

# CHYMOTRIPSIN

## Colorimetric determination of chymotrypsin in faeces

### TEST SUMMARY

Chymotrypsin present in the sample hydrolyses the substratum forming a yellow compound.

### SAMPLES

Faeces. Stability: 10 days at 15-25°C.

### REAGENTS

Reagent  
Chromogen: Succ-Ala-Ala-Pro-Phe-pNA 0.5 mM,  
Tris 100 mM pH 9,0 CaCl<sub>2</sub> 20 mM;  
NaCl 250 mM.

Diluent  
for samples: Chloride of lauril-trimethyl ammonium  
NaCl 500 mM CaCl<sub>2</sub> 100 mM.

### MATERIAL REQUIRED BUT NOT SUPPLIED

Normal laboratory equipment. Spectrophotometer UV/VIS. Automatic Micropipette. Cuvette in optical glass or monouse in optical polystyrene. Physiologic solution.

### PRECAUTIONS

Reagent may contain non reactive and conservative components. It is opportune to avoid contacts with the skin and do not swallow.  
Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

### REAGENTS PREPARATION

Dissolve a vial of Chromogen with 2 ml of distilled water.  
Reagents are stable until expiration date on label if stored at 2-8 °C  
Reagent A reconstituted is stable 5 days at 2-8 °C.

### SAMPLES PREPARATION

Dilute the sample 1 at 100 with the diluent (ex. 100 mg with 10 ml), homogenize, centrifuge and use the supernatant for the analysis.  
Chymotrypsin into supernatant is stable 3 hours at 15-25 °C and 24 hours at 2-8 °C.

### PROCEDURE

Method: Kinetic  
Reading time: 1,2,3 minutes  
Wavelength: 405 nm (400-420)  
Temperature: 25, 30, 37°C  
Pathlength: 1 cm  
Zero: air

REAGENTS	CONTROL	SAMPLE
Sample Chromogen	-- 2 ml	100 µl 2 ml

Mix carefully, wait for 1 minute and read absorbances after 1, 2, 3 minutes.

### CALCULATION

Calculate average value of extinction differences for min. ( $\Delta E/\text{min}$ ).

$U/g (25/30/37^\circ C) \text{ faeces} = 212 \times \Delta E/\text{min}$

### EXPECTED VALUES

	25°C U/g	30°C U/g	37°C U/g
Normal Interval	>6	>8,4	>13,2
Interval that needs a control	3-6	4.2-8.4	6.6-13.2
Pathologic Interval	< 3	< 4.2	< 6.6

Every laboratory should establish own reference intervals in accordance with own population.

### NOTE

- For faeces particularly solid, it is advisable to let rest sample with diluent before homogenisation for 10-15 minutes.
- Suspend supply of medicine containing pancreatic enzymes at least 5 days before test execution.
- Don't give laxative.
- If the results are incompatible with clinical presentation, they have to be evaluated within a total clinical study.
- Only for IVD use.

### CALIBRATION/QUALITY CONTROL

It is suggested to perform an internal quality control. For this purpose on request are available the following control sera.

**CC03730** 2 x 1 ml  
Chymotrypsin-Control Set  
(2 levels)

### TEST PERFORMANCE

#### Precision

In series (n = 10)	Mean (U/g)	SD (U/g)	CV%
Sample 1	7.40	0.51	7.0
Sample 2	18.23	0.87	4.8
Sample 3	56.62	1.64	2.9

Among series (n = 20)	Mean (U/g)	SD (U/g)	CV%
Sample 1	2.32	0.10	4.3
Sample 2	17.15	1.50	8.7
Sample 3	55.15	2.60	4.7

#### Sensitivity

The method is sensitive until 0.2 U/g.

#### Linearity

If  $\Delta$  absorbance/min is > 0.030 dilute 100 µl of supernatant with 400 µl of diluent and repeat the determination. Multiply the result by 5.

#### Methods comparison

A comparison with an available commercial method gave following results on 20 samples compared:

LTA = x  
Competitor = y  
n = 20

$$y = 0,409 + 0,941x \quad r = 0,98$$

#### WASTE DISPOSAL

Product is intended for professional laboratories. Waste products must be handled as per relevant security cards and local regulations.

#### PACKAGING

**CODE CC03700 (20 TESTS)**  
Chromogen 20 x 2 ml (liophile)  
Diluent for samples 2 x 100 ml (liquid)

**CODE CC03720**  
Diluent for samples 6 x 100 ml (liquid)

#### REFERENCES

Kasper, P., G. Moeller, A. W. Wahlefeld e. F. Staehler, Fresenius Z. Anal. Chem. 311 (1982) 391-392.  
OelMar, E.G., C. Largman, J. Brodrick e M. Geokas, Anal. Biochem. 99 (1979) 316-320.  
Ammann, R., Fortschritte in der Pankreasfunktionsdiagnostik, Springer Verlag, Berlin, Heidelberg, New York, 1967.

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### SYMBOLS

- Only for IVD use
- Lot of manufacturing
- Code number
- Storage temperature interval
- Expiration date (year - month)
- Warning, read enclosed documents
- Read the directions
- Biological risk

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