay. *Clinical Chemistry*, **48**(6, Supplement), p.A169-70.

For Reference Use Only

Used symbols:

 Temperature limitation

In Vitro Diagnostic Medical Device

 Manufacturer

Lot number

 Attention

Consult specification data sheet

Catalogue number

To be used with

Contains sufficient for <n> tests

 Use by

 Consult instructions for use

 Irritant

For Reference Use Only

For Reference Use Only

Immundiagnostik AG
Stubenwald-Allee 8a
64625 Bensheim, Germany
Tel.: +49 6251 70190-0
Fax: +49 6251 70190-363
info@immundiagnostik.com
www.immundiagnostik.com