**Manual**

# sIgA ELISA Kit

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

*EU: IVD / CE*

*US: Research Use Only. Not for use in diagnostic procedures.*

Valid from 02.09.2014

REF **K 8870**

Σ 96

+8 °C / +2 °C

IVD

CE

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# Table of Contents

## 1. INTENDED USE

This Immundiagnostik Assay is 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 immunized against gastrointestinal infections.

**Indications**

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

## 3. MATERIAL SUPPLIED

| Cat. No. | Label | Kit components | Quantity |
|---|---|---|---|
| K 8870MTP | PLATE | Holder with precoated strips | 12 x 8 wells |
| K 8870WP | WASHBUF | ELISA wash buffer concentrate 10 x | 2 x 100 ml |
| K 8870K | CONJ | Conjugate concentrate (mouse anti-sIgA, peroxidase-labeled) | 1 x 200 µl |
| K 8870ST | STD | Standards, lyophilized (0; 22.2; 66.6; 200; 600 ng/ml) | 2 x 5 vials |
| K 8870KO1 | CTRL | Control, lyophilized | 2 x 1 vial |
| K 8870KO2 | CTRL | Control, lyophilized | 2 x 1 vial |
| K 8870TMB | SUB | TMB substrate (tetramethylbenzidine), ready to use | 1 x 15 ml |
| K 8870AC | STOP | ELISA stop solution, ready to use | 1 x 15 ml |
| K 8870EP | IDK Extract® | Extraction buffer concentrate *IDK Extract®*, 2,5 x | 2 x 100 ml |

## 4. MATERIAL REQUIRED BUT NOT SUPPLIED

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

* Immundiagnostik AG recommends the use of Ultra Pure 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).

## 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.
- The **ELISA wash buffer concentrate** (WASHBUF) should be diluted **1:10 in ultra pure water** before use (100 ml concentrate + 900 ml ultra pure water), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be redissolved at room temperature or 37 °C before dilution of the buffer solutions. The **buffer concentrate** is stable at **2–8 °C** until the expiry date stated on the label. **Diluted buffer solution** (wash buffer) can be stored in a closed flask at **2–8 °C for one month**.
- The **extraction buffer concentrate *IDK Extract*®** must be diluted with ultra pure water **1:2.5** before use (100 ml concentrate + 150 ml ultra pure water ), mix well. Crystals could occur due to high salt concentration in the stock solutions. Before dilution, the crystals must be redissolved at 37°C in a water bath. The **extraction buffer concentrate *IDK Extract*®** is stable at **2 - 8 °C** until the expiry date stated on the label. Diluted **buffer solution** (extraction buffer) can be stored in a closed flask at **2 - 8 °C for three months**.
- The **lyophilized standards** (STD) and **controls** (CTRL) must be reconstituted with **500 µl of ultra pure water**. Allow the vial content to dissolve for 10 mi-

nutes and mix thoroughly by gentle inversion to ensure complete reconstitution. **Reconstituted standards and control are stable at -20 °C until the expiry date stated on the label and can be subjected to a maximum of two freeze-thaw cycles**

- The **conjugate concentrate** (CONJ) must be diluted **1:101 in wash buffer** (100 µl CONJ + 10 ml wash buffer). The concentrate is stable at **2–8 °C** until the expiry date stated on the label. **Diluted conjugate is not stable and cannot be stored.**
- All other test reagents are ready to use. Test reagents are stable until the expiry date (see label of test package) when stored at **2–8 °C**.

## 6. STORAGE AND PREPARATION OF SAMPLES

### *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 rpm for 10 min.

For analysis, the **saliva supernatant** is diluted **1:2000 in ELISA 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**

Use **100 µl of the final dilution** per well.

### *Extraction of the stool samples*

**Diluted extraction buffer *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 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:

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 sample tube** with **1.5 ml** of ready to use *IDK Extract®* extraction buffer before using it with the sample. Important: Allow the extraction buffer to reach room temperature.

c) Unscrew the tube (orange part of cap) to open. Insert the orange 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) Shake 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 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: 1:12500**

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

## *Stability of stool samples*

The **sample stability** is as follows:

**Raw stool**: 24 hours at 4 °C, 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

## 7. ASSAY PROCEDURE

### *Principle of the test*

This Enzyme-Linked-Immuno-Sorbent-Assay (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 immobilized 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-labeled conjugate (mouse anti-sIgA) is added which recognizes 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 color converts from blue to yellow. The intensity of the yellow color 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 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.

Take as many **microtiter strips** as needed from kit. Store unused strips covered at 2–8 ° C. Strips are stable until expiry date stated on the label.

We recommend to carry out the tests in duplicate.

| | |
|---|---|
| 1. | Wash the pre-coated microtiter plate **5 x with 250 µl ELISA wash buffer before use**. After the final washing step, the inverted microtiter plate should be tapped on absorbent paper. |
| 2. | Add **100 µl STD** (standards), **CTRL** (controls) and **diluted patient samples** into respective wells. |
| 3. | Incubate for **1 hour** shaking on a horizontal mixer at room temperature (15–30 °C). |
| 4. | Aspirate and wash the wells **5 x with 250 µl ELISA wash buffer**. After the final washing step, the inverted microtiter plate should be tapped on absorbent paper. |

| | |
|---|---|
| 5. | Add **100 µl diluted CONJ** (conjugate). |
| 6. | Incubate for **1 hour** shaking on a horizontal mixer at room temperature (15–30 °C). |
| 7. | Aspirate and wash the wells **5 x with 250 µl ELISA wash buffer**. After the final washing step, the inverted microtiter plate should be tapped on absorbent paper. |
| 8. | Add **100 µl SUB** (TMB substrate). |
| 9. | Incubate for **10–20 minutes*** in the dark at room temperature (15–30 °C). |
| 10. | Add **100 µl STOP** (ELISA stop solution) and mix shortly. |
| last | 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. |

* The intensity of the color change is temperature sensitive. We recommend observing the color 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.

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**

For the calculation of the saliva values, the results from the microplate reader must be multiplied by the dilution factor of **2000**.

**Stool**

For the calculation of the stool values, the results from the microplate reader must be multiplied by the dilution factor of **12500**.

## 9. LIMITATIONS

Samples with concentrations above the measurement range must be further diluted and re-assayed. Please consider this greater dilution when calculating the results.

Samples with concentrations lower than the measurement range 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:

*detection limit × sample dilution factor to be used*

## 10. QUALITY CONTROL

Immundiagnostik 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 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 - 237µg/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

### *Precision and reproducibility*

**Intra-Assay (n = 20)**

The precision (intra-assay variation) of the Immundiagnostik anti-sIgA ELISA test was calculated from 20 replicate determinations on each of one samples.

| Sample | sIgA [ng/ml] | CV [%] |
|---|---|---|
| 1 | 77.7 | 5 |
| 2 | 92.5 | 9 |

**Inter-Assay (n = 20)**

The total precision (inter-assay variation) of the Immundiagnostik anti-sIgA ELISA test was calculated from data on 2 samples obtained in 20 different assays by three technicians on two different lots of reagents over a period of three months.

| Sample | sIgA [ng/ml] | CV [%] |
|---|---|---|
| 1 | 102.4 | 8 |
| 2 | 1277.4 | 7.4 |

### *Spiking Recovery*

Two samples were spiked with sIgA calibrator and measured with this assay (n = 2).

| Sample | Unspiked Sample [ng/ml] | Spike [ng/ml] | sIgA expected [ng/ml] | sIgA measured [ng/ml] |
|---|---|---|---|---|
| A | 103.7 | 150 | 253.7 | 279.7 |
| | 103.7 | 75 | 178.7 | 194.7 |
| | 103.7 | 50 | 153.7 | 158.7 |
| | 103.7 | 25 | 128.7 | 141.2 |

| Sample | Unspiked Sample [ng/ml] | Spike [ng/ml] | sIgA expected [ng/ml] | sIgA measured [ng/ml] |
|---|---|---|---|---|
| B | 100.3 | 150 | 250.3 | 272.4 |
| | 100.3 | 75 | 175.3 | 212.9 |
| | 100.3 | 50 | 150.3 | 165.4 |
| | 100.3 | 25 | 125.3 | 126.5 |

## *Dilution recovery*

Two patient samples were diluted with ELISA wash buffer. The results are shown below (n = 2):

| Sample | Dilution | sIgA expected [ng/ml] | sIgA measured [ng/ml] |
|---|---|---|---|
| A | undiluted | 126.8 | 126.8 |
| | 1:2 | 63.4 | 65.5 |
| | 1:4 | 31.7 | 35.1 |
| | 1:8 | 15.9 | 25.6 |
| B | undiluted | 184.9 | 184.9 |
| | 1:2 | 92.5 | 93.7 |
| | 1:4 | 46.2 | 52.1 |
| | 1:8 | 23.1 | 21.9 |

## *Analytical Sensitivity*

The Zero-standard was measured 20 times. The detection limit was set as $B_0$ + 2 SD and estimated to be 13.4 ng/ml.

## *Specificity*

No cross reactivity to other proteins in stool and saliva.

## 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 and Proclin are toxic. Substrates for the enzymatic color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- 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.
- Control samples should be analyzed 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 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.

- 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*.

### *Literature using the Immundiagnostik sIgA ELISA*

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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.
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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.
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*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.

***Used symbols:***

| | | | |
|---|---|---|---|
|  | Temperature limitation |  | Catalogue Number |
|  | In Vitro Diagnostic Medical Device |  | Contains sufficient for <n> tests |
|  | Manufacturer |  | Use by |
|  | Lot number | | |