Manual

IDK® Gluten Fecal

For the determination of gliadin 33mer in stool

Valid from 2022-07-22

REF **KR9309**

Σ 96

+2 °C +8 °C

RUO

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

1. INTENDED USE

This Immundiagnostik AG assay is an enzyme immunoassay intended for the quantitative determination of gliadin 33mer in stool. For research use only. Not for use in diagnostic procedures.

2. INTRODUCTION

Gliadins are 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 alcohol-soluble component of gluten. After being taken up with food, gliadin is digested resulting in smaller fragments, one of which is gliadin 33mer. This molecule passes the intestinal epithelium and forms a complex with transglutaminase. This complex binds to the epithelium where it is attacked by lymphocytes.

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
KR9309	PLATE	Microtiter plate, pre-coated	12 x 8 wells
KR0001.C.100	WASHBUF	Wash buffer concentrate, 10 x	1 x 100 ml
KR9309	SAMPLEBUF	Sample dilution buffer, ready-to-use	1 x 100 ml
KR9309	CONJ	Conjugate, peroxidase-labelled, ready-to-use	1 x 15 ml
KR9309	STD	Standards, lyophilised (see specification for concentrations)	2 x 6 vials
KR9309	CTRL1	Control, lyophilised (see specification for range)	2 x 1 vial
KR9309	CTRL2	Control, lyophilised (see specification for range)	2 x 1 vial

Cat. No.	Label	Kit components	Quantity
KR9309	EXBUF	Extraction buffer, ready-to-use	2 x 100 ml
KR0002.15	SUB	Substrate (tetramethyl-benzidine), ready-to-use	1 x 15 ml
KR0003.15	STOP	Stop solution, 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*
- Calibrated precision pipettors and 10–1000 µl single-use tips
- Foil to cover the microtiter plate
- Multi-channel pipets or repeater pipets
- Centrifuge, 3000 *g*
- Refrigerator (2–8 °C)
- Vortex
- Standard 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. 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** 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**.

- The **lyophilised standards** (STD) and **controls** (CTRL) are stable at **2–8 °C** until the expiry date stated on the label. **Reconstitution** details are given in the **specification data sheet**. **Standards and controls** (reconstituted STD and CTRL) are stable for **7 days** at **2–8 °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. PREPARATION OF SAMPLES

Extraction of the stool samples

Extraction buffer (EXBUF) 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** (***EXBUF)*** 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) 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 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 supernatant of the sample preparation procedure (dilution I) is diluted **1:4 in sample dilution buffer**. For example:

- **100 µl** supernatant (dilution I) + **300 µl** sample dilution buffer, mix well = **1:4** (**dilution II**)
  This results in a final dilution of 1:400.

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

7. ASSAY PROCEDURE

Principle of the test

This ELISA is designed for the quantitative determination of gliadin 33mer.

The assay utilises the sandwich technique with two selected polyclonal antibodies that bind to gliadin-33mer.

Standards, controls and extracted samples which are assayed for gliadin 33mer are added into the wells of a micro plate coated with a high affine polyclonal gliadin 33mer antibody. During the first incubation step, gliadin 33mer is bound by the immobilised antibody. Then a peroxidase-conjugated polyclonal gliadin 33mer antibody is added into each microtiter well and a sandwich of capture antibody – gliadin 33mer – peroxidase-conjugate is formed. Tetramethylbenzidine is used as peroxidase substrate. Finally, an acidic stop solution is added to terminate the reaction. The colour changes from blue to yellow. The intensity of the yellow colour is directly proportional to the concentration of gliadin 33mer. A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from the standard. Gliadin 33mer, present in the 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/samples/controls 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 standards/controls/prepared 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 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.

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.

Stool

The obtained results have to be multplied by the **dilution factor of 400** 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 calibration curve × sample dilution factor to be used

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

LoQ × sample dilution factor to be used

LoQ 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 samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

We recommend each laboratory to establish its own reference range.

11. PERFORMANCE CHARACTERISTICS

Accuracy - Precision

Repeatability (Intra-Assay)

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

Sample	Mean value [ng/ml]	CV [%]
1 (n = 26)	437.01	4.1
2 (n = 27)	55.68	4.1

Reproducibility (Inter-Assay); n = 48

The reproducibility was assessed with 3 stool samples under **varying** parameters (7 operators, 2 instruments, 2 days and 2 kit lots).

Sample	Mean value [ng/ml]	CV [%]
1	120.07	14.0
2	47.44	9.9
3	50.97	13.1

Accuracy – Trueness

The trueness states the closeness of the agreement between the result of a measurement and the true value of the measurand. Therefore, gluten spikes with known concentrations were added to 4 negative stool-samples. The table below shows the results (mean values) without considering possibly used sample dilution factors:

Spike [ng/ml]	Expected [ng/ml]	Obtained [ng/ml]	Recovery [%]
0	-	0	-
0.08	0.08	0.09	108.0
0.16	0.16	0.16	97.5
0.24	0.24	0.23	94.5
0.32	0.32	0.30	93.7
0.41	0.41	0.39	95.3
0.49	0.49	0.46	95.2
0.57	0.57	0.53	93.8

Analytical sensitivity

The following values have been estimated based on the concentrations of the standard curve without considering possibly used sample dilution factors:

Limit of blank, LoB 0.015 ng/ml

Limit of detection, LoD 0.024 ng/ml

Limit of quantitation, LoQ 0.029 ng/ml

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

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 EP06-A by a serial dilution of 6 different stool-samples.

For gluten in stool, the method has been demonstrated to be linear from 0.044 to 1.399 ng/ml without considering possibly used sample dilution factors, showing a non-linear behaviour of less than ± 20 % in this interval. The table below shows 3 exemplary samples:

Sample	Dilution	Expected [ng/ml]	Obtained [ng/ml]	Recovery [%]
1	1:400	1.090	1.090	100.00
	1:800	0.545	0.570	104.62
	1:1 600	0.273	0.285	104.57
	1:3 200	0.136	0.149	109.69
	1:6 400	0.068	0.082	119.97
2	1:1 600	1.203	1.203	100.00
	1:3 200	0.601	0.555	92.35
	1:6 400	0.301	0.280	93.13
	1:12 800	0.150	0.152	101.02
	1:25 600	0.075	0.071	94.88
3	1:800	1.399	1.399	100.00
	1:1 600	0.699	0.650	92.92
	1:3 200	0.350	0.342	97.88
	1:6 400	0.175	0.184	105.50
	1:12 800	0.087	0.102	117.00
	1:25 600	0.044	0.053	121.34

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.
- 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

- The guidelines for laboratories should be followed.
- *IDK*® is a trademark 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.

Used symbols:


Temperature limitation

Catalogue number

For research use only

To be used with


Manufacturer

Contains sufficient for <n> tests

Lot number


Use by


Attention


Consult instructions for use

Consult specification data sheet


Irritant