Pancreatic Elastase (PE) ELISA

For the quantitative determination of pancreatic elastase in human stool

For Research Use Only. Not for Use in Diagnostic Procedures.

Catalog Number: 31-PANHU-E01

Size: 96 determinations

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INTENDED USE

The Pancreatic Elastase ELISA is intended for the quantitative determination of pancreatic elastase (PE) in human stool. For Research Use Only. Not for Use in Diagnostic Procedures.

INTRODUCTION

Pancreatic elastase belongs to a class of serine proteases. It is a digestive enzyme that is produced in the pancreas of all vertebrates as an inactive zymogen (proelastase) and activated in the small intestine by cleavage with trypsin. The elastase is excreted unmodified in the stool.

PRINCIPLE OF THE ASSAY

The Pancreatic Elastase ELISA test determines human pancreatic elastase according to the "sandwich" principle. Pancreatic elastase in samples, standards, and controls binds to antibodies which are coated to the microtiter plate. After a wash step, a peroxidase-labeled detection antibody is added. A second wash step is followed by the addition of the substrate which is converted to a colored product by the peroxidase. The reaction is terminated by the addition of an acidic stop solution. The optical densities are read at 450 nm (against the reference wavelength of 620 nm) in a microtiter plate reader. The pancreatic elastase concentration can be calculated from the standard curve.

The test system was calibrated using a reference preparation of recombinant and purified human pancreatic elastase.

MATERIALS SUPPLIED

31-PANHU-E01			
Component	**Quantity**	**Preparation**	**Storage**
Microtiter plate, coated	1 plate: 12 x 8-well strips	Ready-to-use	2-8˚C
Universal extraction buffer	150 mL	Ready-to-use	2-8˚C
Wash Buffer concentrate, 10X	100 mL	Dilute 1:10	2-8˚C
Standards*	5 vials, 1.5 mL each	Ready-to-use	2-8˚C
Control 1 and 2*	2 vials, 1.5 mL each	Ready-to-use	2-8˚C
Conjugate: peroxidase-labeled antibody	15 mL	Ready-to-use	2-8˚C
Sample Buffer	120 mL	Ready-to-use	2-8˚C
TMB Substrate: tetramethylbenzidine	15 mL	Ready-to-use	2-8˚C
Stop solution	10 mL	Ready-to-use	2-8˚C
Plate Sealers	2	Ready-to-use	RT

*Please refer to the Certificate of Analysis enclosed with each kit for Standard and Control concentrations.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

- Centrifuge, 3000 x g
- Plastic vials
- Stool sample extraction vials (80-EXDEV2-100)

- Various pipettes
- Multichannel or multipipette
- Foil to cover the microtiter plate (substrate step)
- Distilled or deionized water
- ELISA reader with 450 nm filter (620 nm reference filter)
- Microtiter plate shaker (2 mm orbital shaker capable of 400 rpm)
- Vortex mixer
- Absorbent paper (lint-free)
- Timer

PRECAUTIONS

- This assay is for Research Use Only. Not for use in diagnostic procedures.
- Individual components from different batches and test kits should not be interchanged. The expiry dates stated on the relevant packaging must be observed.
- The test kit reagents contain preservatives to protect against bacterial growth. Contact with the skin and/or mucous membranes should be avoided.
- Standards and controls contain recombinant human pancreatic elastase expressed in human HEK293 cells. As a precautionary measure, all test kit reagents must be handled as potentially infectious material in accordance with regulations of health care accident prevention.
- The substrate TMB (tetramethylbenzidine) is toxic by ingestion and skin contact. In the event of contact with the skin, the affected area must be washed immediately with plenty of water and soap.
- Avoid contact of the stop solution, which consists of acid, with the skin. It causes burns on contact. Work with protective gloves and goggles. In the event of contact, the burned area must be immediately and thoroughly rinsed with plenty of water. If necessary, a doctor should be consulted.
- Adherence to the test protocol is essential. ALPCO assumes no liability for any damage caused by unauthorized changes in the test procedure.
- The guidelines for carrying out quality control in testing laboratories must be observed. Appropriate controls must be tested.
- The reagents must not be used after the expiration date.
- Wear disposable gloves when handling samples or kit reagents and wash hands thoroughly afterwards. Do not pipette by mouth. Do not eat, drink, smoke, or apply makeup in areas where samples or kit reagents are being handled.
- Samples may contain unknown interfering substances. This can lead to false high or false low results. Aqueous stool samples can show falsely low concentrations even though there is no pancreatic insufficiency.

REAGENT PREPARATION

Microtiter plate. Assemble the required number of strips in the holder. Allow the plate to reach 20-30°C before use. Unused strips must be stored at 2-8°C in the pouch with desiccant. Please do not dispose of the holder until all strips are used.

Wash buffer. Dilute the wash buffer concentrate 1:10 with distilled or deionized water (1 part buffer + 9 parts DI water). Please note: When storing the wash buffer concentrate at 2-8 °C crystallization may occur. Before dilution, all crystals must be dissolved. The 1X working wash buffer is stable for 14 days at 2-8°C. It is recommended to dilute only the amount of buffer needed to process the given samples.

All other test reagents are stable at 2-8 °C up to the date of expiry stated on the label, unless otherwise specified.

SAMPLE PREPARATION

Stool samples must be extracted with Universal Extraction Buffer at a ratio of 1:100 before testing in the Pancreatic Elastase ELISA. Manual weighing or an approved stool extraction device (80-EXDEV2-100) may be used.

For manual weighing mix **15 mg** stool with **1.5 ml** universal extraction buffer (or greater amount of stool diluted 1:100 with universal extraction buffer), then vortex until the mixture is homogenous. Transfer the resulting slurry to a plastic vial and centrifuge for 10 min at 3000 x g.

The supernatant is diluted 1:50 in sample buffer, e.g. 5µL supernatant + 245µL sample buffer. 40 µL of this dilution is used per well in the Pancreatic Elastase ELISA.

ASSAY PROCEDURE

All reagents and samples should be equilibrated to 20-30°C and mixed well before use.
The position of standards, controls, and samples are noted on a protocol sheet.

1. **Wash step**

   Pick out the pre-assembled microtiter plate with the needed number of strips and wash them 1x with 250 µl 1X working wash buffer per well. Remove residual buffer by tapping the plate on absorbent paper after the wash step.

2. **Sample incubation**

   Pipette **100 µl Standards and Controls, and 40 µl diluted samples** in duplicate in the microtiter plate.

   The strips are covered and incubated by shaking for **60 min** at room temperature (20-30 °C; 400 rpm, 2 mm orbit).

3. **Wash step**

   Discard the contents of the microwells and wash 5x with 250 µl 1X working wash buffer per well. Remove residual buffer by tapping the plate **gently** on absorbent paper after the last wash step.

4. **Conjugate incubation**

Pipette **100 µl Conjugate** in each microwell.

Cover the strips and incubate with shaking for **60 min** at room temperature (20-30°C; 400 rpm, 2 mm orbit).

5. **Wash step**

Discard the contents of the microwells and wash 5x with 250 µl 1X working wash buffer per well. Remove residual buffer by tapping the plate **gently** on absorbent paper after the last wash step.

6. **Substrate incubation**

Pipette **100 µl TMB substrate** in each microwell.

Incubate for **10-15 min** at room temperature (20-30°C; 400 rpm, 2 mm orbit) in the dark.

7. **Stopping the reaction**

Pipette **50 µl stop solution** in each microwell. Mix well by tapping the side of the plate frame.

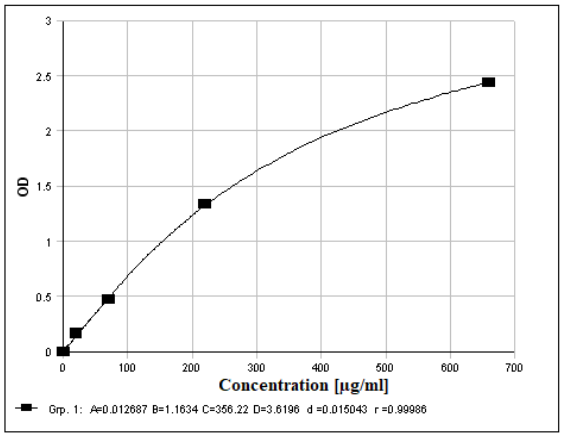
8. **Reading**

Read the absorbance at 450 nm. If available, use 620 nm as reference wavelength. Reading should be done within **5 minutes** after stopping the reaction.

CALCULATION OF RESULTS

The use of the 4-parameter-Marquardt algorithm is recommended for calculation of results. The pancreatic elastase concentration is read from the standard curve and multiplied by the factor **2.5** to generate results for pancreatic elastase in stool, due to the applied sample amount of 40µL.

TYPICAL STANDARD CURVE



The curve at left is for demonstration purposes only. It must not be used for calculation of sample values.

PERFORMANCE CHARACTERISTICS

Measuring Range

The measuring range of pancreatic elastase is 15 – 1,650 µg/ml (or µg/g).

Sensitivity

Limit of Detection (LOD): 1.3 µg/mL (or µg/g)

For the determination of the detection limit, 20 replicates of Standard 0 were measured. After addition of 3x the standard deviation to the mean value, the concentration was read from the standard curve.

Limit of Quantification (LOQ): 2.4 µg/mL (or µg/g)

For the determination of the detection limit, 20 replicates of Standard 0 were measured. After addition of 10x the standard deviation to the mean value, the concentration was read from the standard curve.

Precision: Within run (intra-assay) variation

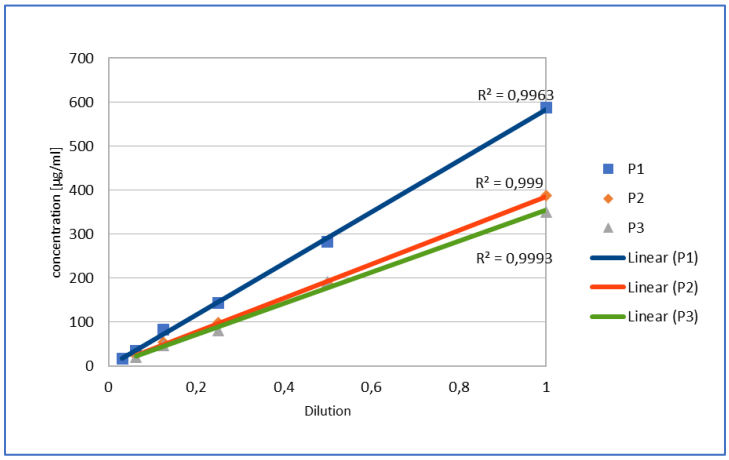
Intra-Assay CV Stool:	3.8 % (279 µg/mL)	[n = 10]
	1.6 % (186 µg/mL)	[n = 10]
	3.1 % (41 µg/mL)	[n = 10]

Precision: Between run (inter-assay) variation

Inter-Assay CV stool:	8.0 % (626 µg/ml)	[n = 10]
	10.7 % (203 µg/ml)	[n = 10]
	11.7 % (64 µg/ml)	[n = 10]

Linearity

Dilution of the samples was performed with Sample Buffer.



Sample	Dilution Factor	Expected (µg/mL)	Measured (µg/mL)	Recovery (%)
1	--	587	--	--
	1:2	293	282	96.1
	1:4	147	143	97.4
	1:8	73.4	83.2	113
	1:16	36.7	34.5	94.0
	1:32	18.3	16.6	90.5
2	--	388	--	--
	1:2	194	188	96.9
	1:4	97.0	98.4	101
	1:8	48.5	53.9	111
	1:16	24.3	23.5	96.9
3	--	351	--	--
	1:2	175	191	109
	1:4	87.8	80.9	92.2
	1:8	43.9	47.5	108
	1:16	21.9	20.7	94.4

Spike and Recovery

Sample	Endogenous (µg/mL)	Added Conc. (µg/mL)	Expected (µg/mL)	Measured (µg/mL)	Recovery (%)	Mean Recovery (%)
1	26.1	10	36.1	36.2	100	100
		50	76.1	80.4	106	
		200	226	213	94.2	
2	148	10	158	159	101	98.3
		50	198	187	94.4	
		200	348	347	99.7	
3	104	10	114	115	101	98.5
		50	154	153	99.3	
		200	304	290	95.4	

Cross-Reactivity

Cross reactivity to pancreatin at a concentration of 100 mg/l could not be detected in stool samples.

Limitations of the Method

Stool samples with pancreatic elastase concentrations above the standard curve should be diluted with sample buffer and measured again.

Disposal

The substrate must be disposed as non-halogenated solvent. The stop solution can be neutralized with NaOH and, if the pH value is neutral, it can be disposed of as salt solution (**important:** this reaction produces heat and should be handled carefully). Please refer to local and national guidelines.

REFERENCES

1. Beharry, S., Ellis, L., Corey, M., Marcon, M. & Durie, P. How useful is fecal pancreatic elastase 1 as a marker of exocrine pancreatic disease? *The Journal of Pediatrics* **141**, 84–90 (2002).
2. Bode, W., Meyer, E. & Powers, J. C. Human leukocyte and porcine pancreatic elastase: x-ray crystal structures, mechanism, substrate specificity, and mechanism-based inhibitors. *Biochemistry* **28**, 1951–1963 (1989).
3. Stein, J. *et al.* Immunoreactive elastase I: clinical evaluation of a new noninvasive test of pancreatic function. *Clin. Chem.* **42**, 222 (1996).
4. Whitcomb, D. C. & Lowe, M. E. Human Pancreatic Digestive Enzymes. *Digestive Diseases and Sciences* **52**, 1–17 (2007).