Manual

IDK® Pancreatic Elastase ELISA Kit

RUO for the determination of human pancreatic elastase in stool

Valid from 2015-12-29

REF K 6915

Σ 96

+8 °C +2 °C

IVD

CE

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For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.

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1. INTENDED USE

The described assay is intended for the quantitative determination of human pancre-atic elastase in stool. For *research* use only.

2. INTRODUCTION

Pancreatic elastase is an anionic endoprotease of the serine protease family with a molecular weight of 26 kDa. Together with other digestive enzymes it is synthesized as an inactive pro-enzyme in the acinar cells of the pancreas and is secreted into the duodenum. After its activation, pancreas elastase cleaves peptides after neutral amino acids.

Pancreas elastase is mainly bound to bile salts during intestinal passage and is not degraded. In human feces it is 5–6 fold more concentrated than in pancreatic juice. The stool concentration reflects the secretory capacity of the pancreas.

Indications:

- Diagnosis/exclusion of exocrine pancreas insufficiency in case of unexplained diarrhea, constipation, steatorrhea, flatulence, weight loss, upper abdominal pain, and food intolerances
- Monitoring of exocrine pancreas function in cystic fibrosis, diabetes mellitus, or chronic pancreatitis

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
K 6915	PLATE	Microtiter plate, pre-coated	12 x 8 wells
K 6915	WASHBUF	ELISA wash buffer concentrate, 10 x	2 x 100 ml
K 6915	IDK Extract®	Extraction buffer concentrate *IDK Extract®*, 2.5 x	1 x 100 ml
K 6915	CONJ	Conjugate concentrate (mouse anti pancreatic elastase)	1 x 200 µl
K 6915	STD	Standard, lyophilized	4 x 5 vials
K 6915	CTRL1	Control, lyophilized	4 x 1 vial
K 6915	CTRL2	Control, lyophilized	4 x 1 vial
K 6915	SUB	TMB substrate (Tetramethylbenzidine), ready to use	1 x 15 ml
K 6915	STOP	ELISA 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

- Ultra pure water*
- Calibrated precision pipettors and 10–1000 µl tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- 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.
- **Preparation of the wash buffer:** The **wash buffer concentrate (WASHBUF)** has to be diluted with ultra pure water **1:10** before use (100 ml WASHBUF + 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 in a water bath at 37 °C before dilution of the buffer solutions. 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 one month**.
- **Preparation of the extraction buffer:** The **extraction buffer concentrate *IDK Extract®*** has to be diluted with ultra pure water **1:2.5** before use (100 ml *IDK Extract®* + 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 ***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 three months**.
- The **lyophilized 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 **data sheet**. **Reconstituted standards and controls are not stable and cannot be stored.**

- **Preparation of the conjugate:** Before use, the **conjugate concentrate (CONJ)** has to be diluted **1:101** in wash buffer (100 µl CONJ + 10 ml wash buffer). The CONJ is stable at **2–8 °C** until 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 of test package) when stored at **2–8°C**.

6. STORAGE AND PREPARATION OF SAMPLES

Sample stability and storage

According to literature, the stability of pancreatic elastase in **raw stool** is 3 days at room temperature [5], 3 days at 4–8 °C [1], and up to a year at -20 °C [1].

Stool extract is stable at room temperature (15–30 °C) for three days, at 2–8 °C as well as at -20 °C for seven days. Avoid more than one freeze-thaw cycle.

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 samples

After centrifugation, the supernatant of the sample preparation procedure (dilution I) is diluted **1:100 in wash buffer**. For this purpose, one of the two following dilution procedure variants can be used:

Variant A (recommended by Immundiagnostik):

- **100 µl** supernatant (dilution I) + **900 µl** wash buffer, mix well = **1:10** (**dilution IIa**)
- **100 µl** dilution IIa + **900 µl** wash buffer, mix well = **1:10** (**dilution IIIa**). This results in a final dilution of 1:10 000.

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

Variant B:

Alternatively, the 1:100 dilution can be done in one step. For example:

- **10 µl** supernatant (dilution I) + **990 µl** wash buffer, mix well = **1:100** (**dilution IIb**). This results in a final dilution of 1:10 000.

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

7. ASSAY PROCEDURE

Principle of the test

This ELISA is intended for the quantitative determination of pancreatic elastase in stool. In a first incubation step, the pancreatic elastase in the samples is bound to monoclonal antibodies, 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 pancreatic elastase) is added which recognizes specifically the bound pancreatic elastase. After another washing step to remove all unbound substances, the solid phase is incubated with the substrate, tetramethyl-benzidine (TMB), which reacts with the peroxidase. An acidic stop solution is added to stop the reaction. The color changes from blue to yellow. The intensity of the yellow color is directly proportional to the concentration of pancreatic elastase. A dose response curve of absorbance unit (optical density, OD at 450 nm) vs. concentration is generated using the values obtained from the standards. Pancreatic elastase present 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.
Mark positions for **STD/SAMPLE/CTRL** (standard / sample / controls) in the protocol sheet.

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.

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.	Add **100 µl of STD/SAMPLE/CTRL** (standard / sample / controls) into respective well.
2.	Cover the strips and incubate for **30 min** at room temperature (15-30 °C) on a horizontal shaker.
3.	Discard the contents of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
4.	Add **100 µl conjugate** to each well.

5.	Cover the strips and incubate for **30 min** at room temperature (15-30 °C) on a horizontal shaker.
6.	Discard the contents of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
7.	Add **100 µl** SUB (TMB substrate) in each well.
8.	Incubate for **10-20 minutes*** at room temperature (15-30 °C) in the dark.
9.	Add **100 µl STOP** (ELISA stop solution) and mix well.
10.	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 to observe the color change and to stop 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

Multiply the result by the **dilution factor of 10000** to obtain the concentration of pancreatic elastase in the sample.

In case **another dilution factor** has been used, multiply the obtained result with the dilution factor used.

9. LIMITATIONS

Samples with concentrations above the measurement range (see definition below) must be further diluted and re-assayed. Please consider this greater 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*

Liquid stools may lead to false pancreatic elastase results. In such cases, we recommend to also consider clinical symptoms and other diagnostic tests for the final diagnosis and/or to request another patient sample.

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

We recommend each laboratory to establish its own reference concentration range.

Reference range in stool samples[4]

1 g stool is equivalent to 1 ml.

> 200 µg/ml	normal value
100 - 200 µg/ml	slight to moderate exocrine pancreatic insufficiency
< 100 µg/ml	exocrine pancreatic insufficiency

11. PERFORMANCE CHARACTERISTICS

Analytical Sensitivity

The LoB (limit of blank) was evaluated without considering sample dilution factor according to the guideline CLSI EP17-A2 and resulted in 0.66 µg/ml.

Spiking Recovery

Two samples were spiked with different pancreatic elastase concentrations and measured using this assay (n = 2).

Sample	Unspiked Sample [ng/ml]	Spike [ng/ml]	expected [ng/ml]	measured [ng/ml]
A	21.3	35.7	57.0	55.5
	21.3	26.6	47.9	42.4
	21.3	18.4	39.7	34.0
B	33.2	35.7	68.9	61.6
	33.2	26.6	59.8	51.0
	33.2	18.4	51.6	47.4

Specificity

No cross reactivity to the following proteins was observed:

- Pancreatic lipase
- Chymotrypsin
- Pankreatic amylase
- Pancreatin
- Calprotectin

Precision and reproducibility

Intra-Assay (n = 20)

Sample	Pancreatic elastase [µg/ml]	CV [%]
1	568.4	4.6
2	424.9	5.6

Inter-Assay (n = 12)

Sample	Pancreatic elastase [µg/ml]	CV [%]
1	449.1	7.7
2	379.7	9.2

Dilution recovery

Three samples were diluted and analysed. The results are shown in the table below (n = 3):

Sample	Dilution	expected [µg/ml]	measured [µg/ml]
A	–	235.0	235.0
	1:2	117.5	115.0
	1:4	58.8	64.9
	1:8	29.4	36.3
B	–	171.0	171.0
	1:2	85.5	92.3
	1:4	42.8	49.4
	1:8	21.4	28.3
C	–	177.0	177.0
	1:2	88.5	90.1
	1:4	44.3	52.2
	1:8	22.1	27.4

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 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.
- *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 send to Immundiagnostik AG along with a written complaint.

15. REFERENCES

1. Nandhakumar, N. & Green, M.R., 2010. Interpretations: How to use faecal elastase testing. Archives of disease in childhood. *Education and practice edition*, **95**(4), pp.119–23.
2. Whitcomb, D.C. & Lowe, M.E., 2007. Human pancreatic digestive enzymes. *Digestive diseases and sciences*, **52**(1), pp.1–17.
3. Dominici, R. & Franzini, C., 2002. Fecal elastase-1 as a test for pancreatic function: a review. *Clinical chemistry and laboratory medicine : CCLM / FESCC*, **40**(4), pp.325–32.
4. Löser, C., Möllgaard, A. & Fölsch, U.R., 1996. Faecal elastase 1: a novel, highly sensitive, and specific tubeless pancreatic function test. *Gut*, **39**(4), pp.580–6.
5. Stein, J. et al., 1996. Immunoreactive elastase I: clinical evaluation of a new noninvasive test of pancreatic function. *Clinical chemistry*, **42**(2), pp.222–6.

Used symbols:


Temperature limitation

REF Catalogue Number

IVD In Vitro Diagnostic Medical Device

→REF To be used with


Manufacturer

Contains sufficient for <n> tests

LOT Lot number


Use by


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