

# IDK<sup>®</sup> $\alpha_1$ -Antitrypsin ELISA

*For the determination of  $\alpha_1$ -antitrypsin in stool*

Valid from 2022-02-15

**REF** KR6750

$\Sigma$   
96

$\pm 8^{\circ}\text{C}$   
 $+2^{\circ}\text{C}$

**RUO**



**Immundiagnostik AG**, Stubenwald-Allee 8a, 64625 Bensheim, Germany

Tel.: +49 6251 70190-0

Fax: + 49 6251 70190-363

e.mail: [info@immundiagnostik.com](mailto:info@immundiagnostik.com)

[www.immundiagnostik.com](http://www.immundiagnostik.com)

# Table of Contents

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

## 1. INTENDED USE

This Immundiagnostik AG assay is an enzyme immunoassay intended for the quantitative determination of  $\alpha_1$ -antitrypsin in stool.

For research use only. Not for use in diagnostic procedures.

## 2. INTRODUCTION

In serum,  $\alpha_1$ -antitrypsin represents the majority of serine protease inhibitors and protects tissues from protease damages during inflammation. The protein is synthesised primarily in the liver but also to a small extent in intestinal macrophages, monocytes, and intestinal epithelial cells. Since  $\alpha_1$ -antitrypsin is relatively resistant against enzymatic digestion, the secreted amount in stool reflects the internal concentration of the protein. An elevated  $\alpha_1$ -antitrypsin stool concentration is therefore a widely recognised marker for intestinal protein loss and for an increased mucosal permeability.

## 3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
KR6750	PLATE	Microtiter plate, pre-coated	12 x 8 wells
KR0001.C.100	WASHBUF	Wash buffer concentrate, 10x	2 x 100 ml
KR6750	CONJ	Conjugate concentrate, (goat-anti- $\alpha_1$ -antitrypsin, peroxidase-labelled)	1 x 200 $\mu$ l
KR6750	STD	Standards, lyophilised (0; 3.3; 10; 30; 90 $\mu$ g/l)	2 x 5 vials
KR6750	CTRL1	Control, lyophilised (see specification for range)	2 x 1 vial
KR6750	CTRL2	Control, lyophilised (see specification for range)	2 x 1 vial
KR0002.15	SUB	Substrate (tetramethylbenzidine), 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 <i>IDK Extract</i> ® 2.5x	2 x 100 ml

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

\* The used standards have been calibrated on the WHO reference material CRM 470.

#### 4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water\*
- Calibrated precision pipettors and 10–1000  $\mu$ l single-use tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- 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  $\mu$ m) with an electrical conductivity of 0.055  $\mu$ S/cm at 25 °C ( $\geq$  18.2 M $\Omega$ 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  $\mu$ l** 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 standards (STD)** and **controls (CTRL)** are stable at **2–8 °C** until the expiry date stated on the label. Before use, the STD and CTRL have to be reconstituted with **500  $\mu$ l 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 2–8 °C for 4 weeks.**

- **Preparation of the conjugate:** Before use, the **conjugate concentrate (CONJ)** has to be diluted **1:101** in wash buffer (100  $\mu$ l CONJ + 10 ml wash buffer). The CONJ is stable 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 are stable until the expiry date (see label) when stored at **2–8 °C.**

## 6. STORAGE AND PREPARATION OF SAMPLES

### *Storage*

The **sample stability** is as follows:

**Raw stool:** 3 days at room temperature (15–30 °C), 3 days at 2–8 °C or at least 4 weeks at -20 °C

**Stool extracts:** 9 days at room temperature, 2–8 °C or -20 °C, maximum 3 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.



by the immobilised antibody. Then a peroxidase-conjugated polyclonal anti-human  $\alpha_1$ -antitrypsin antibody is added into each microtiter well and a sandwich of capture antibody – human  $\alpha_1$ -antitrypsin – 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  $\alpha_1$ -antitrypsin. 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.  $\alpha_1$ -antitrypsin 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/controls/samples on a protocol sheet.

Take as many microtiter strips as needed from the 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 standards/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> (diluted 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 <math>\mu</math>l wash buffer</b> . After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
8.	Add <b>100 <math>\mu</math>l 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 <math>\mu</math>l 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 the highest standard 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

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 samples

The obtained results have to be multiplied by the **dilution factor of 25 000** 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 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 = 35**

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

Sample	Mean value [ $\mu\text{g/l}$ ]	CV [%]
1	11.97	5.4
2	32.36	9.1

#### **Reproducibility (Inter-Assay); n = 20**

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

Sample	Mean value [ $\mu\text{g/l}$ ]	CV [%]
1	13.36	9.6
2	41.99	11.9

### *Analytical sensitivity*

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

Limit of blank, LoB

0.359  $\mu\text{g/l}$

## 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.
- 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®* 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 sent to Immunodiagnostik AG along with a written complaint.

## 15. REFERENCES

1. Amarri, S. et al., 2006. Changes of gut microbiota and immune markers during the complementary feeding period in healthy breast-fed infants. *Journal of pediatric gastroenterology and nutrition*, **42**(5), pp.488–95.
2. Faust, D et al., 2001. Determination of alpha1-proteinase inhibitor by a new enzyme linked immunosorbant assay in feces, serum and an enterocyte-like cell line. *Zeitschrift für Gastroenterologie*, **39**(9), pp.769–74.
3. Faust, D. et al., 2002. Regulation of alpha1-proteinase inhibitor release by proinflammatory cytokines in human intestinal epithelial cells. *Clinical and experimental immunology*, **128**(2), pp.279–84.
4. Hsu, P.-I. et al., 2010. Diagnosis of gastric malignancy using gastric juice alpha1-antitrypsin. *Cancer epidemiology, biomarkers & prevention*, **19**(2), pp.405–11.
5. Lamprecht, M. et al., 2012. Probiotic supplementation affects markers of intestinal barrier, oxidation, and inflammation in trained men; a randomized, double-blinded, placebo-controlled trial. *Journal of the International Society of Sports Nutrition*, **9**(1), p.45.
6. Muss, C., Schütz, B. & Kirkamm, R., 2002. Alpha1-Antitrypsin - ein objektiver Verlaufsparemeter bei entzündlichen Darmerkrankungen. *Ärztzeitschrift für Naturheilverfahren*, 43(4).
7. Oswari, H. et al., 2013. Comparison of stool microbiota compositions, stool alpha1-antitrypsin and calprotectin concentrations, and diarrhoeal morbidity of Indonesian infants fed breast milk or probiotic/prebiotic-supplemented formula. *Journal of paediatrics and child health*, Epub ahead of print.
8. Quint, J.K. et al., 2011. SERPINA1 11478G>A variant, serum  $\alpha_1$ -antitrypsin, exacerbation frequency and FEV1 decline in COPD. *Thorax*, **66**(5), pp.418–24.
9. Ragab, H.M. et al., 2009. Clinical utility of serum TNF alpha and alpha-1 anti-trypsin in predicting the stage and progression of lung cancer. *International Journal of Integrative Biology*, **7**(1), pp.45–52.
10. Roeckel, N. et al., 2009. High frequency of LMAN1 abnormalities in colorectal tumors with microsatellite instability. *Cancer research*, **69**(1), pp.292–9.
11. Strygler, B. et al., 1990.  $\alpha_1$ -Antitrypsin Excretion in Stool in Normal Subjects and in Patients With Gastrointestinal Disorders. *Gastroenterology*, **99**(5), pp.1380–1387.

12. Török, E. et al., 2011. Primary human hepatocytes on biodegradable poly(l-lactic acid) matrices: a promising model for improving transplantation efficiency with tissue engineering. *Liver transplantation*, **17**(2), pp.104–14

**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