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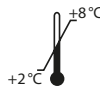
**Manual**

# anti-Gliadin sIgA ELISA

*For the in vitro determination of  
anti-Gliadin sIgA antibodies in stool*

Valid from 2019-12-05

**REF** K 9311



**IVD** **CE**



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# Table of Contents

<b>1. INTENDED USE</b>	<b>17</b>
<b>2. INTRODUCTION</b>	<b>17</b>
<b>3. MATERIAL SUPPLIED</b>	<b>19</b>
<b>4. MATERIAL REQUIRED BUT NOT SUPPLIED</b>	<b>19</b>
<b>5. PREPARATION AND STORAGE OF REAGENTS</b>	<b>20</b>
<b>6. STORAGE AND PREPARATION OF SAMPLES</b>	<b>20</b>
<i>Sample storage</i>	20
<i>Extraction of the stool samples</i>	21
<i>Dilution of samples</i>	22
<b>7. ASSAY PROCEDURE</b>	<b>22</b>
<i>Principle of the test</i>	22
<i>Test procedure</i>	22
<b>8. RESULTS</b>	<b>24</b>
<b>9. LIMITATIONS</b>	<b>24</b>
<b>10. QUALITY CONTROL</b>	<b>24</b>
<i>Reference range</i>	24
<b>11. PERFORMANCE CHARACTERISTICS</b>	<b>25</b>
<i>Accuracy – Precision</i>	25
<i>Linearity</i>	25
<i>Analytical sensitivity</i>	26
<b>12. PRECAUTIONS</b>	<b>26</b>
<b>13. TECHNICAL HINTS</b>	<b>26</b>
<b>14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE</b>	<b>27</b>
<b>15. REFERENCES</b>	<b>27</b>

## 1. INTENDED USE

This Immundiagnostik AG assay is an enzyme immunoassay intended for the determination of anti-gliadin-sIgA antibodies in stool. For *in vitro* diagnostic use only.

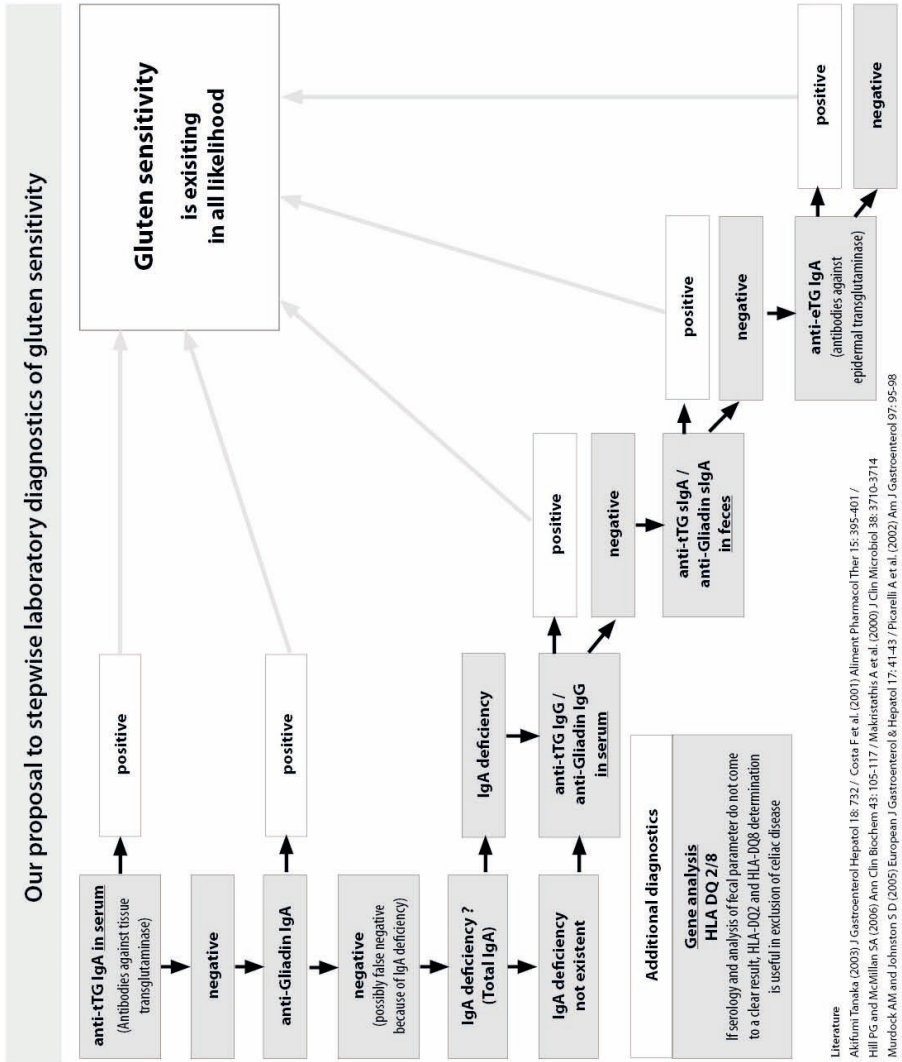
## 2. INTRODUCTION

The celiac/coeliac disease is caused by the gliadin fraction of wheat gluten and similar proteins of rye and barley. The disease is mainly manifested as chronic digestion insufficiency in children or young adults. In addition, patients with celiac disease have a greatly increased risk of developing malignant T-cell lymphoma of the small bowel, as T-cell lymphoma was found in 6 of 10 patients with coeliac sprue. Early diagnosis of celiac disease is important, because there is evidence that a gluten-free diet might help to prevent complications and malignancies (including intestinal lymphoma).

The measurement of anti-gliadin-sIgA-antibodies may be useful as a screening criterion before jejunal biopsy, the „Gold Standard“, and for the monitoring of the gluten-free diet treatment.

### Indication

- Celiac disease
- Food intolerance
- See also our proposal for stepwise diagnosis of gluten sensitivity on page 18.



### 3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
K 9311	PLATE	Microtiter plate, pre-coated	12 x 8 wells
K 0001.C.100	WASHBUF	Wash buffer concentrate, 10x	2 x 100 ml
K 9311	CONJ	Conjugate (peroxidase-labelled), ready-to-use	1 x 15 ml
K 9311	CTRL NEG	Control negative, lyophilised (see specification for range)	4x 1 vial
K 9311	CTRL POS	Control positive, lyophilised (see specification for range)	4x 1 vial
K 9311	CTRL CUT-OFF	Cut-off control, lyophilised (see specification for concentration)	4x 1 vial
K 0002.15	SUB	Substrate, ready-to-use	1 x 15 ml
K 0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml
K 6999.C.100	IDK Extract®	Extraction buffer concentrate <i>IDK Extract® 2.5x</i>	1 x 100 ml
K 9311	DIL	Dilution buffer, ready-to-use	1 x 15 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\*
- Stool sample application system such as Cat. No.: K 6998SAS
- Horizontal microtiter plate shaker
- Calibrated precision pipettors and 5–1000 µl single-use tips
- Foil to cover the microtiter plate
- Multi-channel pipets or repeater pipets
- 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).

## 5. PREPARATION AND STORAGE 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** is stable at **2–8 °C** until the expiry date stated on the label. **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®* is stable at **2–8 °C** until the expiry date stated on the label. Extraction buffer (1:2.5 diluted *IDK Extract®*) can be stored in a closed flask at **2–8 °C for 4 months**.
- The **lyophilised controls (CTRL CUT-OFF, CTRL POS and CTRL NEG)** are stable at **2–8 °C** until the expiry date stated on the label. Reconstitution details are given in the specification data sheet. **Controls** (reconstituted CTRL NEG, CTRL POS and CTRL CUT-OFF) **are stable for 3 days at -20 °C**.
- All other test reagents are ready-to-use. Test reagents are stable until the expiry date (see label) when stored at **2–8 °C**.

## 6. STORAGE AND PREPARATION OF SAMPLES

### *Sample storage*

#### **Raw stool**

Raw stool samples can be stored for 8 months at -20 °C. Avoid more than 3 freeze-thaw-cycles.

## Stool suspensions

Stool extract can be stored for 7 days at 2–8°C or -20°C or for one day at room temperature (15–30°C). Avoid more than three freeze-thaw cycles.

### *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:

- 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 samples*

The suspension from the sample extraction (dilution I) is further diluted **1:50 with wash buffer**. For example:

- **20 µl** dilution I + **980 µl** wash buffer, mix well = **1:50 (dilution II)**  
This results in a final dilution of **1:5 000**.

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

## **7. ASSAY PROCEDURE**

### *Principle of the test*

The antigen gliadin is immobilised on the microtiter plate. During the first incubation step, the human anti-gliadin-sIgA antibodies in the samples are bound by the immobilised antigen. After a washing step, anti-gliadin-sIgA antibody presence is determined by the addition of a peroxidase-labelled anti-sIgA antibody. Tetramethylbenzidine is used as a peroxidase substrate. The enzymatic reaction is stopped by an acidic stop solution. The absorbance of the colour compound is determined photometrically at 450 nm. The measured absorbance indicates the presence of bound anti-gliadin-sIgA antibodies. The results are evaluated by comparison with a cut-off value.

### *Test procedure*

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

Mark the positions of 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.	<b>Before use</b> , wash the wells <b>5 times</b> with <b>250 µl wash buffer</b> . After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
2.	Add each <b>100 µl controls/diluted samples</b> into the respective wells.
3.	Cover the strips and incubate for <b>1 hour</b> at room temperature (15–30 °C) on a <b>horizontal shaker*</b> .
4.	Discard the content of each well and wash <b>5 times</b> with <b>250 µl wash buffer</b> . After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
5.	Add <b>100 µl conjugate</b> (CONJ) into each well.
6.	Cover the strips and incubate for <b>1 hour</b> at room temperature (15–30 °C) on a <b>horizontal shaker*</b> .
7.	Discard the content of each well and wash <b>5 times</b> with <b>250 µl wash buffer</b> . After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
8.	Add <b>100 µl substrate</b> (SUB) into each well.
9.	Incubate for <b>10–20 min**</b> at room temperature (15–30 °C) in the <b>dark</b> .
10.	Add <b>100 µl stop solution</b> (STOP) into each well and mix well.
11.	Determine <b>absorption immediately</b> with an ELISA reader at <b>450 nm</b> against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of one sample exceeds the range of the photometer, absorption must be measured immediately at <b>405 nm</b> 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

Samples with an optical density higher than the average optical density of the cut off control are positive.

$$\text{Cut off} = \text{OD}_{\text{cut-off control}} = 100 \text{ U/l}$$

### Example

$$\text{OD}_{\text{patient sample}} = 0.685$$

$$\text{OD}_{\text{cut-off control}} = 0.321 = 100 \text{ U/l}$$

$$\text{Concentration patient sample} = \frac{0.685 * 100 \text{ U/l}}{0.321} = 213.4 \text{ U/l}$$

**Attention:** Calculation is only valid for a sample dilution factor of 1:5 000.

## 9. LIMITATIONS

The lower limit of the measurement range is the LoB.

LoB see chapter "Performance Characteristics".

Samples with concentrations lower than the measurement range cannot be clearly quantified.

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

### *Reference range*

Based on an internal study with apparently healthy adults (n = 45) resulted in a reference value of < **100 U/l**.

We recommend each laboratory to establish its own reference range.

## 11. PERFORMANCE CHARACTERISTICS

### *Accuracy – Precision*

#### **Repeatability (Intra-Assay); n = 40**

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

Sample	Mean value [U/l]	CV [%]
1	503.37	3.0
2	332.94	4.0

#### **Reproducibility (Inter-Assay); n = 24**

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

Sample	Mean value [U/l]	CV [%]
1	441.04	11.6
2	241.78	9.1

### *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 anti-gliadin sIgA in stool, the method has been demonstrated to be linear from 57.16 to 342.93 U/l, showing a non-linear behaviour of less than  $\pm 20\%$  in this interval.

Sample	Dilution	Expected [U/l]	Obtained [U/l]	Recovery [%]
A	1:15000	342.94	342.94	100.00
	1:30000	171.47	170.93	99.68
	1:45000	114.31	115.91	101.39
	1:60000	85.73	87.71	102.31
	1:90000	57.16	65.73	115.00

Sample	Dilution	Expected [U/l]	Obtained [U/l]	Recovery [%]
B	1:20000	233.74	233.74	100.00
	1:22000	212.49	208.28	98.02
	1:24000	194.78	183.58	94.25
	1:26000	179.80	172.96	96.20
	1:28000	166.96	165.10	98.89
	1:30000	155.83	160.07	102.73

### Analytical sensitivity

Limit of blank, LoB

37.53 U/l

## 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 colour 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 as wells from already opened microtiter plates are exposed to different conditions than sealed ones.
- 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.
- Control samples should be analysed with each run.
- 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

1. Dieterich et al.: 1997; *Nat. Med.* **7** (3), 797
2. Rieken et al.: 1998; *Dtsch. med. Wschr.* **123**, 1454
3. Green et al.: 1998; *Clin. Persp. Gastroenterol.* November, **133**
4. Mothes, Th.: 1997; *Münsch. Med. Wschr.* **139**, 111

**Used symbols:**

	Temperature limitation		Catalogue Number
	In Vitro Diagnostic Medical Device		To be used with
	Manufacturer		Contains sufficient for <n> tests
	Lot number		Use by
	Attention		Consult instructions for use
	Consult specification data sheet		