

# COACHROM® $\alpha$ 2-Macroglobulin

## Chromogenic assay for $\alpha$ 2-Macroglobulin

Art.No. COA0041



For Research Use Only

Not For Use in Diagnostic Procedures

For *in vitro* Use Only

This kit is designed for the determination of  $\alpha$ 2-Macroglobulin ( $\alpha$ 2-M) in human plasma. Diluted plasma is mixed with excess trypsin, and the  $\alpha$ 2-M becomes complexed with the trypsin. The remaining, non-complexed trypsin is inhibited with soybean trypsin inhibitor. The trypsin complexed with  $\alpha$ 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  $\alpha$ 2-M concentration.

### REAGENTS

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

**1. Porcine Trypsin, 10 mL** 1 vial  
Dissolve in 10 ml of 1 mmol/L HCl. Stable for 4 hours at 2-8°C and 6 months at -20°C.

**2. Chromogenic Trypsin Substrate, 10 mL** 1 vial  
10  $\mu$ mol/vial Bz-Val-Gly-Arg-pNA, plus mannitol. Reconstitute with 10.0 mL sterile distilled water. Stable for 6 months at 2-8°C if kept free from contamination. It may also be stored in aliquots at below -20°C.

**3. Soybean Trypsin Inhibitor, 10 mL** 1 vial  
Reconstitute with 10 mL sterile distilled water. This solution is stable for 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 distilled water. This gives an *assay buffer* of 0.05M Tris, 0.1M NaCl, pH 8.0. Diluted buffer should be used within 24 hours.

**5. Standard Plasma, 1 mL** 1 vial  
Add 1.0 mL distilled water, leave for 5 minutes at room temperature and then mix gently until dissolved. Stable for 4 hours at 2-8°C.

### Reagents required, but not provided

1mmol/L HCl, 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 (Std, Reagent 5) is diluted with assay buffer as follows:

Standard (%)	Std Plasma ( $\mu$ L)	Buffer ( $\mu$ L)
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  $\mu$ L of each **test plasma** with 3975  $\mu$ L 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  $\mu$ L

*Incubate at 37°C for 2 minutes, add:*

Porcine Trypsin 200  $\mu$ L

*Mix and incubate at 37°C for 2 minutes, add:*

Soybean Trypsin Inhibitor 200  $\mu$ L

*Mix and incubate at 37°C for 2 minutes, add:*

Chromogenic Substrate 200  $\mu$ L

*Mix and record the change in optical density per*

minute at 405 nm (rate assay), or incubate for exactly 5 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).

#### MICROTITRE METHOD

Follow the manual method above, but pipette 50 µL volumes of each plasma dilution and reagent into the wells of a 96 well polystyrene microtitre plate. 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 buffer for Chromogenic Substrate. Subtract the  $A_{405}$  values for the blanks from the test values before reading the  $\alpha_2$ -M values from the standard curve.

Plot the results as  $A_{405}$  against percentage  $\alpha_2$ -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:

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

\*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.

#### INTERPRETATION

Normal Range 0.70 - 1.50 U/mL was obtained testing citrated plasma samples from 50 healthy individuals.

$\alpha_2$ -M may be reduced in adult respiratory distress syndrome<sup>3</sup> and septic shock<sup>4</sup>. Levels are higher in adult females than in males. Highest levels are found between birth and three years of age<sup>5</sup>.

#### 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

1. 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.
2. Gallimore MJ, Aurell L, Friberger P, Gustavsson S. Chromogenic peptide substrate assays for determining functional activities of alpha-2-macroglobulin and alpha-1-antitrypsin using a new trypsin substrate. Thrombos Haemost 1983; 50: 230.
3. Velasco F, Torres A, Guerrero A, Andres P, Guerrero R, Aljama P, Alvarez F. Behaviour of the contact phase of blood coagulation in the adult respiratory distress syndrome (ARDS). Thrombos Haemostas 1986; 55: 357-360.
4. Martinez-Brotons F, Oncins JR, Mestres J, Amargos V, Reynaldo C. Plasma kallikrein-kinin system in patients with uncomplicated sepsis and septic shock - Comparison with cardiogenic shock. Thrombos Haemostas 1987; 58: 709-713.
5. Ganrot PO & Schersten B. Serum alpha-2-macroglobulin concentration and its variation with age and sex. Clin Chim Acta 1967; 15: 113-120.

#### 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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