Manual

Chymotrypsin ELISA

For the determination of chymotrypsin in stool

Valid from 2019-03-26

REF **KR6910**

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+2°C +8°C

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1. INTENDED USE

This Immundiagnostik assay is an enzyme immunoassay intended for the quantitative determination of chymotrypsin in stool. For research use only. Not for use in diagnostic procedures.

2. INTRODUCTION

Chymotrypsin is a serine protease secreted from the pancreas into the duodenum after food intake. Chymotrypsin cleaves food proteins preferentially next to aromatic residues. A small part of the active enzyme form is excreted within the stool. The advantage of this assay is that the concentration of the enzyme is accurately determined and not the enzyme's activity. Many studies have shown a longer stability of chymotrypsin (12 days at room temperature) compared to pancreatic elastase. Therefore, the chymotrypsin ELISA is a reliable and low-priced alternative to the pancreatic elastase assay.

Possible research areas

- Chronic pancreatitis
- Exocrine pancreatic insufficiency

3. MATERIAL SUPPLIED

Art.-Nr.	Bezeich-nung	Kit-Komponenten	Menge
KR6910	PLATE	Microtiter plate, pre-coated	12 x 8 wells
KR0001.C.100	WASHBUF	Wash buffer concentrate, 10x	2 x 100 ml
KR6910	AB	Detection antibody concentrate, (mouse anti-chymotrypsin)	1 x 150 µl
KR6910	CONJ	Conjugate concentrate (streptavidin, peroxidase-labelled)	1 x 150 µl
KR6910	ABBUF	Detection antibody dilution buffer	1 x 15 ml
KR6910	STD	Standards, ready-to-use (1000; 250; 125; 62.5; 15.6; 0 ng/ml)	6 x 250 µl
KR6910	CTRL1	Control, ready-to-use (see specification for range)	1 x 250 µl
KR6910	CTRL2	Control, ready-to-use (see specification for range)	1 x 250 µl

Art.-Nr.	Bezeich-nung	Kit-Komponenten	Menge
KR6910	DIL	Dilution buffer, ready-to-use	1 x 15 ml
KR0002.15	SUB	Substrate (tetramethylbenzidine), ready-to-use	1 x 15 ml
KR0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml

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

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Stool sample application system such as cat. no.: KR6998SAS
- Calibrated precision pipettors and 10–1000 µl single-use tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Centrifuge, 3000 *g*
- Vortex
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

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

- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. **Prepare only the appropriate amount necessary for each run.** The kit can be used up to 4 times within the expiry date stated on the label.
- Reagents with a volume less than **100 µl** should be centrifuged before use to avoid loss of volume.
- **Preparation of the wash buffer:** The **wash buffer concentrate (WASHBUF)** has to be diluted with ultrapure water **1:10** before use (100 ml WASHBUF + 900 ml ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37 °C. The **WASHBUF** is

stable at **2–8 °C** until the expiry date stated on the label. **Wash buffer** (1:10 diluted WASHBUF) can be stored in a closed flask at **2–8 °C for 1 month**.

- **Preparation of the detection antibody:** The **detection antibody concentrate (AB)** has to be diluted **1:101** with antibody dilution buffer (ABBUF) before use (e. g. 100 µl AB + 10 ml ABBUF). The **AB** is stable at **2–8 °C** until the expiry date stated on the label. **Detection antibody** (1:101 diluted AB) **is not stable and can not be stored.**
- **Preparation of the conjugate:** Before use, the **conjugate concentrate (CONJ)** has to be diluted **1:101** in **wash buffer** (100 µl CONJ + 10 ml wash buffer). The **CONJ** is stable at **2–8 °C** until the expiry date stated on the label. **Conjugate** (1:101 diluted CONJ) **is not stable and cannot be stored.**
- All other test reagents are ready-to-use. Test reagents are stable until the expiry date (see label) when stored at **2–8 °C**.

6. STORAGE AND PREPARATION OF SAMPLES

Sample stability

Chymotrypsin is stable in raw stool for 6 months at -20 °C or for 4 days at 2–8 °C. Storage at elevated temperatures should be avoided.

Extraction of the stool samples

The **wash buffer** is used as a sample extraction buffer. We recommend the following sample preparation:

Stool Sample Application System (SAS) (Cat. No.: KR6998SAS)

Stool sample tube – Instructions for use

Please note that the dilution factor of the final stool suspension depends on the amount of stool sample used and the volume of the buffer.

SAS with 0.75 ml sample extraction buffer:

Applied amount of stool:	15 mg
Buffer Volume:	0.75 ml
Dilution Factor:	1:50

Please follow the instructions for the preparation of stool samples using the SAS as follows:

a) The raw stool sample has to be thawed. For particularly heterogeneous samples we recommend a mechanical homogenisation using an applicator, inoculation loop or similar device.

b) Fill the **empty sample tube** with **0.75 ml** of ready-to-use wash buffer before using it with the sample. Important: Allow the sample extraction buffer to reach room temperature.

c) Unscrew the tube (yellow part of cap) to open. Insert the yellow dipstick into the sample. The lower part of the dipstick has notches which need to be covered completely with stool after inserting it into the sample. Place dipstick back into the tube. When putting the stick back into the tube, excess material will be stripped off, leaving 15 mg of sample to be diluted. Screw tightly to close the tube.

d) Shake the tube well until no stool sample remains in the notches. Important: Please make sure that you have a maximally homogenous suspension after shaking. Especially with more solid samples, soaking the sample in the tube with sample extraction buffer for ~ 10 minutes improves the result.

e) Allow sample to stand for ~10 minutes until sediment has settled. Floating material like shells of grains can be neglected.

f) Carefully unscrew the complete cap of the tube including the blue ring plus the dipstick. Discard cap and dipstick. Make sure that the sediment will not be dispersed again.

Dilution : 1:50

For analysis, pipet **20 µl** of this **dilution** per well.

The supernatant can be stored for one month at -20 °C.

7. ASSAY PROCEDURE

Principle of the test

This ELISA is designed for the quantitative determination of chymotrypsin in stool samples. The wells of the microtiter plate are coated with polyclonal anti-chymotrypsin antibodies. In a first incubation step, the chymotrypsin in the samples is bound to the coated antibodies (in excess). To remove all unbound substances, a washing step is carried out.

In a second incubation step, a second anti-chymotrypsin antibody is bound to the antigen. After another washing step, the solid phase is incubated with a peroxidase labelled antibody and then with the peroxidase substrate, tetramethylbenzidine. The

colour converts to yellow by the addition of an acidic stop solution. The intensity of the yellow color is directly proportional to the chymotrypsin concentration of the sample. A dose response curve of the absorbance (at 450 nm) unit vs. concentration is generated. Chymotrypsin, present in the samples, is determined directly from this calibration curve.

Test procedure

Bring all **reagents and samples to room temperature** (15–30 °C) and mix well.

Mark the positions of standards/controls/samples on a protocol sheet.

Take as many microtiter strips as needed from the kit. Store unused strips together with the desiccant bag in the closed aluminium packaging at 2–8 °C. Strips are stable until the expiry date stated on the label.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact your supplier or Immundiagnostik AG.

We recommend to carry out the tests in duplicate.

1.	**Before use**, wash the wells **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
2.	Add **100 µl dilution buffer** (DIL) into each well.
3.	Add **20 µl standards/controls/samples** into the respective wells.
4.	Cover the strips and incubate for **1 hour** at room temperature (15–30 °C) **shaking on a horizontal mixer***.
5.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
6.	Add **100 µl detection antibody** (diluted AB) into each well.
7.	Cover the strips and incubate for **1 hour** at room temperature (15–30 °C) **shaking on a horizontal mixer***.
8.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
9.	Add **100 µl conjugate** (diluted CONJ) into each well.

10.	Cover the strips and incubate for **1 hour** at room temperature (15–30 °C) **shaking on a horizontal mixer***.
11.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
12.	Add **100 µl substrate** (SUB) into each well.
13.	Incubate for **5–15 min**** at room temperature (15–30 °C) **in the dark**.
14.	Add **100 µl stop solution** (STOP) and mix shortly.
15.	Determine **absorption immediately** with an ELISA reader at **450 nm** against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at **405 nm** against 620 nm as a reference.

* We recommend shaking the strips at 550 rpm with an orbit of 2 mm.

** The intensity of the colour change is temperature sensitive. We recommend observing the colour change and stopping the reaction upon good differentiation.

8. RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend using the "4 parameter algorithm".

1. 4 parameter algorithm

It is recommended to use a linear ordinate for the optical density and a logarithmic abscissa for the concentration. When using a logarithmic abscissa, the zero standard must be specified with a value less than 1 (e.g. 0.001).

2. Point-to-point calculation

We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

3. Spline algorithm

We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

The plausibility of the duplicate values should be examined before the automatic evaluation of the results. If this option is not available with the programme used, the duplicate values should be evaluated manually.

Stool

The obtained chymotrypsin levels of stool samples have to be multplied by the dilution factor of **50**.

In case **another dilution factor** has been used, multiply the obtained result by the dilution factor used.

9. LIMITATIONS

Samples with concentrations above the measurement range can be further diluted and re-assayed. Please consider this higher dilution when calculating the results.

Samples with concentrations lower than the measurement range cannot be clearly quantified.

The upper limit of the measurement range can be calculated as:

highest concentration of the standard curve × *sample dilution factor to be used*

The lower limit of the measurement range can be calculated as:

analytical sensitivity × *sample dilution factor to be used*

Analytical sensitivity see chapter "Performance Characteristics".

10. QUALITY CONTROL

Immundiagnostik AG 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 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

We recommend each laboratory to establish its own reference range.

11. PERFORMANCE CHARACTERISTICS

Accuracy – Precision

Repeatability (Intra-Assay); n=24

The repeatability was assessed with 3 stool samples under constant parameters (same operator, measurement system, day and kit lot). The following values have been estimated without considering possibly used sample dilution factors:

Sample	Mean value [ng/ml]	CV [%]
1	45.07	6.8
2	96.41	9.6
3	335.57	3.8

Reproducibility (Inter-Assay); n=47

The reproducibility was assessed with 2 control samples under varying parameters (different operators, measurement systems, days and kit lots).

Sample	Mean value [ng/ml]	CV [%]
1	54.65	4.7
2	122.13	9.1

Analytical sensitivity

The following value has been estimated based on the concentrations of the standards without considering possibly used sample dilution factors.

Limit of blank, LoB 8.366 ng/ml

12. PRECAUTIONS

- All reagents in the kit package are for research use only.
- 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.
- Kit reagents contain sodium azide or ProClin as bactericides. Sodium azide and ProClin are toxic. Substrates for the enzymatic colour reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.

- The stop solution consists of diluted sulphuric acid, a strong acid. Although diluted, it still should be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped up immediately with copious quantities of water. Do not breath vapour and avoid inhalation.

13. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch.
- Control samples should be analysed with each run.
- Reagents should not be used beyond the expiration date stated on the kit label.
- Substrate solution should remain colourless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- The guidelines for laboratories 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.

Used symbols:


Temperature limitation

Catalogue Number

For research use only

To be used with


Manufacturer

Contains sufficient for <n> tests

Lot number


Use by


Attention


Consult instructions for use

Consult specification data sheet