Alpha-1-Antitrypsin ELISA

For the quantitative determination of alpha-1-antitrypsin in human stool

For Research Use Only. Not for Use in Diagnostic Procedures.

Catalog Number: 31-A1AHU-E01

Size: 96 determinations

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INTENDED USE

The Alpha-1-Antitrypsin ELISA is intended for the quantitative determination of alpha-1-antitrypsin (A1A) in human stool. For Research Use Only. Not for Use in Diagnostic Procedures.

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). Deficiency leads to increased proteolysis. Since alpha-1-antitrypsin is not appreciably broken down or reabsorbed in the intestine, it can be detected in the stool if the intestinal mucosa has increased permeability ("leaky gut" syndrome) (1,2).

PRINCIPLE OF THE ASSAY

The Alpha-1-Antitrypsin ELISA determines human alpha-1-antitrypsin according to the "sandwich" principle. A1A in samples, standards, and controls binds to antibodies which are coated to the microtiter plate. After a wash step a peroxidase-labeled detection antibody is added. A second wash 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 microtiter plate reader. The A1A concentration can be calculated from the standard curve.

MATERIALS SUPPLIED

31-A1AHU-E01			
Component	**Quantity**	**Preparation**	**Storage**
Microtiter plate, coated	1 plate: 12 x 8-well strips	Ready-to-use	2-8˚C
Universal extraction buffer	150 mL	Ready-to-use	2-8˚C
Wash Buffer concentrate, 10X	100 mL	Dilute 1:10	2-8˚C
Standards*	5 vials, 1 mL each	Ready-to-use	2-8˚C
Control 1 and 2*	2 vials, 1 mL each	Ready-to-use	2-8˚C
Conjugate: peroxidase-labeled antibody	15 mL	Ready-to-use	2-8˚C
TMB Substrate: tetramethylbenzidine	15 mL	Ready-to-use	2-8˚C
Stop solution	10 mL	Ready-to-use	2-8˚C
Plate Sealers	2	Ready-to-use	RT

*Please refer to the Certificate of Analysis enclosed with each kit for Standard and Control concentrations.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

- Centrifuge, 3000 x g
- Plastic vials
- Stool sample extraction vials (80-EXDEV2-100)
- Vortex mixer

- Various pipettes
- Multichannel or multipipette
- Foil to cover the microtiter plate (substrate step)
- Distilled or deionized water
- ELISA reader with 450 nm filter (620 nm reference filter)
- Microtiter plate shaker (2 mm orbital shaker capable of 400 rpm)
- Absorbent paper (lint-free)
- Timer

PRECAUTIONS

- This assay is for Research Use Only. Not for use in diagnostic procedures.
- 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. Avoid contact with the skin and/or mucous membranes.
- 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. 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 test protocol is essential. ALPCO assumes no liability for any damage caused by unauthorized changes in the test procedure.
- The guidelines for carrying out quality control in testing laboratories must be observed. Appropriate controls must be tested.
- The reagents must not be used after the expiration date.
- Wear disposable gloves when handling samples or kit reagents and wash hands thoroughly afterwards. Do not pipette by mouth. Do not eat, drink, smoke, or apply makeup in areas where samples or kit reagents are being handled.
- Samples may contain unknown interfering substances. This can lead to false high or false low results.

REAGENT PREPARATION

Microtiter plate - Assemble the required number of strips in the holder. Allow the plate to reach 20-30°C before use. Unused strips must be stored at 2-8°C in the pouch with desiccant. Please do not dispose of the holder until all strips are used.

Wash buffer - Please note: When storing the wash buffer concentrate at 2-8 °C crystallization may occur. Before dilution, all crystals must be dissolved. Dilute the wash buffer concentrate 1:10 with distilled or deionized water (1 part buffer + 9 parts DI water). The 1X working wash buffer is stable for 14 days at 2-8°C. Dilute only the amount of buffer needed 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.

SAMPLE PREPARATION

Stool samples must be extracted with Universal Extraction Buffer at a ratio of 1:100 (e.g. 10 mg/ml) before testing in the Alpha-1-Antitrypsin ELISA. Manual weighing or an approved stool extraction device (80-EXDEV2-100) may be used.

For manual weighing, mix **15 mg** stool with **1.5 ml** universal extraction buffer (or greater amount of stool diluted **1:100** with universal extraction buffer), then vortex until the mixture is homogenous. Transfer the resulting slurry to a plastic vial and centrifuge for 10 min at 3000 x g.

Dilute the supernatant **1:125** in 1x working wash buffer, (e.g. 8 µl supernatant + 992 µl 1x working wash buffer).

ASSAY PROCEDURE

All reagents and samples should be equilibrated to 20-30°C and mixed well before use.
The position of standards, controls, and samples are noted on a protocol sheet.

1. **Wash step**

   Pick out the pre-assembled microtiter plate with the needed number of strips and wash them 1x with 250 µl 1X working wash buffer per well. Remove residual buffer by tapping the plate on absorbent paper after the wash step.

2. **Sample incubation**

   Pipette **100 µl STD, CTRL, and diluted samples** in duplicate in the microtiter plate.

   Cover the strips and incubate by shaking for **60 min** at room temperature (20-30 °C; 400 rpm, 2 mm orbit).

3. **Wash step**

   Discard the contents of the microwells and wash 5x with 250 µl 1X working wash buffer per well. Remove residual buffer by tapping the plate **gently** on absorbent paper after the last wash step.

4. **Conjugate incubation**

   Pipette **100 µl CONJ** in each microwell.

   Cover the strips and incubate with shaking for **60 min** at room temperature (20-30°C; 400 rpm, 2 mm orbit).

5. **Wash step**

Discard the contents of the microwells and wash 5x with 250 µl 1X working wash buffer per well. Remove residual buffer by tapping the plate on absorbent paper after the last wash step.

6. **Substrate incubation**

Pipette **100 µl TMB substrate** in each microwell.

Incubate by shaking for **10-15 min** at room temperature (20-30°C; 400 rpm, 2 mm orbit) **in the dark**.

7. **Stopping the reaction**

Pipette **50 µl stop solution** in each microwell. Mix well.

8. **Reading**

Read the absorbance at 450 nm. If available, use 620 nm as reference wavelength. Reading should be done within **5 minutes** after stopping the reaction.

CALCULATION OF RESULTS

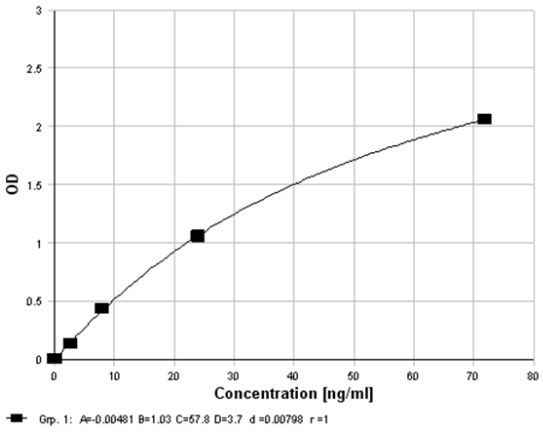
The use of the 4-parameter-Marquardt algorithm is recommended for calculation of results. The Alpha-1-Antitrypsin concentration is multiplied by **12.5.**

Dilution 1: 15 mg in 1.5 ml corresponds to a factor of **100** (assumption: 1 g stool = 1 ml)

Dilution 2: Factor **125** (8 µl supernatant + 992 µl 1x working wash buffer)

Calculation: Sample concentration [µg/ml] = obtained conc. [ng/ml] x 100 x 125 / 1000

TYPICAL STANDARD CURVE



The curve at left is for demonstration purposes only. It must not be used for calculation of sample values.

PERFORMANCE CHARACTERISTICS

Measuring Range
The measuring range of the Alpha-1-Antitrypsin ELISA is 33 - 900 µg/ml.

Sensitivity
Limit of Detection (LOD): 0.3 ng/ml

For the determination of the detection limit, 20 replicates of Standard 0 were measured. The 3x standard deviation was added to the mean value of the optical density. The respective concentration was read from the standard curve.
Limit of Quantification (LOQ): 0.6 ng/ml
For the determination, 20 replicates of Standard 0 were measured. The 10x standard deviation was added to the mean value of the optical density. The respective concentration was read from the standard curve.

Precision: Within run (intra-assay) variation

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]

Precision: Between run (inter-assay) variation

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
Sample dilution was performed with 1x working 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

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 samples.

Limitations of the Method
Stool samples with A1A concentrations above the standard curve should be diluted with 1x working wash buffer and measured again. Samples impacted by diarrhea may show normal values.

Disposal
The substrate must be disposed of as non-halogenated solvent. The stop solution can be neutralized with NaOH and, if the pH value is neutral, it can be disposed of as salt solution (**Important:** this reaction produces heat and should be handled carefully). Please refer to local and national guidelines.

REFERENCES

1. Braamskamp (2010). Protein-losing enteropathy in children. Eur J Pediatr (2010) 169:1179–1185

2. Beckmann, Rüffer (2000). Mikroökologie des Darmes. ISBN 3-87706-521-X D. 262-264

3. Martin et al (2000). Gastroenterologische Aspekte in der Naturheilkunde. ISBN 3-930620-29-4 S 213-214.

4. Karbach et al (1983). Alpha 1-antitrypsin, a reliable endogenous marker for intestinal protein loss and its application in patients with Crohn's disease. Gut. 1983 Aug;24(8):718-23

5. Martin (Hrsg.), Das Standardlabor in der naturheilkundlichen Praxis, 4. Auflage, 2014 ISBN 978-3-437-56303-4

6. Kirkamm, Martin (Hrgs.), Spezielle Labordiagnostik in der naturheilkundlichen Praxis 2014, ISBN 978-3-437-56323-2

7. Stein (Hrgs), Labordiagnostik in der Gastroenterologie und Hepatologie, 2018, ISBN 978-3-8374-1562-9

8. Tibble JA, et al. 1999. High prevalence of NSAID