

Human Alpha-1-antitrypsin in stool ELISA

Cat. No.: RIC6200R

1. Intended use

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

2. Introduction

Alpha-1-antitrypsin is a 52 kD glycoprotein, which is produced by the liver, intestinal macrophages, monocytes and mucous membrane cells of the gut. It belongs to the group of acute phase proteins and is one of the most important proteinase inhibitor. Alpha-1-antitrypsin inhibits, beside others, the proteinases trypsin and the elastase of neutrophils. A lack of α -1-AT leads to an enhanced proteolysis. Only a very small amount of alpha-1-antitrypsin is cleaved or resorbed in the gut. Therefore the measurement of α -1-AT in stool reflects the permeability of the gut during inflammatory processes.

Applications

- Enteral loss of proteins- syndrome
- "Leaky gut" syndrome
- Enteroclitides of several origin
- Morbus Crohn / Colitis ulcerosa

The complete kit allows an easy, rapid and precise quantitative determination of alpha-1-antitrypsin in biological samples. The kit includes all reagents ready to use for preparation of the samples.

3. Warnings and precautions

All reagents of this kit are strictly intended for in vitro diagnostic use only.

This assay was produced and put on the market according to the IVD guidelines of 98/79/EC.

Do not interchange kit components from different lots.

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 out immediately with copious quantities of water. Do not breath vapor and avoid inhalation. In case of an accident or indisposition contact immediately a physician. The substrate TMB (tetramethyl benzidine) is toxic by ingestion and contact with the skin. Any spill be wiped out 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 testkit 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.	Compont	Description	Amount
IC6200mtp	MTP	Mikrotiterplate coated	12 x 8 wells
IC6200wp	WASHBUF	ELISA waschbuffer conc. 10 fold	100 ml
IC6200st	STD	Standard (1 ml) (0; 3.3; 10; 30; 90 ng/ml)	5 vials
IC6200ko	CTRL	Control 1 and 2(1 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

- Laboratory balance
- Centrifuge, 3000xg
- Glas or plastic vials
- Various pipettes
- Foil to cover the microtiterplate
- Multichannel or multipipette
- ELISA reader with filter 450 nm (reference filter 620 or 690 nm)
- Microtiterplate shaker
- Vortex mixer

6. Reagent preparation

Microtiterplate (MTP). Take the needed strips out of the bag and mount them on the holder. Please take care that the package has reached room temperature before opening the bag. Stripes which are not needed yet could be stored at 2-8°C. Please dispose the holder when all stripes are used.

Washbuffer (WASHBUF). Dilute the washbuffer concentrate 1:10 with aqua bidest. (1 part buffer + 9 parts aqua bidest.) The dilution is stable for 14 days at 2-8°C.

Important: When storing the washbuffer concentrate at 2-8°C crystalization could 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.

7. Specimen

Stool samples

Alpha-1-antitrypsin is extracted by the sample dilution buffer out of the stool sample.

100 mg stool are mixed with 5 ml WASHBUF on a vortex mixer until the mixture is homogenous.

1 ml of the mixture is transferred into an "Eppendorf" reaction vial and centrifuged for 10 min at 10000xg.

Dilute the supernatant 1:250 with WASHBUF (4 µl + 996 µl WASHBUF)

100 µl of the dilution are used in the test per well.

Serum/ plasma samples

Serum or EDTA-plasma drawn from venous fasting blood could be used in this test system. The sample should be centrifuged (2000 g, 10 min., 2-8°C) within 60 min after venipuncture and stored directly at 2-8°C. For long time storage the samples should be frozen at -20°C.

Patients suffering Morbus Crohn showing very high concentrations of α-1-AT in blood. These samples should be diluted 1:1,000,000 with washbuffer.

The samples of all other patients should be diluted 1: 250,000 with washbuffer.

100 µl of the dilution are used in the test per well.

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 microtiterplate. 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 have room temperature (18-26°C) and mixed well before use.

The position of standards, controls and 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. 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 microtiterplate.

The stripes are covered and incubated by shaking for 60 min at room temperature (18-26 °C).

The reaction starts on pipetting to the antibody coated microwell. Pipetting should be as quickly as possible. When processing many samples at once the samples should be pipetted to a separate microtiterplate (150 µl) and transferred simultaneously using a multichannel pipette.

3. Washing step

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

The stripes are covered and incubated by shaking for 60 min at room temperature (18-26 °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 on absorbent paper after the last washing step.

6. Incubation substrate
Pipette 100 µl SUB in each microwell. Incubate for 10-15 min at room temperature (18-26 °C) in the dark.
7. Stopping reaction
Pipette 50 µl STOPP in each microwell, mix well.
8. Reading
Read the absorbance at 450 nm. If the microtiterplate reader allows to use a reference wavelength use 620 or 690 nm as reference wavelength.
Reading should be done within 5 min after stopping reaction.
In case that the highest standard exceeds the range of the reader the reading should be done at 405 nm against 620 nm (690 nm).

9. Calculation of analytical results

For calculating the results we recommend to use the 4-parameter algorithm. If this algorithm not available a “point to point” or a “spline” function can be used.

Stool samples

The obtained α-1-AT concentration is multiplied with 12,5

Dilution 1: 100 mg in 5 ml corresponds to a factor 50 (assumption: 1 g stool = 1 ml)

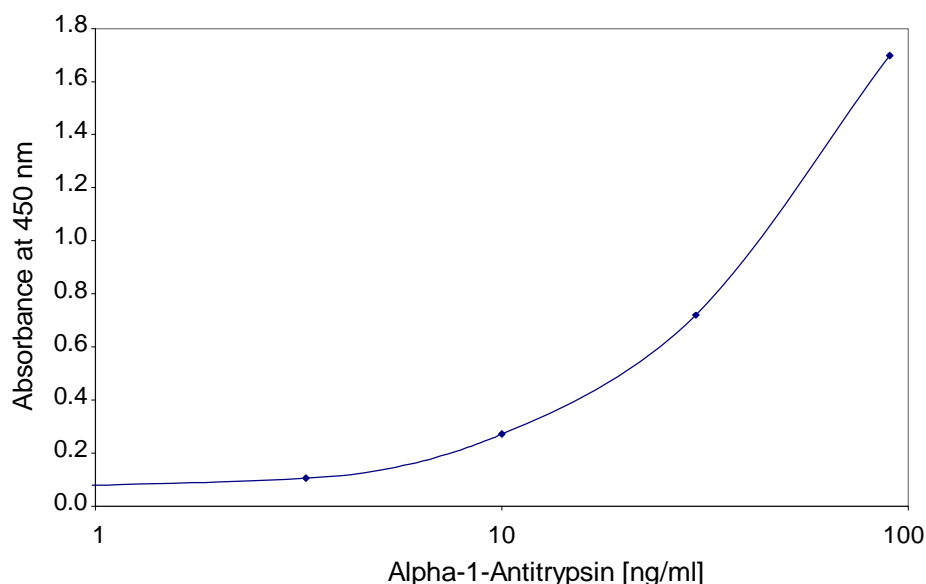
Dilution 2: Factor 250

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

Serum/plasma samples

The obtained α-1-AT concentration [ng/ml] is multiplied with with the used dilution factor (250,000 or 1,000,000).

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: < 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 published data.

11. Validation data

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]

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

1.5 ng/ml

For the determination of the detection limit 20 replicates of the standard 0 were measured. After addition of the twofold 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 washbuffer (WASHBUF) and measured again.

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

Blood samples with α -1-AT concentrations above the standard curve should be diluted with washbuffer (WASHBUF) and measured again. Haemolytic and lupenic samples should not be measured.

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

G. Beckmann (Hrsg.). Mikroökologie des Darmes ISBN 3-87706-521-X;

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BioVendor - Laboratorní medicína a.s.

CTPark Modrice
Evropska 873
664 42 Modrice
CZECH REPUBLIC
Phone: +420-549 124 185
Fax: +420-549 211 460
e-mail: info@biovendor.com
<http://www.biovendor.com>

European Union:

BioVendor GmbH

Im Neuenheimer Feld 583
D-69120 Heidelberg, GERMANY
Phone: +49-6221-433-9100
Fax: +49-6221-433-9111
e mail: roth@biovendor.com
e mail: abels@biovendor.com

USA, Canada and Mexico:

BioVendor, LLC

1463 Sand Hill Road, Suite 227
Candler, NC 28715, USA
Phone: +1-828-670-7807, +1-800-404-7807
Fax: +1-828-670-7809
e mail: info-us@biovendor.com

China - Hong Kong Office:

BioVendor Laboratories Ltd.
Room 4008, Hong Kong Plaza, No.188
Connaught Road West
Hong Kong, CHINA
Phone: +852 28030523
Fax: +852 28030525
e-mail: lu@biovendor.com

China – Mainland Office:

BioVendor Laboratories Ltd.
Room 2405, YiYa Tower, TianYu Garden, No.150
Lihe Zhong Road, Guang Zhou, CHINA
Phone: +86-20-87063029
Fax: +86-20-87063016
e-mail: jhsu@biovendor.com

Manufactured by



Tiergartenstr. 7
D-64646 Heppenheim, GERMANY
<http://www.immuchrom.de>
email: info@immuchrom.de
