Manual

anti-Gliadin sIgA ELISA

For the determination of anti-Gliadin sIgA antibodies in stool

Valid from 2022-03-01

REF **KR9311**

Σ 96

+2 °C ... +8 °C

RUO

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1. INTENDED USE

This Immundiagnostik AG assay is an enzyme immunoassay intended for the determination of anti-gliadin-sIgA antibodies in stool. For research use only. Not for use in diagnostic procedures.

2. INTRODUCTION

Gliadin is a class of proteins present in wheat and several other cereals within the grass genus *Triticum*. Gliadins, which are a component of gluten, are essential for giving bread the ability to rise properly during baking. Gliadins and glutenins are the two main components of the gluten fraction of the wheat seed. This gluten is found in products such as wheat flour. Gluten is split about evenly between the gliadins and glutenins, although there are variations found in different sources.

Gliadin is the water-soluble component of gluten, while glutenin is insoluble.

This assay allows for the measurement of soluble IgA (sIgA) antibodies from human stool samples.

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
KR9311	PLATE	Microtiter plate, pre-coated	12 x 8 wells
KR0001.C.100	WASHBUF	Wash buffer concentrate, 10 x	2 x 100 ml
KR9311	CONJ	Conjugate (peroxidase-labelled), ready-to-use	1 x 15 ml
KR9311	CTRL NEG	Control negative, lyophilised (see specification for range)	4x 1 vial
KR9311	CTRL POS	Control positive, lyophilised (see specification for range)	4x 1 vial
KR9311	CTRL CUT OFF	Cut-off control, lyophilised (see specification for concentration)	4x 1 vial
KR0002.15	SUB	Substrate, ready-to-use	1 x 15 ml
KR0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml
KR6999.C.100	IDK Extract®	Extraction buffer concentrate *IDK Extract®* 2.5 x	1 x 100 ml

Cat. No.	Label	Kit components	Quantity
KR9311	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*
- 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 NEG, CTRL POS and CTRL CUT OFF)** 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 not stable and cannot be stored.**
- 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.: KR6998SAS)

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) ist 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.	**Before use**, wash the wells **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
2.	Add each **100 µl controls/diluted samples** into the respective wells.
3.	Cover the strips and incubate for **1 hour** at room temperature (15–30 °C) on a **horizontal shaker***.
4.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
5.	Add **100 µl conjugate** (CONJ) into each well.
6.	Cover the strips and incubate for **1 hour** at room temperature (15–30 °C) on a **horizontal shaker***.

7.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
8.	Add **100 µl substrate** (SUB) into each well.
9.	Incubate for **10–20 min**** at room temperature (15–30 °C) in the **dark**.
10.	Add **100 µl stop solution** (STOP) into each well 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 one sample 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

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

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

Example

$OD_{\text{patient sample}}$ = 0.685

$OD_{\text{cut-off control}}$ = 0.321 = 100 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

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, instrument, 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, instruments, days and kit lots).

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

Analytical sensitivity

Limit of blank, LoB 37.53 U/l

12. PRECAUTIONS

- All reagents in the kit package are for research 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 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

- Control samples should be analysed with each run.
- The guidelines for 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

REF Catalogue number

RUO For research use only

→REF To be used with

 Manufacturer

 Contains sufficient for <n> tests

LOT Lot number

 Use by

 Attention

 Consult instructions for use

 Consult specification data sheet

 Irritant