CoaChrom® α1-Antitrypsin

Chromogenic assay for α1-Antitrypsin

Art.No. COA0016

For Research Use Only
Not For Use in Diagnostic Procedures
For *in vitro* Use Only

This kit is designed for the assay of α1-Antitrypsin (α1-AT) in human plasma. Plasma is diluted in buffer containing Methylamine, which inactivates α2-Macroglobulin. Excess Trypsin is added and during an incubation period, it complexes with α1-AT. The remaining free Trypsin is measured by its ability to cleave a chromogenic peptide substrate. The release of p-nitroaniline (pNA) from the substrate is measured at 405nm, and the log absorbance is inversely proportional to the plasma α1-AT concentration.

REAGENTS

The reagents should be stored at 2-8°C until reconstituted.

1. Chromogenic Trypsin Substrate, 10 mL 1 vial

10 µmol chromogenic Trypsin substrate plus mannitol. Reconstitute with 10 mL sterile distilled water. Stable for at least 6 months at 2-8°C if kept free from contamination.

2. Porcine Trypsin, 10 mL 1 vial

0.2 mg Trypsin plus stabilizers. Reconstitute with 10 mL of 1 mmol/L HCl, then further dilute 1 volume with 14 volumes of HCl (1 mmol/L). Stable for at least 8 hours at 2-8°C and 6 months at -20°C.

3. Buffer Concentrate, 10 mL 2 vials

Dilute the buffer concentrate 1+9 with distilled water. This gives an assay buffer of 0.05M Tris, 0.15M methylamine, pH 8.0. Store at 2-8°C. Diluted buffer should be used within 24 hours.

4. Standard Plasma, 1.0 mL 1 vial

Add 1.0 mL distilled water, leave for 5 minutes at room temperature and then mix gently until completely dissolved. The labelled potency is assigned against a plasma pool from 50 healthy donors. Stable for 8 hours at 2-8°C.

Reagents required, but not provided

1 mmol/L Hydrochloric acid (HCl) for Trypsin reagent preparation, 20% Acetic acid or 2% Citric acid for the endpoint method.

BLOOD COLLECTION AND PLASMA PREPARATION

Blood (9 mL) is mixed with 0.106M Tri-sodium citrate (1 mL) and centrifuged at 2000g for 15 minutes at room temperature. The plasma should be removed with plastic pipettes within two hours of blood collection and should be assayed immediately or stored frozen at -20°C.

PREPARATION OF THE STANDARD CURVE

Dilute 50 µL Standard Plasma (Reagent 4) with 950 µL diluted buffer and then further dilute as follows:

STANDARD %	DIL PLASMA	BUFFER
150	75 µL	1925 µL
100	50 µL	1950 µL
From the 100% standard prepare:		
75	300 µL	100 µL
50	200 µL	200 µL
25	100 µL	300 µL
12.5	50 µL	350 µL
0	Use buffer alone	

SAMPLE PREPARATION

Dilute 50 µL of test plasma with 950 µL buffer and then further dilute 50 µL with 1950 µL of buffer.

ASSAY METHOD

Have the chromogenic substrate at 37°C. Into plastic tubes or siliconised glass tubes pipette:

Buffer or plasma dilutions	200 µL
Incubate at 37°C for 2 minutes, add:	
Trypsin (Reagent 2)	200 µL
Mix and incubate at 37°C for 5 minutes, add:	
Chromogenic Substrate (Reagent 1)	200 µL
Mix and record the change in optical density at 405nm (rate assay), or incubate for exactly 2 minutes at 37°C, add	
Acetic acid (20%) or Citric acid (2%)	200 µL
Read optical density at 405 nm (endpoint assay).	

Microplate method
Follow the manual method above, but pipette 50 µL volumes of each plasma dilution and reagent into the wells of a microplate. Care must be taken to ensure adequate mixing after each reagent addition.

CALCULATION
With the end point assay, if the test plasmas have high Bilirubin levels, are lipaemic or have visible haemolysis, blanks must be performed. For the blanks, take 200 µL volumes of diluted plasma, add 400 µL buffer and 200 µL Acetic or Citric acid, and mix (for the microplate method, reduce these volumes by a factor of four). The A_{405} values for the blanks are subtracted from the test values before reading the α1-AT values from the standard curve.
Plot the results as log A_{405} against percentage α1-AT for the standard plasma dilutions and read the values for the test plasmas from the standard curve.
The values can be expressed either as a percentage or in units per mL (U/mL) by applying the formula:

$$\alpha\text{1-Antitrypsin (U/mL)} = \frac{\text{\% Activity x Potency of Standard*}}{100}$$

* value printed on the standard plasma vial label

PERFORMANCE CHARACTERISTICS
The assay is linear up to 150%, with a sensitivity limit of 10% (0.10 U/mL). The intra-assay coefficient of variation is < 5% at 1.00 U/mL.

INTERPRETATION
Usual Range 0.70 - 1.50 U/mL.

α1-Antitrypsin accounts for 90% of the inhibitory capacity in plasma for Neutrophil elastase. It is also an important inhibitor of Factor XIa and activated Protein C.

HAZARD WARNING
All materials of human origin were tested and found negative for the presence of HBsAg, anti-HB core, HCV antibodies and anti-HIV antibody. However, as with all preparations of human origin, these products cannot be assumed to be free from infectious agents and suitable precautions should be taken in their use and disposal.

NOTE
The recommended standard and test sample dilutions may vary between different batches of this kit owing to differences in the specific activity of some batches of reagents.

REFERENCES
Friberger P. Chromogenic peptide substrates. Their use for the assay of factors in the fibrinolytic and plasma kallikrein-kinin systems. Scand J Clin Lab Invest 1982; 42: Suppl 162: pp 84-85.

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