
BIOXYTECH® α_1 AP-410™ Assay**Spectrophotometric Assay for α_1 -Antiproteinase Activity****For Research Use Only. Not For Use In Diagnostic Procedures.**Catalog Number 21047

INTRODUCTION

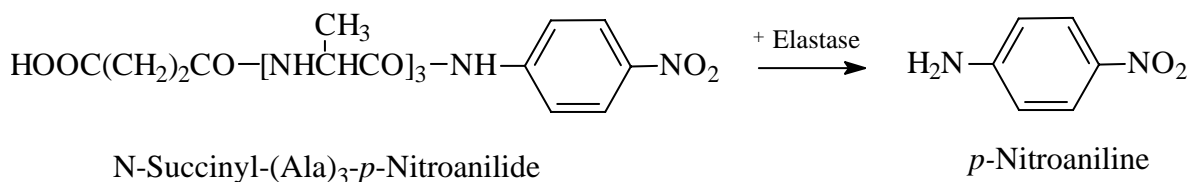
The Analyte

Human plasma α_1 -Antiproteinase (α_1 AP), also known as α_1 -antitrypsin or α_1 -proteinase inhibitor, is a 53-kDa glycoprotein formed of a single chain of 394 amino acid residues. α_1 AP is produced by the liver and diffuses from the blood into other tissues. It is a member of the serine proteinase inhibitor (Seprin) superfamily, and is mainly responsible for the inhibition of trypsin-, chymotrypsin-, or elastase-proteolytic capacity.¹ Proteolytic activity is lost after the formation of a proteinase-antiproteinase complex. Imbalance of proteinase-antiproteinase is involved in several diseases including pulmonary emphysema, chronic bronchitis, cystic fibrosis and rheumatoid arthritis.^{2,3} α_1 AP deficiency is an autosomal co-dominant genetic disorder and is seen in some patients with obstructive lung disease and hepatic disease.

Reactive oxygen species and reactive nitrogen species generated from oxidative stress can inactivate α_1 AP by oxidizing an essential methionine at position 1, 8 or 358 of the active site loop to methionine sulfoxide.³ A study by Whiteman and Halliwell⁴ showed the inactivation of α_1 AP by peroxynitrite and hypochlorous acid generated by myeloperoxidase from neutrophils at sites of inflammation. Because α_1 AP is very sensitive to oxidant agents, such as peroxynitrites from cigarette smoke and hydroxyl radicals, the measurement of active α_1 AP in body fluids may be a good biomarker to assess oxidative damage in biological systems.

Principles of the Procedure

The BIOXYTECH® α_1 AP-410™ Assay is based on the inactivation of elastase by α_1 AP. Since α_1 AP is an irreversible, equimolar inhibitor of elastase, the concentration of active α_1 AP in a sample is defined as the molar decrease of elastase activity. The activity of α_1 AP is therefore reported as a concentration equivalent to the concentration of elastase inactivated. The difference between the elastase activity of a Control (elastase alone) and Test (elastase + α_1 AP) in the presence of substrate is used to calculate the α_1 AP activity in samples.^{1,6} The kinetic method of measuring the elastase activity is based on monitoring the rate of cleavage of N-succinyl-(Ala)₃-*p*-nitroanilide (NSAN) by elastase,⁷ which results in the production of the chromogen, *p*-nitroaniline, with a maximum absorbance at 410 nm.⁷



Because of its simplicity, accuracy and sensitivity, the BIOXYTECH® α_1 AP-410™ Assay can be used to measure α_1 AP activity in biological samples. Serum, plasma and other body fluids, such as sputum, synovial fluid, and fluid from sites of inflammation, are sources of α_1 AP. Lung, liver and pancreas also contain α_1 AP activity. The concentration of active α_1 AP in plasma is about 20-50 μM in normal populations.¹

REAGENTS

Materials Provided (for 100 tests)

- Assay Buffer Tris•HCl-phosphate buffer, pH 8.0, 120 mL.
- Substrate N-Succinyl-(Ala)₃-p-Nitroanilide (NSAN) in Assay Buffer, 45 mL.
- Enzyme Elastase in Assay Buffer, 5.5 mL.

Materials Required But Not Provided

1. Spectrophotometer, preferably equipped with a 25°C temperature controlled cuvette holder and the ability to record the absorbance at 410 ± 2 nm over time.
2. Semi-micro (1 mL) cuvettes with 1 cm path length.
3. Adjustable Pipettes, 50-500 µL, with disposable tips.
4. Glass test tubes.

Warnings and Precautions

Use established laboratory precautions when handling or disposing any chemical contained in this product. Refer to the Material Safety Data Sheet for risk, hazard, and safety information. If any of the components come in contact with skin or eyes, rinse immediately with plenty of water. Seek medical advice.

Reagent Storage and Handling

Store reagent bottles tightly sealed at 2-8°C.
Unopened reagents are stable until the indicated expiration date.

PROCEDURE

Reagent Preparation

All reagents are ready to use.

Sample Preparation

All samples should be stored at -70°C until use. A 40-50 times dilution in Assay Buffer is recommended for plasma or serum samples.

Assay

1. Prepare test tubes for each Sample and 2-5 for CONTROLS.
2. Add 500 µL of Assay Buffer into all test tubes.
3. Add 50 µL of Enzyme into all test tubes.
4. Add 50 µL of Assay Buffer (for CONTROL) or Sample (for TEST) into all test tubes.
5. Incubate at 37°C for 5 minutes.
6. Incubate at room temperature for an additional 5 minutes or longer (up to 60 minutes).
7. Add 400 µL of Substrate, vortex and immediately transfer to a cuvette.
8. Place the cuvette in a spectrophotometer and record the absorbance at 410 nm for at least 1 minute.
9. Determine the rate of change of the absorbance at 410 nm per minute (ΔA_{410}) from the slope of the line or the net change over one minute.

Calculations

The calculation of α_1 AP activity (μ M) is as follows:

$$\alpha_1\text{AP} = \left(\frac{(\Delta A_{410}\text{Control} - \Delta A_{410}\text{Test})}{\Delta A_{410}\text{Control}} \right) \times 2d$$

Where: d = Dilution factor of the sample prior to addition to the assay test tube.
 The factor of 2 represents the 2 μM elastase concentration in the Enzyme reagent.

Sample Calculation

The following rates were measured for a CONTROL and TEST sample assayed in duplicate. The TEST sample is a plasma sample that was diluted 50x in Assay Buffer before running it in the assay.

Sample	ΔA_{410}
CONTROL	0.1254
CONTROL	0.1195
TEST	0.0805
TEST	0.0751

The average ΔA_{410} for the CONTROL is 0.1224, for the sample 0.0778.

$$\alpha_1\text{AP} = \left(\frac{(0.1224 - 0.0778)}{0.1224} \right) \times 2(50) = 36.44\mu\text{M}$$

PERFORMANCE CHARACTERISTICS

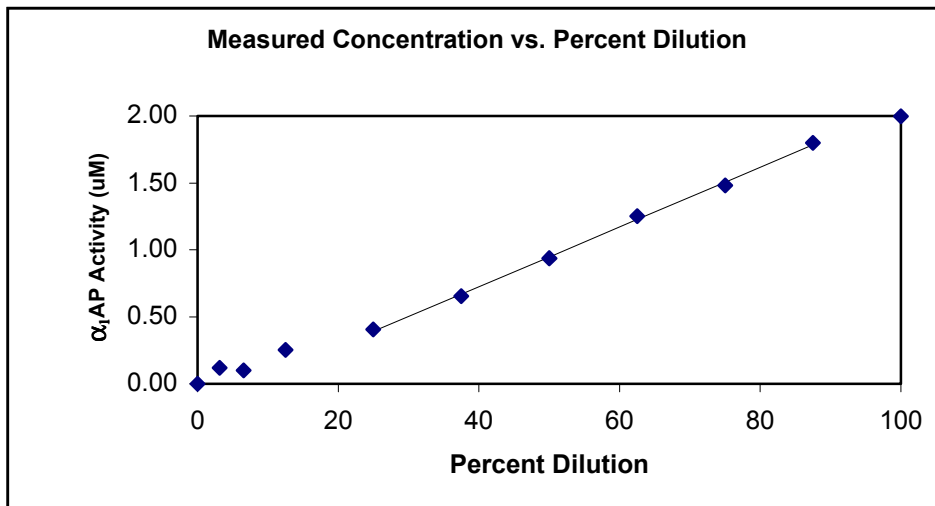
Total Precision Estimate

Three concentrations of diluted plasma were prepared in Assay Buffer and stored at -70°C in single use aliquots. The precision of the assay was determined by measuring the sample concentrations in duplicate at 23 timepoints (twice a day on multiple days) for a total of N=46 values. The intra-assay (between duplicates), inter-assay (between timepoints) and total precision were calculated.

	Low	Medium	High
Mean μM	0.50	0.79	1.29
Intra-Assay %CV	5.88	5.30	1.08
Inter-Assay %CV	4.46	4.00	0.23
Total %CV	6.67	6.01	1.09

Linearity

Dilutions of $\alpha_1\text{AP}$ were made in Assay Buffer and tested using the $\alpha_1\text{AP-410}^{\text{TM}}$ Assay. The result is a sigmoidal curve with a "pseudo linear" region across the middle concentrations. Samples with a concentration outside of this region must be diluted to obtain a concentration in the linear range of the assay.



Assay Range

The linear region of the assay defines the assay range. Samples with a concentration above 1.8 μM must be diluted with Assay Buffer before assay. The assay range is 0.5 - 1.8 μM .

Accuracy by Addition Recovery

The recovery of α_1 AP from a spiked diluted plasma sample was tested. First, spiking a stock solution into Assay Buffer tested the concentration of the added α_1 AP. Then the concentration of a diluted plasma sample was determined. The plasma sample was spiked with the stock solution and the recovery of the added α_1 AP calculated. The recovery of α_1 AP added to a biological sample was 103.5%.

Average Concentration of the Spiked Assay Buffer: 0.57

Average Concentration of the Diluted Plasma: 0.74

Average Concentration of the Spiked Plasma Sample: 1.33

Recovered Concentration of Spike: $1.33 - 0.74 = 0.59$

Percent Recovery of Spike: $(0.59 / 0.57) \times 100 = 103.5$

Specificity

Several components of biological tissues and other possible interferences were added to both a pure system (buffer) and biological system (plasma) and then tested in the assay. In the pure system, the interference is calculated by comparing to elastase concentration (no α_1 AP present). In the biological system, the interference is calculated by comparing with the plasma sample's α_1 AP activity. The values are expressed as percentage change in measured concentration.

In Pure System:

Test Compound	Concentration Added	Measured Elastase (μM)	Percent Interference
Control (Elastase)	1.40	1.40	0.00
Glucose	5 mM	1.41	0.75
Human Albumin	5%	1.36	-2.58
Human γ -Globulin	3%	1.40	0.43
Cholesterol	2 mg/mL	1.25	-10.91
GSH	1 mM	1.38	-1.29
Cysteine	100 μ M	1.52	8.17
Acetylcysteine	100 μ M	1.36	-2.58
Dithiothreitol	100 μ M	1.38	-1.24
Penicillamine	100 μ M	1.38	-1.68
Acetaminophen	100 μ M	1.39	-0.54
Ibuprofen	100 μ M	1.38	-1.77
Sucrose	100 mM	1.36	-3.12
EDTA	100 μ M	1.48	5.43
Fe ⁺⁺	500 μ M	1.46	4.68
Heparin	14.3 U/mL	1.33	-5.27
Chymotrypsin	10 μ g/L	1.44	3.13
Trypsin	1.0 mg/dL	1.46	4.30
Methionine	0.5 mg/dL	1.34	-4.02
Serine	0.5 mg/dL	1.40	-0.28

There is no significant interference on the elastase assay system from any tested compounds.

In Biological System:

Test Compound	Concentration Added	Measured α_1 AP (μ M)	Percent Interference
Plasma alone	N/A	16.24	N/A
Glucose	5 mM	14.92	8.12
Human Albumin	5%	17.16	-5.67
Human γ -Globulin	3%	17.24	-6.14
Chloesterol	2 mg/mL	17.80	-9.61
GSH	1 mM	16.67	-2.66
Cysteine	100 μ M	14.75	9.16
Acetylcysteine	100 μ M	14.19	4.76
Dithiothreitol	100 μ M	15.92	1.97
Penicillamine	100 μ M	15.34	5.57
Acetaminophen	100 μ M	15.47	-6.60
Ibuprofen	100 μ M	17.31	-1.73
Sucrose	100 mM	16.52	12.63
EDTA	100 μ M	15.07	7.19
Fe ⁺⁺	500 μ M	15.67	-4.05
Heparin	14.3 U/mL	16.90	3.48
Plasma alone	N/A	31.03	N/A
Chymotrypsin	10 μ g/L	31.07	-0.13
Trypsin	1.0 mg/dL	30.65	1.20
Methionine	0.5 mg/dL	31.22	-0.64
Serine	0.5 mg/dL	30.50	1.77

In a biological system, only a high concentration of sucrose shows interference greater than 10%.

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