Manual

Pancreatic Elastase

ELISA

For the determination of pancreatic elastase in stool

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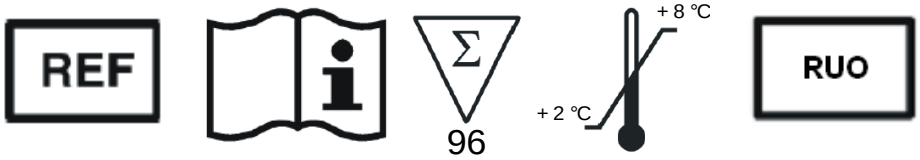

IC7400

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1. Intended use

The *ImmuChrom* ELISA Kit is intended for the quantitative determination of pancreatic elastase in stool. For research use 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 for use, except the washing buffer.

3. Warnings and precautions

All reagents of this kit are strictly intended for research use only.

Do not interchange kit components from different lots.

The test kit contains components of human origin. It was tested and found negative for HBsAg, anti-HIV-1/2, and anti-HCV. No test can guarantee the absence of HBsAg or HIV, and so all reagents of human origin in this kit must be handled as though capable of transmitting infection.

The stop solution (STOP) contains acid and has to be handled carefully. It is corrosive and causes burns. It should be handled with gloves, eye protection and appropriate protective clothing in a hood. Any spill should be wiped up immediately with copious quantities of water. Do not breath vapor and avoid inhalation. In case of an accident or indisposition contact a physician immediately.

The substrate TMB (tetramethyl benzidine) is toxic by ingestion and contact with the skin. Any spill should be wiped up immediately with copious quantities of water.

Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.

Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.

The reagents of the test kit contain bactericides to protect against bacterial growth. Avoid the contact with the skin or mucous membrane.

Reagents should not be used beyond the expiration date shown on kit label.

Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera. During handling of all kit reagents, controls and serum samples observe the existing legal regulations.

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) (0; 24.4; 73.3; 220; 660 µg/ml)	5 vials
IC7410ko	CTRL	Controls (2 levels, 1.5 ml)	1 vial each
IC7400kg	CONJ	Conjugate, peroxidase labeled antibody	15 ml
IC7400vp	SAMPLEBUF	Sample buffer	24 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 room temperature 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 with the extraction buffer (EXT) from the stool sample.

Extraction in stick vials

In a stool sample extraction vial 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).

(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 is calibrated using a reference preparation of recombinant and purified human pancreatic elastase.

Sample preparation

All reagents and samples should be warmed up 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. 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).

3. Washing step

Discard the content of the microwells and wash 5x with 250 µl diluted WASHBUF. 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).

5. Washing step

Discard the content of the microwells and wash 5x with 250 µl diluted WASHBUF. 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).

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 stopping reaction.

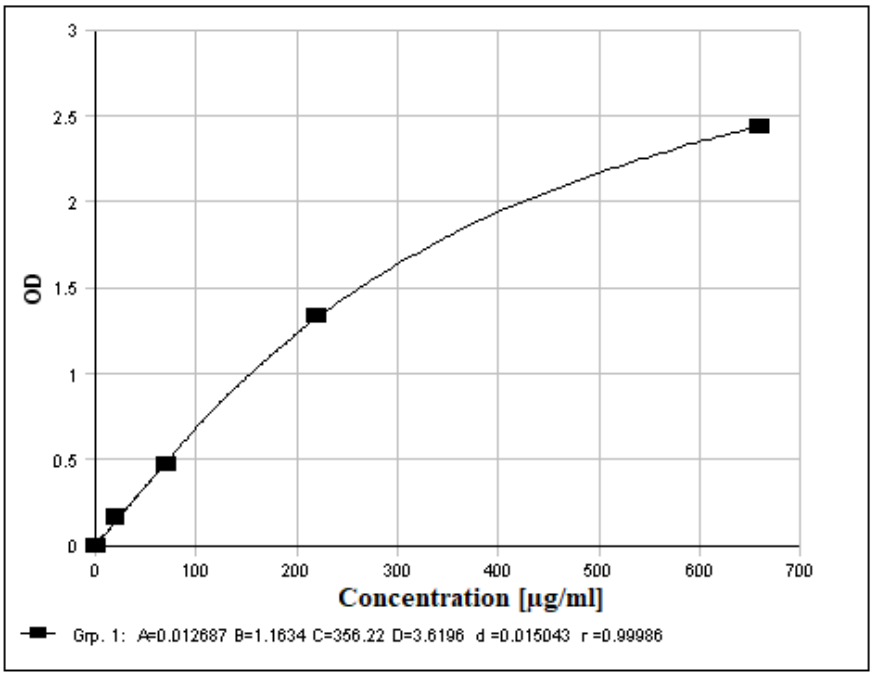
9. Calculation of analytical results

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 multiplied 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:

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 publicized data.

11. Validation data

Precision and reproducibility

Intra-Assay CV:	< 10 % (279 µg/ml)	[n = 10]
	< 10 % (186 µg/ml)	[n = 10]
	< 10 % (41 µg/ml)	[n = 10]
Inter-Assay CV:	< 15 % (626 µg/ml)	[n = 10]
	< 15 % (203 µg/ml)	[n = 10]
	< 15 % (64 µg/ml)	[n = 10]

Detection limit

Stool 1.25 µg/ml

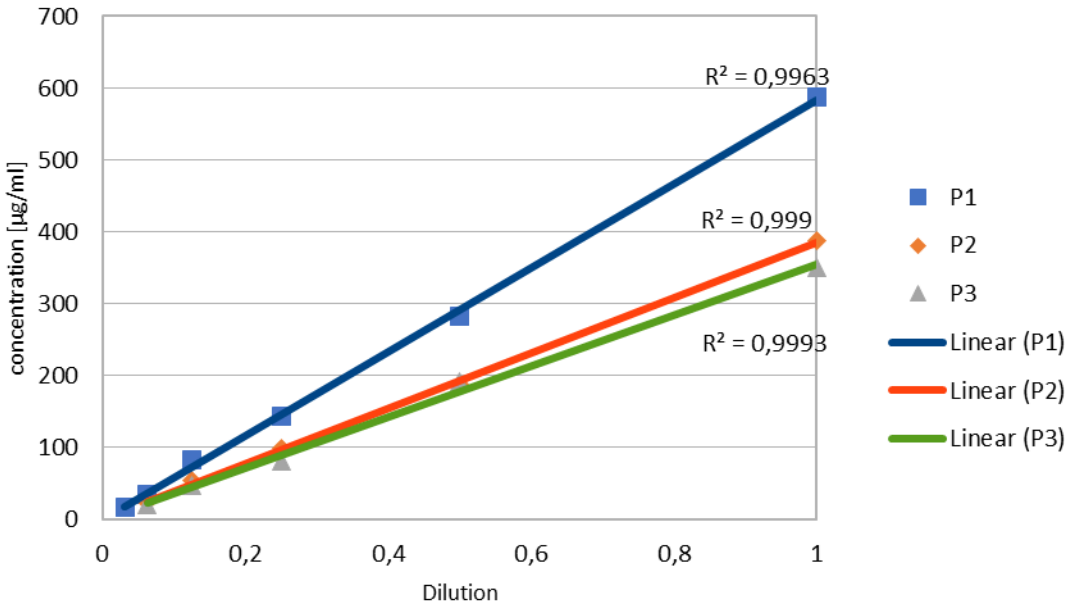
For the determination the zero-standard was measured 20 times. The 3-fold standard deviation was added to the mean value of the optical density. The respective concentration was read from the standard curve.

Recovery

Sample	Endogenous concentration [µg/ml]	Added concentration [µg/ml]	Expected concentration [µg/ml]	Measured concentration [µg/ml]	Recovery [%]
1	26.1	10	36.1	36.2	100
		50	76.1	80.4	106
		200	226	213	94.2
2	148	10	158	159	101
		50	198	187	94.4
		200	348	347	99.7
3	104	10	114	115	101
		50	154	153	99.3
		200	304	290	95.4

Linearity

The dilution of the samples was 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 substances 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

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).
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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).