IMMUCHROM
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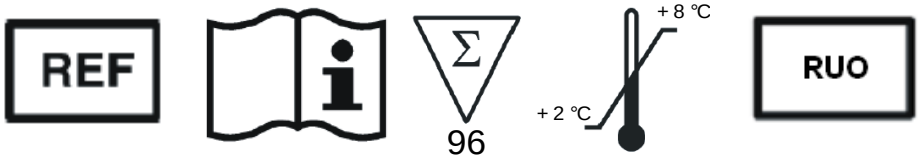
Manual

Alpha-1-Antitrypsin

ELISA

For the determination of alpha-1-antitrypsin in stool

Valid from 28.12.2022



IC6200

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1. Intended use

The *ImmuChrom* ELISA Kit is intended for the quantitative determination of alpha-1-antitrypsin stool. For research use only.

2. Introduction

Alpha-1-antitrypsin is a 52 kD glycoprotein produced in the liver, intestinal macrophages, monocytes and intestinal epithelial cells. It belongs to the group of acute-phase proteins and is one of the most important proteinase inhibitors in serum. Alpha-1-antitrypsin inhibits the proteinases trypsin and neutrophil elastase (1). A deficiency leads to increased proteolysis. Since alpha-1-antitrypsin is not appreciably broken down or resorbed in the intestine, it can be detected in the stool if the intestinal mucosa has increased permeability ("leaky gut" syndrome) (1,2). Patients with inflammatory bowel disease show elevated levels of alpha-1-antitrypsin in their stools (4).

Taking non-steroidal anti-inflammatory drugs (e.g. aspirin, ibuprofen, diclofenac) or COX-2 inhibitors (e.g. celecoxib) can lead to enteropathies, which result in an increase in the alpha-1-antitrypsin value in the stool. Before carrying out the determination, the corresponding medication should therefore not be taken for a period of 14 days, so as not to influence the measurement of the degree of intestinal inflammation.

In the case of diarrhea, due to the dilution effect, normal values can be measured in the stool despite the presence of inflammatory processes in the intestine.

Applications

- Enteral loss of proteins- syndrome
- "Leaky gut" syndrome
- Enterocolitides of several origin

3. General notes, warnings and precautions

All reagents of this kit are strictly intended for research 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.

The test kit contains components of human origin. The starting reagents were tested for antibodies against HIV1/2, hepatitis B and anti-HCV using immunoassay methods. All parameters tested were found negative. As a precautionary measure, all test kit reagents should always be treated as potentially infectious material in accordance with health care accident prevention regulations.

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 wear 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.

4. Material delivered in the test package

Article no.	Compont	Description	Amount
IC6200mtp	MTP	Mikrotiter plate coated	12 x 8 wells
IC6200wp	WASHBUF	ELISA wash buffer conc. 10 fold	100 ml
IC6200st	STD	Standards (1.0 ml)	5 vials
IC6200ko	CTRL	Control 1 and 2 (1.0 ml)	1 vial each
IC6200kg	CONJ	Conjugate, peroxidase labeled antibody	15 ml
IC6200su	SUB	TMB substrate (tetramethylbenzidine)	15 ml
IC6200sp	STOPP	Stop solution	7 ml

5. Additional special equipment

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

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

Alpha-1-antitrypsin is extracted by the diluted wash buffer out of the stool sample in a ratio of 1:50 (e.g. 20 mg/ml).

Extraction in Stool extraction vials

For the extraction stick vials could be used.

We recommend to mix **15 mg** stool with **0.75 ml** diluted wash buffer, then vortex it until the mixture is homogenous. Transfer the resulting slurry to a plastic vial and centrifuge it for 10 min at 3000 x g.

Dilute the supernatant **1:250** with diluted wash buffer (e.g. 4 µl supernatant + 996 µl diluted wash buffer).

8. Procedure

Principle of the method

The α-1-AT ELISA test determines human alpha-1-antitrypsin according to the “sandwich”-principle. α-1-AT 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 microtiterplate reader. The α-1-AT concentration can be calculated from the standard curve.

Sample preparation

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

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

1. Washing step

Take out the needed strips of the microtiter plate and wash 1x with 250 µl diluted WASHBUF per cavity. Remove residual buffer by tapping the plate on absorbent paper after the washing step.

2. Incubation samples

Pipette **100 µl STD**, **CTRL** and **samples** in double values in 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 on absorbent paper after the last washing step.

4. Incubation conjugate

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 on absorbent paper after the last washing step.

6. Incubation substrate

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 stopping reaction.

9. Calculation of analytical results

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

Stool samples

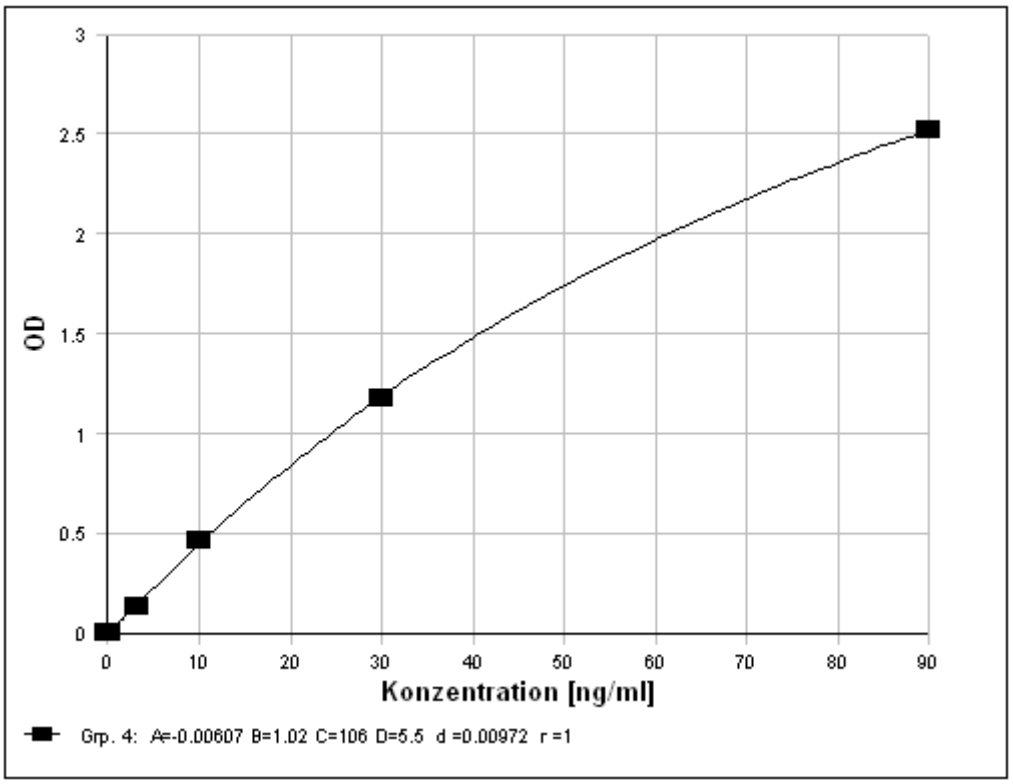
The obtained α-1-AT concentration is multiplied with **12.5**

Dilution 1: 15 mg in 0.75 ml corresponds to a factor **50** (assumption: 1 g stool = 1 ml)

Dilution 2: Factor **250** (4 µl supernatant + 996 µl diluted wash buffer)

Calculation: Conc. Patient [µg/ml] = obtained conc. [ng/ml] x 50 x 250 / 1000

Standard curve



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

10. Internal quality control

Reference values

Stool: < 0.27 mg/g stool

Ref: G. Beckmann (Hrsg.). Mikroökologie des Darmes

ISBN 3-87706-521-X; S.263

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

11. Validation data

Measuring range

The measuring range of alpha-1-antitrypsin is between a sample concentration of 41.3 - 1125 µg/ml.

Precision and reproducibility

Intra-Assay CV:	9.6 % (77.0 ng/ml)	[n = 6]
	8.7 % (42.6 ng/ml)	[n = 6]
	11.2 % (13.0 ng/ml)	[n = 6]
Inter-Assay CV:	11.9 % (75.1 ng/ml)	[n = 6]
	9.7 % (44.3 ng/ml)	[n = 6]
	14.4 % (14.6 ng/ml)	[n = 6]

Linearity

The dilution of the samples was done with diluted wash buffer.

Sample	Dilution factor	Expected [ng/ml]	Measured [ng/ml]	Recovery [%]
1	--	--	72.8	
	1:2	36.4	31.4	86.3
	1:4	18.2	15.1	83.0
	1:8	9.1	8.3	91.2
	1:16	4.8	3.5	76.1
2	--	--	46.4	
	1:2	23.2	19.9	85.8
	1:4	11.6	10.7	92.2
	1:8	5.8	4.0	77.6
3	--	--	15.7	
	1:2	7.9	7.2	91.1
	1:4	3.9	3.2	82.1

Detection limit

0.4 ng/ml

For the determination of the detection limit 20 replicates of the standard 0 were measured. After addition of the 3 fold standard deviation to the mean value the concentration was read from the standard curve.

Limit of quantification

0.7 ng/ml

For the determination of the detection limit 20 replicates of the standard 0 were measured. After addition of the 10 fold standard deviation to the mean value the concentration was read from the standard curve.

Recovery

The recovery was found between 80.3 and 105.2 %

Cross reactivity

Cross reactivity to other plasma proteins could not be detected in stool and serum/plasma samples.

12. Limitations of the method

Stool samples with α-1-AT concentrations above the standard curve should be diluted with wash buffer (WASHBUF) and measured again.

In case of diarrhea it is possible that even patients with an inflammation in the gut show normal values.

13. Disposal

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

Please refer to the appropriate national guidelines.

14. Literature references

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