CHROMOGENIC SUBSTRATE ASSAY FOR α_2-MACROGLOBULIN

This kit is designed for the determination of α_2-macroglobulin (α_2-M) in human plasma. Dilute plasma is mixed with excess trypsin, and the α_2-M becomes complexed with the trypsin. The remaining, non-complexed trypsin is inhibited with soy bean trypsin inhibitor. The trypsin complexed with α_2-M is still able to cleave small substrates[1,2] and releases p-nitroaniline (pNA) from a suitable chromogenic peptide substrate. The pNA concentration may be measured photometrically and is proportional to the α_2-M concentration.

REAGENTS

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

1. *Porcine Trypsin*
Dissolve in 10ml of 1mmol/l hydrochloric acid. Stable for at least 4 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. *Soybean Trypsin Inhibitor*
Reconstitute with 10ml sterile distilled water. Stable for 8 hours at 4°C and 6 months at -20°C.

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

5. *Standard Plasma*
Add 1.0ml distilled water, leave for 5 minutes at room temperature and then mix gently until completely dissolved. Stable for 8 hours at 4°C.

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

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

The Standard Plasma is diluted with buffer as follows:

STANDARD (% Activity)	PLASMA (µl)	BUFFER (µl)
200	50	3950
150	25	2642
100	25	3975
From the 100% standard prepare:		
75	300	100
50	200	200
25	100	300
0	Use buffer alone	

Dilute 25µl of test plasma with 3975µl buffer.

ASSAY METHOD

Have the Thrombin 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 2 minutes, add:

Soybean Trypsin Inhibitor 200µl

Mix and incubate at 37°C for 2 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, add the reagents in reverse order and substitute 200µl of buffer for Trypsin Substrate. Subtract the A_{405} values for the blanks from the test values before reading the α_2-M values from the standard curve.

Plot the results as A_{405} against percentage α_2-M 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:

$$\alpha_2\text{-M (U/ml)} = \frac{\%\ \text{Activity x Potency of Standard}^*}{100}$$

*The potency of the Standard Plasma for α_2-M (lot UD-0137-0148) is 0.95 U/ml.

PERFORMANCE CHARACTERISTICS

The assay is linear up to 150%, and the intra-assay coefficient of variation is 4% at 1.00 U/ml.

INTERPRETATION

Normal Range 0.70 - 1.50 U/ml.

α_2-M may be reduced in adult respiratory distress syndrome[3] and septic shock[4]. Levels are higher in adult females than in adult males. Highest levels are found between birth and three years of age[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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