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User's Manual

Pancreatic Elastase ELISA Kit

REF **DEIA-NS2408-11**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

This assay is an enzyme immunoassay intended for the quantitative determination of human pancreatic elastase in stool.

General Description

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.

Principles of Testing

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, immobilised 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-labelled conjugate (mouse anti pancreatic elastase) is added which recognises 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 solutionis added to stop the reaction. The colour changes from blue to yellow. The intensityof the yellow colour 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.

Reagents And Materials Provided

1. PLATE Microtiter plate, pre-coated, 12 × 8 wells
2. WASHBUF Wash buffer concentrate 10×, 2 × 100 ml
3. CONJ Conjugate concentrate, peroxidase-labelled (mouse anti pancreatic elastase), 1 × 200 µl
4. STD Standards, lyophilised (see specification for concentrations), 4 × 5 vials.
5. CTRL1 Control, lyophilised (see specification for range), 4 × 1 vial
6. CTRL2 Control, lyophilised (see specification for range), 4 × 1 vial
7. SUB Substrate, (tetramethylbenzidine),ready-to-use 1 × 15 ml
8. STOP Stop solution, ready-to-use, 1 × 15 ml
9. Extraction buffer concentrate 2.5×, 1 × 100 ml

Materials Required But Not Supplied

1. Ultrapure water*
2. Calibrated precision pipettors and 10–1000 µl single-use tips
3. Foil to cover the microtiter plate
4. Horizontal microtiter plate shaker
5. Multi-channel pipets or repeater pipets
6. Vortex
7. Standard single-use laboratory glass or plastic vials, cups, etc.
8. Microtiter plate reader

* CD 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).

Storage

2-8°C

Specimen Collection And Preparation

1. Sample stability and storage

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

2. Extraction of the stool samples

Extraction buffer (1:2.5 diluted) is used as a sample extraction buffer.

We recommend the following sample preparation:

It is recommended to purchase a professional **Stool Sample Application System (SAS)**, please consult CD for details.

Stool sample tube

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:

1) 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.

2) Fill the empty stool sample tube with 1.5 ml sample extraction buffer (1:2.5 diluted) before using it with the sample. Important: Allow the sample extraction buffer to reach room temperature.
3) 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 coveredcompletely 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.
4) 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.
5) Allow sample to stand for ~10 minutes until sediment has settled. Floating material like shells of grains can be neglected.
6) 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

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:

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.

Reagent Preparation

1. 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.
2. Reagents with a volume less than 100 µl should be centrifuged before use to avoid loss of volume.
3. **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.
4. **Preparation of the extraction buffer:** The Extraction Buffer Concentrate has to be diluted with ultrapure water 1:2.5 before use (100 ml Extraction Buffer Concentrate + 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 Extraction Buffer Concentrate is stable at 2–8 °C until the expiry date stated on the label. Extraction buffer (1:2.5 diluted Extraction Buffer Concentrate) can be stored in a closed flask at 2–8 °C for 4 months.

5. 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 not stable and cannot be stored.
6. 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 the expiry date stated on the label. Conjugate (1:101 diluted CONJ) is not stable and cannot be stored.
7. All other test reagents are ready-to-use. Test reagents are stable until the expiry date (see label) when stored at 2–8 °C.

Assay 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.

We recommend to carry out the tests in duplicate.

1. Add each 100 µl standards/controls/diluted samples into the respective wells.
2. Cover the strips and incubate for 30 min at room temperature (15–30 °C) on a horizontal shaker*.
3. 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.
4. Add 100 µl conjugate (diluted CONJ) into each well.
5. Cover the strips and incubate for 30 min at room temperature (15–30 °C) on a horizontal shaker*.
6. 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.
7. Add 100 µl substrate (SUB) into each well.
8. Incubate for 10–20 min** at room temperature (15–30 °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 colour change is temperature sensitive. We recommend observing the colour change and stopping the reaction upon good differentiation.

Quality Control

CD 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 concentration range.

Reference range in stool samples

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

Calculation

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 10000** to get the actual concentrations.

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

Performance Characteristics

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

Precision

Repeatability (Intra-Assay); n = 42

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

Sample	Mean value [µg/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, instruments, days and kit lots).

Sample	Mean value [µg/ml]	CV [%]
1	209.01	9.7
2	349.12	7.4

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.

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
Pankreatic amylase	37 ng/ml	< 0.906	< LoB
Hemoglobin	500 ng/ml	< 0.906	< LoB
Pankreatic 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

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 accordingto 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
	1:640000	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

Precautions

1. 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.
2. 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 CD on request.
3. The 10× 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.

4. 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.

TECHNICAL HINTS

1. 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.
2. Control samples should be analysed with each run.
3. Reagents should not be used beyond the expiration date stated on kit label.
4. Substrate solution should remain colourless until use.
5. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
6. Avoid foaming when mixing reagents.
7. Do not mix plugs and caps from different reagents.
8. The assay should always be performed according to the enclosed manual.

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

Liquid stools may lead to false pancreatic elastase results. In this case, we recommend considering other testing methods or testing another sample.