CHROMOGENIC SUBSTRATE ASSAY FOR α_1-ANTITRYPSIN

This kit is designed for the determination of α_1-antitrypsin (α_1-AT) in human plasma. Plasma is diluted in buffer containing methylamine, which inactivates α_2-macroglobulin. Excess trypsin is added and during an incubation period, it complexes with α_1-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 α_1-AT concentration[1,2].

REAGENTS

The kit reagents should be stored at 4°C until reconstituted.

1. *Porcine Trypsin*
Dissolve in 10ml of 1mmol/l HCl, then further dilute 1 volume with 39 volumes of 1mmol/l HCl. Stable for at least 8 hours at 4°C and 6 months at -20°C.

2. *Trypsin Substrate*
Reconstitute in 10ml sterile distilled water. Stable for 8 hours at 4°C and 6 months at -20°C, if free from contamination.

3. *Buffer Concentrate*
Dilute 1 part of buffer concentrate with 9 parts sterile distilled water.

4. *Standard Plasma*
Dissolve in 1ml distilled water. Stable for 8 hours at 4°C.

Required but not provided: 1mmol/l Hydrochloric Acid (for reagent preparation), acetic acid 50% (end-point assay).

BLOOD COLLECTION AND PREPARATION OF PLASMA

Blood (9ml) is mixed with 0.106M Tri-sodium citrate (1ml) and centrifuged at 2000g for 15 minutes at room temperature. The plasma samples 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 25µl Standard Plasma with 975µl buffer and then further dilute as follows:

STANDARD %	Dil PLASMA	BUFFER
200	100µl	1900µl
150	75µl	1925µl
100	50µl	1950µl
From the 100% standard prepare:		
75	300µl	100µl
50	200µl	200µl
25	100µl	300µl
0	Use buffer alone	

Dilute 25µl of test plasma with 975µl buffer and then further dilute 50µl with 1950µl of buffer.

ASSAY METHOD

Have the Trypsin Substrate at 37°C. Into plastic tubes, siliconised glass tubes or siliconised microcuvettes, pipette:

Buffer or plasma dilutions	200µl

Incubate at 37°C for 2 minutes, add:

Porcine Trypsin	200µl

Mix and incubate at 37°C for 5 minutes, add:

Trypsin Substrate	200µl

Mix and record the change in optical density at 405nm (Rate assay), or Incubate for exactly 2 minutes at 37°C, add

Acetic acid (50%)	200µl

Read A_{405} (end point assay)

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 200µl volumes of diluted plasma, add 400µl buffer and 200µl acetic acid, and mix. The A_{405} values for the blanks are subtracted from the test

values before reading the α_1-AT values from the standard curve.
Plot the results as log A_{405} against percentage α_1-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), assuming that the standard plasma has 1U/ml α_1-AT.

PERFORMANCE CHARACTERISTICS

The assay is linear up to 150%, with a sensitivtiy limit of 10%. The intra-assay coefficient of variation = 5% at 1.00U/ml.

INTERPRETATION

Normal Range 0.70 - 1.50 U/ml.

α_1-antitrypsin accounts for 90% of the inhibitory capacity in plasma for neutrophil elastase[3], and decreased levels may be found in patients with multi-systems organ failure and sepsis. It is also an important inhibitor of FXIa[4] and activated protein C[5].

HAZARD WARNING

All materials of human origin were tested and found negative for the presence of HBsAG 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.

REFERENCES

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CATALOGUE NUMBER: 0076
PRODUCT: α_1-Antitrypsin Kit
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14/10/92