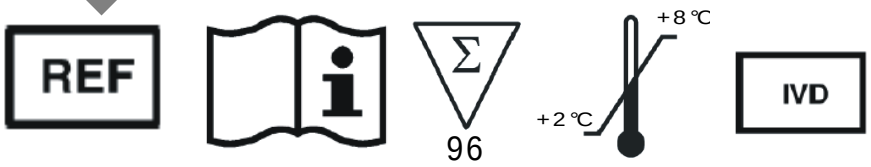
Manual

Pancreatic Elastase

ELISA

For the determination of pancreatic elastase in stool

Valid from 29.06.2023



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1. Intended use

The *ImmuChrom* ELISA Kit is intended for the quantitative determination of pancreatic elastase in stool. For *in vitro* diagnostic use by trained personnel in laboratories only.

2. Introduction

The pancreatic elastase belongs to the class of serine proteases and has a molecular weight of 26 kDa. It is a digestive enzyme that is produced in the pancreas of all vertebrates. The pancreatic elastase is produced in the pancreas as an inactive zymogen (proelastase) and activated in the small intestine by cleavage with trypsin. The elastase is excreted unmodified in the stool. The stool concentration of pancreatic elastase provides information about the performance of the pancreas.

Indications

- Diagnosis / exclusion of exocrine pancreatic insufficiency on the occurence of unclear diarrhea, constipation, steatorrhea, flatulence, weight loss, epigastric pain and food intolerance
- Monitoring of exocrine pancreatic function in cystic fibrosis, diabetes mellitus or chronic pancreatitis

The *ImmuChrom* complete pancreatic elastase kit allows an easy, rapid and precise quantitative determination of pancreatic elastase in biological samples. The kit includes all reagents ready to use, except the washing buffer.

3. General notes, warnings and precautions

This assay was produced and put on the market according to the IVD guidelines of 98/79/EC. All reagents of this kit are strictly intended for in vitro diagnostic use only.

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. Therefore 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. You should therefore 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 prescribed protocol for performing the test is essential. ImmuChrom GmbH assumes no liability for any damage caused by unauthorized changes in the test procedure.

The guidelines for carrying out quality control in medical laboratories must be observed. Appropriate controls must be carried along.

The reagents must not be used after the expiration date.

Wear disposable gloves when handling specimens or kit reagents and wash hands thoroughly afterwards. Do not pipette by mouth. Do not eat, drink, smoke, or put on makeup in areas where specimens or kit reagents are being handled.

Patient 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.

The final clinical diagnosis should not be based on the results of a single test, but should be considered by a physician only after all clinical and laboratory results have been evaluated.

4. Material delivered in the test package

Article no.	Component	Description	Amount
IC7400mtp	MTP	Microtiter plate coated	12 x 8 wells
IC7400wp	WASHBUF	Pancreatic Elastase ELISA wash buffer conc. 10-fold	100 ml
IC7400ex	EXT	Extraction buffer	150 ml
IC7410st	STD	Standards (1.5 ml) The concentrations are given in the specification	5 vials
IC7410ko	CTRL	Controls (2 levels, 1.5 ml) The concentrations are given in the specification	1 vial each
IC7400kg	CONJ	Conjugate, peroxidase labeled antibody	15 ml
IC7400vp	SAMPLEBUF	Sample buffer	50 ml
IC7400su	SUB	TMB substrate (tetramethylbenzidine)	15 ml
IC7400sp	STOPP	Stop solution	10 ml

5. Additional special equipment

- Centrifuge, 3000xg
- Plastic vials
- Stool sample extraction vials
- Various pipettes
- Multichannel- or multipipette
- Foil to cover the microtiter plate
- Bidest. water
- ELISA reader with filter 450 nm (reference filter 620 nm)
- Microtiter plate shaker
- Vortex mixer

6. Reagent preparation

Microtiter plate (MTP). Take the needed number of stripes and assemble them on the holder. Please take care that the plate has reached 20-30 °C before usage. Stripes which are not needed yet must be stored at 2-8°C. Please do not dispose of the holder until all stripes are used.

Wash buffer (WASHBUF). Dilute the wash buffer concentrate 1:10 with bidest. water (1 part buffer + 9 parts bidest. water). The dilution is stable for 14 days at 2-8°C.

Important: When storing the wash buffer concentrate at 2-8°C crystallization may occur. Before dilution, all crystals must be dissolved.

It is recommended to dilute only the amount of buffer which is used 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.

7. Specimen

Stool samples

The pancreatic elastase is extracted by the extraction buffer out of the stool sample in a ration of 1:100 (e.g. 10 mg/ml).

Extraction in stick vials

For the extraction stick vials could be used.

We recommend to mix **15 mg** stool with **1.5 ml** extraction buffer (EXT), then vortex it until the mixture is homogenous. Transfer the resulting slurry to a plastic vial and centrifuge it for 10 min at 3000 xg.

The supernatant is diluted **1:50** in sample buffer (SAMPLEBUF) (e.g. 5 µl supernatant + 245 µl sample buffer).

40 µl of the dilution are used in the test per well.

8. Procedure

Principle of the method

The pancreatic elastase-ELISA test determines human pancreatic elastase according to the “sandwich-principle”. The elastase in sample, standard and controls binds to antibodies, which are coated to the microtiter plate. After a washing step a peroxidase labeled detection antibody is added. A second washing 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 620 nm) in a microtiter plate reader. The pancreatic-elastase concentration can be calculated from the standard curve.

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

Sample preparation

All reagents and samples should be prewarmed to 20-30 °C and mixed well before use.

The position of standards, controls and samples are noted on a protocol sheet.

1. Washing step

Pick out the pre-assembled microtiter plate with the needed number of stripes and wash them 1x with 250 µl diluted WASHBUF per cavity. Remove residual buffer by tapping the plate **gently** on absorbent paper after the washing step.

2. Samples incubation

Pipette **100 µl STD**, **CTRL** and **40 µl diluted sample** in double values into the microtiter plate.

Cover the stripes with a cover film and incubate the microtiter plate by shaking for **60 min** (20-30 °C; 400 rpm, 2 mm orbit diameter).

3. Washing step

Discard the content of the microwells and wash 5x with 250 µl diluted WASHBUF per cavity. Remove residual buffer by tapping the plate **gently** on absorbent paper after the last washing step.

4. Conjugate incubation

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

Cover the stripes with a cover film and incubate the microtiter plate by shaking for **60 min** (20-30 °C; 400 rpm, 2 mm orbit diameter).

5. Washing step

Discard the content of the microwells and wash 5x with 250 µl diluted WASHBUF per cavity. Remove residual buffer by tapping the plate **gently** on absorbent paper after the last washing step.

6. Substrate incubation

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

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

7. Stopping reaction

Pipette **50 µl STOPP** in each microwell. Mix well.

8. Reading

Read the absorbance at 450 nm. If the microtiter plate reader allows to use a reference wavelength use 620 nm as reference wavelength.

Reading should be done within 5 min after the stopping reaction.

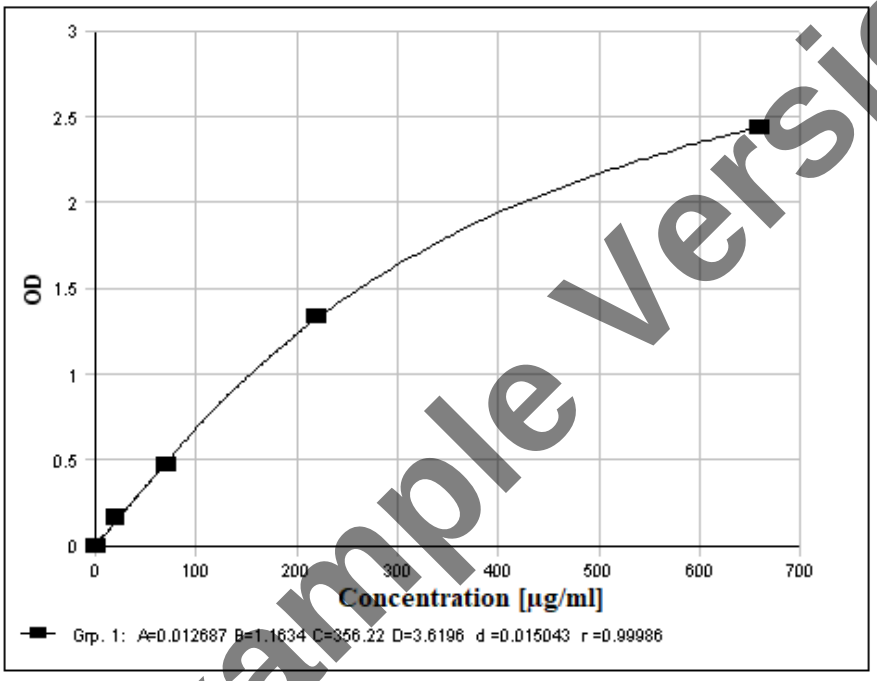
9. Calculation of analytical results

For calculating the results, we recommend to use the 4-parameter Marquardt algorithm.

Stool samples

The pancreatic elastase concentration is read from the standard curve and multiplicated with the factor **2.5** to get the results for pancreatic elastase in stool, due to the applied sample amount of 40 µl.

Standard curve



The curve given above is only for demonstration. It must not be used for calculation of your samples.

10. Internal quality control

Reference values

Stool:

Assumption: 1 g of stool corresponds to 1 ml.

> 200 µg/ml normal value

100 - 200 µg/ml mild to moderate exocrine pancreatic insufficiency

< 100 µg/ml exocrine pancreatic insufficiency

We recommend, that each laboratory should develop their own normal range. The values mentioned above are only for orientation and can deviate from other published data.

11. Validation data

Measuring range

The measuring range is between a sample concentration of 15 µg/ml to 1650 µg/ml.

Precision and reproducibility

Intra-Assay CV:	3.8 % (279 µg/ml)	[n = 10]
	1.6 % (186 µg/ml)	[n = 10]
	3.1 % (41 µg/ml)	[n = 10]
Inter-Assay CV:	8.0 % (626 µg/ml)	[n = 10]
	10.7 % (203 µg/ml)	[n = 10]
	11.7 % (64 µg/ml)	[n = 10]

Detection limit

1.3 µg/ml

For the determination the zero-standard was measured 20 times. The 3-fold standard deviation was added to the mean value of the optical density. The respective concentration was read from the standard curve.

Limit of quantification

2.4 µg/ml

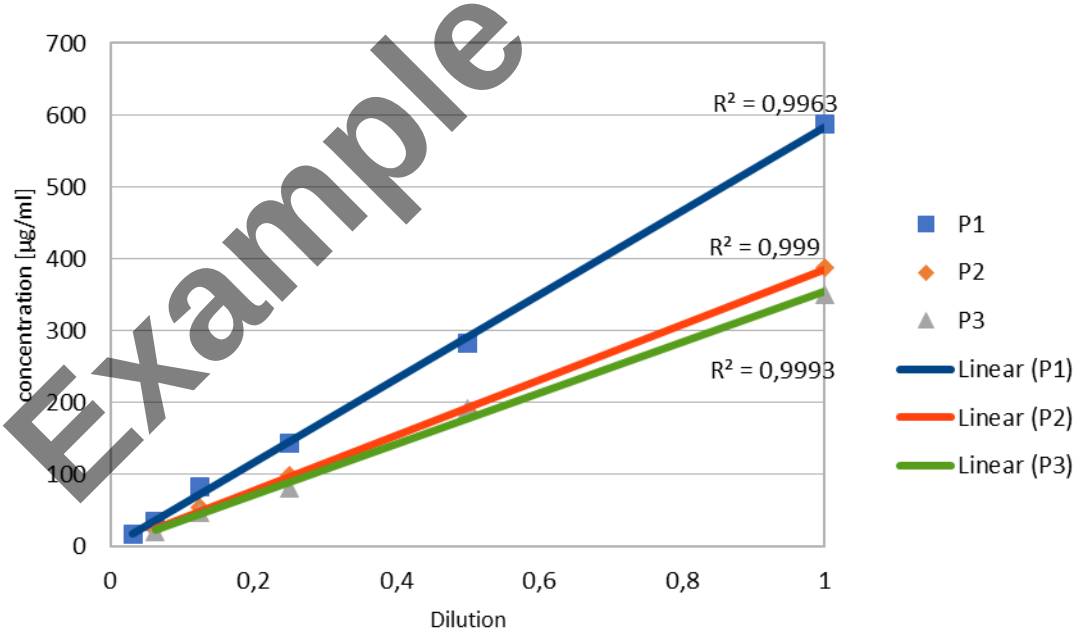
For the determination the zero-standard was measured 20 times. The 10-fold standard deviation was added to the mean value of the optical density. The respective concentration was read from the standard curve.

Recovery

Sample	Endogenous concentration [µg/ml]	Added concentration [µg/ml]	Expected concentration [µg/ml]	Measured concentration [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	

Linearity

The dilution of the samples is performed with sample buffer.



Sample	Dilution	Expected concentration [µg/ml]	Measured concentration [µg/ml]	Recovery [%]
P 1	1	587		
	0.5	293	282	96.1
	0.25	147	143	97.4
	0.125	73.4	83.2	113
	0.0625	36.7	34.5	94.0
	0.03125	18.3	16.6	90.5
P 2	1	388		
	0.5	194	188	96.9
	0.25	97.0	98.4	101
	0.125	48.5	53.9	111
	0.0625	24.3	23.5	96.9
P 3	1	351		
	0.5	175	191	109
	0.25	87.8	80.9	92.2
	0.125	43.9	47.5	108
	0.0625	21.9	20.7	94.4

Cross Reactivity

Cross reactivity to pancreatin could not be detected in stool samples. The used concentration of the substance was 100 mg/l.

12. Limitations of the method

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

13. Disposal

The substrate (SUB) must be disposed as non-halogenated solvent. The stop solution (STOPP) can be neutralized with NaOH and if the pH value is neutral, it can be disposed as salt solution (**important:** Reaction will produce heat and should be handled carefully).

Please refer to the appropriate national guidelines.

14. Literature References

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4. Whitcomb, D. C. & Lowe, M. E. Human Pancreatic Digestive Enzymes. *Digestive Diseases and Sciences* **52**, 1–17 (2007).