Instructions for Use

Pancreatic Elastase ELISA

IVD

CE

REF **EIA-5842**

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Please use only the valid version of the Instructions for Use provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Gebrauchsanweisung.
Si prega di usare la versione valida delle istruzioni per l'uso a disposizione con il kit.
Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.
Utilisez seulement la version valide des Instructions d'utilisation fournies avec le kit.

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1 INTENDED USE

This assay is an enzyme immunoassay intended for the quantitative determination of human pancreatic elastase in stool. For *in vitro* diagnostic 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 synthesised 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 faeces 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

Label	Kit components	Quantity
PLATE	Microtiter plate, pre-coated	12 x 8 wells
WASHBUF	Wash buffer concentrate, 10x	2 x 100 mL
CONJ	Conjugate concentrate, peroxidase-labelled (mouse anti-pancreatic elastase)	1 x 200 µL
STD	Standards, lyophilised (see specification for concentrations)	4 x 5 vials
CTRL1	Control, lyophilised (see specification for range)	4 x 1 vial
CTRL2	Control, lyophilised (see specification for range)	4 x 1 vial
SUB	Substrate (tetramethylbenzidine), ready to use	1 x 15 mL
STOP	Stop solution, ready to use	1 x 15 mL
IDK Extract®	Extraction buffer concentrate IDK Extract®, 2.5x	1 x 100 mL

4 MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Stool sample application system such as Cat. No.: EIA-5674
- Calibrated precision pipettors and 10–1000 µL 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)

* DRG 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 re-dissolved at room temperature or in a water bath at 37 °C.
  The **WASHBUF** is stable at **2 °C - 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 °C - 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 must be re-dissolved at 37 °C in a water bath.
  The ***IDK Extract*®** is stable at **2 °C - 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 °C - 8 °C for 4 months**.
- The **lyophilized standards** (STD) and **controls** (CTRL) are stable at **2 °C - 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 °C - 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 °C - 8 °C**.

6 STORAGE AND PREPARATION OF SAMPLES

6.1 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 °C - 8 °C [1], and
up to a year at -20 °C [1].

Stool extract is stable
at room temperature (15 °C - 30 °C) for three days,
at 2 °C - 8 °C as well as at -20 °C for seven days.

Avoid more than one freeze-thaw cycle.

6.2 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.: EIA-5674)

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 homogenization 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 *IDK Extract®)* 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 dip- stick 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

6.3 Dilution of samples

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 DRG):

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 1:10000.

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 1:10000.

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

7 ASSAY PROCEDURE

7.1 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.

7.2 Test procedure

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

Mark positions for standards / 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 °C - 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.

We recommend to carry out the tests in duplicate.

1. Add **100 µL standards / controls / diluted samples** into the respective well.
2. Cover the strips and incubate for **30 min** at room temperature (15 °C - 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, remove residual wash buffer by firmly tapping the plate on absorbent paper.
4. Add **100 µL conjugate** (diluted CONJ) into each well.
5. Cover the strips and incubate for **30 min** at room temperature (15 °C - 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, remove residual wash buffer by firmly tapping the plate on absorbent paper.
7. Add **100 µL substrate** (SUB) into each well.
8. Incubate for **10-20 minutes**** at room temperature (15 °C - 30 °C) in the **dark**.
9. Add **100 µL stop solution** (STOP) into each well 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.

* We recommend shaking the strips at 550 rpm with an orbit of 2 mm.

** 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

The obtained results have to be multiplied by the **dilution factor of 10 000** to get the actual concentrations.

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) 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”.

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

DRG 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.

10.1 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

11.1 Accuracy – Precision

Repeatability (Intra-Assay); n = 42

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

Sample	Mean value [ng/mL]	CV [%]
1	187.92	5.7
2	308.06	4.0

Reproducibility (Inter-Assay); n=20

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

Sample	Mean value [ng/mL]	CV [%]
1	209.01	9.7
2	349.12	7.4

11.2 Accuracy – Trueness

The trueness states the closeness of the agreement between the result of a measurement and the true value of the measurand. Therefore, pancreatic elastase spikes with known concentrations were added to 3 different stool samples.

Sample [ng/mL]	Spike [ng/mL]	Expected [ng/mL]	Obtained [ng/mL]	Recovery [%]
23.69	4.0	27.69	27.05	97.70
	6.0	29.69	30.12	101.45
	8.0	31.69	32.59	102.82
	9.0	32.69	34.74	106.25
68.63	4.0	72.63	74.16	102.12
	6.0	74.63	76.35	102.31
	8.0	76.63	76.32	99.60
	9.0	77.63	80.11	103.20
22.96	4.0	26.96	29.17	108.19
	6.0	28.96	29.71	102.58
	8.0	30.96	30.96	100.00

11.3 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 0.906 ng/mL

Limit of detection, LoD 1.884 ng/mL

Limit of quantitation, LoQ 2.055 ng/mL

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

11.4 Analytical specificity

The specificity of the antibody was tested by measuring the cross-reactivity against a range of compounds with structural similarity to pancreatic elastase. There was no cross-reactivity observed.

Substance tested	Concentration added	Concentration obtained [ng/mL]	Conclusion
Chymotrypsin	166.67 ng/mL	< 0.906	< LoB
PMN-elastase	10 µg/mL	< 0.906	< LoB
Pancreatic amylase	37 ng/mL	< 0.906	< LoB
Hemoglobin	500 ng/mL	< 0.906	< LoB
Pancreatic lipase	200 U/L	< 0.906	< LoB
Calprotectin	840 ng/mL	< 0.906	< LoB
α1-antitrypsin	90 µg/mL	< 0.906	< LoB
Pancreatin	80 mg/mL	< 0.906	< LoB

11.5 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 with a serial dilution of 3 different stool-samples.

For pancreatic elastase in stool, the method has been demonstrated to be linear from 3.42 to 48.89 ng/mL, showing a non-linear behaviour of less than ±20 % in this interval.

Sample	Dilution	Expected [ng/mL]	Obtained [ng/mL]	Recovery [%]
A	1:40 000	48.89	48.89	100.00
	1:80 000	24.44	28.52	116.69
	1:160 000	12.22	12.69	103.84
	1:320 000	6.11	6.86	112.24
	1:640 000	3.06	3.42	111.92
B	1:80 000	40.10	40.10	100.00
	1:160 000	20.05	22.85	113.97
	1:320 000	10.03	10.95	109.22
	1:640 000	5.01	5.34	106.49
C	1:40 000	40.68	40.68	100.00
	1:80 000	20.34	23.54	115.71
	1:160 000	10.17	11.71	115.10
	1:320 000	5.09	5.73	112.76

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.
- 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 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.
- 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. DRG can therefore not be held responsible for any damage resulting from incorrect use.

15 REFERENCES / LITERATURE

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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.
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SYMBOLS USED

Symbol	English	Deutsch	Italiano	Español	Français
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conformità europea	Conformidad europea	Conformité normes européennes
	Consult instructions for use *	Gebrauchsanweisung beachten *	Consultare le istruzioni per l'uso	Consulte las instrucciones de uso	Consulter les instructions d'utilisation
IVD	*In vitro* diagnostic medical device *	*In-vitro*-Diagnostikum *	Diagnostica in vitro	Diagnóstico in vitro	Diagnostic in vitro
REF	Catalogue number *	Artikelnummer *	No. di Cat.	No de catálogo	Référence
LOT	Batch code *	Chargencode *	Lotto no	Número de lote	No. de lot
	Contains sufficient for <n> tests *	Ausreichend für <n> Prüfungen *	Contenuto sufficiente per "n" saggi	Contenido suficiente para <n> ensayos	Contenu suffisant pour "n" tests
	Temperature limit *	Temperaturbegrenzung *	Temperatura di conservazione	Temperatura de conservacion	Température de conservation
	Use-by date *	Verwendbar bis *	Data di scadenza	Fecha de caducidad	Date limite d'utilisation
	Manufacturer *	Hersteller *	Fabbricante	Fabricante	Fabricant
	Caution *	Achtung *			
RUO	For research use only	Nur für Forschungszwecke	Solo a scopo di ricerca	Sólo para uso en investigación	Seulement dans le cadre de recherches
Distributed by	Distributed by	Vertreiber	Distributore	Distribuidor	Distributeur
Content	Content	Inhalt	Contenuto	Contenido	Conditionnement
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantità	Volumen/Número	Volume/Quantité