CoaChrom® α2-Macroglobulin

Chromogenic Assay for α2-Macroglobulin

Art.No. COA0041

For in vitro research use only

This kit is designed for the determination of α_2-macroglobulin (α_2-M) in human plasma. Diluted plasma is mixed with excess trypsin, and the α_2-M becomes complexed with the trypsin. The remaining, non-complexed trypsin is inhibited with soybean trypsin inhibitor. The trypsin complexed with α_2-M is able to cleave substrates 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 2-8°C until reconstituted.

1. Chromogenic Trypsin Substrate, 10 mL 1 vial
10 µmol/vial plus mannitol. Reconstitute with 10 mL aqua dest. Stable for at least 6 months at 2-8°C if kept free from contamination. It may also be stored in aliquots at below -20°C.

2. Porcine Trypsin, 10 mL 2 vials
Dissolve in 10 mL of 1 mmol/L HCl. Stable for at least 8 hours at 2-8°C and 6 months at -20°C.

3. Soybean Trypsin Inhibitor, 10 mL 1 vial
Reconstitute with 10 mL sterile aqua dest. Stable for at least 8 hours at 2-8°C and 6 months at -20°C.

4. Buffer Concentrate, 10 mL 2 vials
Dilute the buffer concentrate 1+9 with aqua dest. This gives a buffer of 0.05M Tris, 0.1M NaCl, pH 8.0. Diluted buffer should be used within a week.

5. Standard Plasma, 1 mL 1 vial
Add 1.0 mL aqua dest, leave for 5 minutes at room temperature and then mix gently until dissolved. Stable for 8 hours at 2-8°C or 6 months at -20°C.

Reagents required, but not provided

20% acetic acid or 2% citric acid.

BLOOD COLLECTION AND PLASMA PREPARATION

Blood (9 parts) is mixed with 0.106 M Tri-sodium citrate (1 part) and centrifuged at 2000 g 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 (Reagent 5) is diluted with assay buffer as follows:

Standard (%)	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 25 µL of each test plasma with 975 µL of assay buffer.

ASSAY METHOD

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

Buffer or plasma dilutions	200 µL
Incubate at 37°C for 2 minutes, add:	
Porcine Trypsin	200 µL
Mix and incubate at 37°C for 2 minutes, add:	
Soybean Trypsin Inhibitor	200 µL
Mix and incubate at 37°C for 2 minutes, add:	
Chromogenic Substrate	200 µL

Mix and record the change in optical density per minute at 405 nm (rate assay), or incubate for exactly 4 minutes at 37°C, add:

Acetic acid (20%) or Citric acid (2%)	200 µL

Mix and read optical density at 405 nm (end point assay).

Microplate Method
Follow the manual method above, but pipette 50 µL of each plasma dilution and reagent into the wells of a polystyrene 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, add the reagents in reverse order and substitute 200 µL of assay 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:

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

*see vial label

PERFORMANCE CHARACTERISTICS
The assay is linear up to 150 % (1.5 U/mL), and the detection limit is 10 % (0.10 U/mL). The intra-assay coefficient of variation is 5 % at 1.0 U/mL.

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.

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.

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.

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