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Manual

Chymotrypsin Activity

For the in vitro determination of chymotrypsin activity in stool

Valid from 2016-06-06

REF **K 6990**

Σ 20

+8°C
+2°C

IVD

CE

Table of Contents

1. INTENDED USE

The described photometric kit is intended for the activity determination of chymotrypsin in stool. It is for in vitro diagnostic use only.

2. INTRODUCTION

Chymotrypsin is a pancreas specific proteolytic enzyme. It is produced in the pancreas and excreted in the small intestine. Chymotrypsin is involved in **exocrine pancreas insufficiency in the case of a chronic pancreatitis**. The detection of chymotrypsin in stool is a strong, indicative parameter for pancreas function disorder and has the advantage of only requiring a non-invasive sampling. The time, effort and expense in a chymotrypsin determination is significantly low when compared to the pancreolauryl or pancreozymin-secretin test.

The **chymotrypsin activity** can be determined with our spectral-photometric chymotrypsin test. Patients with digestive disturbances as a result of pancreas function disorder are often supplemented with pancreatic enzymes. Such medication must be discontinued at least 5 days before the stool sample is collected. Laxatives should also not be taken before the sample collection. False low chymotrypsin activity levels can be expected in the case of diarrhoea, low-protein diet or obstructive jaundice, whereas false normal level by non-discontinued enzyme substitution. Very high levels are indicative of a fermentation or an increased intestinal fluid secretion (e. g. by enterocolitis).

Indication

- Exocrine pancreas insufficiency in the case of a chronic pancreatitis

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
K 6990	SUB	Substrate, lyophilised	20 x 1 vial
K 6990	CTRL 1	Control, lyophilised (see specification for range)	4 x 1 vial
K 6990	CTRL 2	Control, lyophilised (see specification for range)	4 x 1 vial

For reorders of single components, use the catalogue number followed by the label as product number.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultra pure water*
- Calibrated precision pipettors and 10-1000 µl tips
- Multi-channel pipets or repeater pipets
- Centrifuge, 3000 *g*
- Vortex
- 10 mm one way cuvettes
- Spectral photometer for kinetics measurements at 405 nm (Hg)
- Standard laboratory glass or plastic vials, cups, etc.
- Sample preparation system (e. g. Roche Catalog No. 745 804)

* Immundiagnostik AG recommends the use of Ultra Pure 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. PREPARATION AND STORAGE OF REAGENTS

- **Lyophilised substrate** (SUB) and **lyophilised controls** (CTRL 1 and 2) are stable at **2–8 °C** until the expiry date stated on the label.
- **Lyophilised substrate** (SUB) must be reconstituted in each **2.1 ml ultra pure water** before use. **Substrate** (reconstituted SUB) **cannot be stored**.
- **Lyophilised controls** (CTRL 1 and 2) must be reconstituted in each **0.5 ml ultra pure water** before use. **Controls** (reconstituted CTRL 1 and 2) **cannot be stored**.

6. SAMPLE PREPARATION

Extraction of the stool sample

We recommend to use the solvens buffer (catalogue no. K 6990 SOL) as a **sample extraction buffer** and a stool sample preparation kit for dosing 100 mg of stool sample (e. g. sample preparation kit from Roche Diagnostics, Mannheim, Germany; cat #745804). The stool sample must be suspended in 10 ml solvens buffer.

Constant buffer volume: 10 ml

Constant dilution factor: 1:100

If the sample preparation kit is not used, the weighed stool sample must be diluted 100-fold with solvens buffer (e. g. 120 mg stool sample + 12.0 ml of solvens buffer).

Mix the stool suspension with the solvens on a vortexer for 30 sec.

Transfer the suspension into an Eppendorf tube and centrifuge for 5 min.

Dilution of samples

Stool samples

Pipet **100 µl** of the supernatant per cuvette for analysis.

The stool suspension is not stable and can not be stored. We recommend to weigh fresh sample if the analysis should be repeated.

The chymotrypsin activity in stool is constant for 10 days at room temperature.

7. ASSAY PROCEDURE

Principle of the test

Succ-Ala-Ala-Pro-Phe-pNA + H_2O $\xrightarrow{\text{Chymotrypsin}}$ Succ-Ala-Ala-Pro-Phe + p-Nitroanilin

Test procedure

1.	Run the assay either at 25 °C, 30 °C or 37 °C (see data sheet)
2.	Label a cuvette for each control and sample and note on a protocol sheet
3.	Add 2000 µl SUB (substrate) into each cuvette **Note: Add controls and samples only after the desired temperature has been reached!**
4.	Add 100 µl of controls or sample into the respective cuvette, mix well
5.	Determine the extinction after approximate 1 min at 405 nm and start the stop watch simultaneously. Determine the extinction again after exactly 1, 2 and 3 min.

8. RESULTS

The mean is calculated from the extinction difference pro min (ΔE/min)

Calculation:

The chymotrypsin activity of the sample is given in the table below. It can be calculated as described in the following example:

U/g Stool = 212 x ΔE_{405}/min

Chymotrypsin activity values for measurement at Hg 405 nm

ΔE/min	U/g	ΔE/min	U/g	ΔE/min	U/g
0,001	0,2	0,013	2,8	0,045	9,5
0,002	0,4	0,014	3,0	0,050	10,6
0,003	0,6	0,015	3,2	0,055	11,7
0,004	0,8	0,016	3,4	0,060	12,7
0,005	1,1	0,017	3,6	0,065	13,8
0,006	1,3	0,018	3,8	0,070	14,8
0,007	1,5	0,019	4,0	0,075	15,9
0,008	1,7	0,020	4,2	0,080	17,0
0,009	1,9	0,025	5,3	0,085	18,0
0,010	2,1	0,030	6,4	0,090	19,0
0,011	2,3	0,035	7,4	0,095	20,1
0,012	2,5	0,040	8,5	0,100	21,2

9. LIMITATIONS

The dilution limit is defined by extinction difference of ΔE/min = 0,100 for detection time of 3 min. If the sample activity is higher, mix 0.1 ml suspension with 0.4 ml of solvens buffer and re-assay the sample. To calculate the chymotrypsin activity of the sample **multiply the obtained value by 5**.

10. QUALITY CONTROL

Immundiagnostik 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.

Reference range

1 g stool corresponds to 1 ml.

	25 °C	37 °C
Normal range	> 6 U/g	> 13,2 U/g
Grey area	3–6 U/g	6,6–13,2 U/g
Pathological range	< 3 U/g	< 6,6 U/g

H. Goebbel et al. *Labormedizin*, Supplement 1 (1985) 8-10

We recommend each laboratory to establish its own reference concentration range.

11. PERFORMANCE CHARACTERISTICS

Dilution recovery

Control [µl]	Solvens [µl]	value expected [U/g]	value measured [U/g]
1000	0	50	52.21
900	100	45	45.16
800	200	40	42.41
700	300	35	38.96
600	400	30	29.04
500	500	25	22.07

12. PRECAUTIONS

- All reagents in the kit package are for *in vitro* diagnostic use only.
- Control samples should be analyzed with each run.
- 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.

13. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay.
- Control samples should be analyzed with each run.

- Reagents should not be used beyond the expiration date stated on the kit label.
- Substrate solution should remain colourless until use.
- 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.
- Quality control guidelines 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. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.
- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.

15. REFERENCES

H. Goebbel et al. *Labormedizin*, Supplement 1 (1985) 8-10.

Used symbols:

 Temperature limitation

REF Catalogue Number

IVD In Vitro Diagnostic Medical Device

→REF To be used with

 Manufacturer

 Contains sufficient for <n> tests

LOT Lot number

 Use by

 Attention

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