

Quantitative determination of human α₂-macroglobulin

 (α₂-macro)

IVD

Store 2 - 8°C.

PRINCIPLE OF THE METHOD

α₂-MACRO is a quantitative turbidimetric test for the measurement of α₂-macro in human serum or plasma.

Anti-human α₂-macro antibodies when mixed with samples containing α₂-macro, form insoluble complexes. These complexes cause an absorbance change, dependent upon the α₂-macro concentration of the patient sample, that can be quantified by comparison from a calibrator of known α₂-macro concentration.

CLINICAL SIGNIFICANCE¹

α₂-macroglobulin is the major plasma proteinase inhibitor. It is a very large molecule (725 kD), that doesn't diffuse out of the plasma space in significant amounts, acts as a substrate for proteases and inhibits many different classes of proteinases. Primarily hepatic cells synthesize α₂-macro and the normal half-life of the intact protein is several days. This molecule is the most important physiologically in the primary or secondary inhibition of enzymes in the kinin, complement, coagulation and fibrinolytic pathways. Also, α₂-macro is believed to modulate immunologic and inflammation reactions.

Levels of α₂-macro are decreased in plasma as a consequence of acute-phase response, pancreatitis and prostatic carcinoma.

Levels of α₂-macro are increased in plasma as a result of hormonal effects (estrogens may contribute to increase the level of α₂-macro), the age (infants and children have 2-3 times adult levels), and nephritic syndrome.

REAGENTS

R1	Tris buffer 20 mmol/L, PEG 8000, pH 8.2. Sodium azide 0.95 g/L.
R2	Goat serum, anti-human α ₂ -macroglobulin, pH 7.5. Sodium azide 0.95 g/L.
Saline Solution	NaCl 9% (for samples pre-dilution)

CALIBRATION

The assay is calibrated to the Reference Material CRM 470/RPPHS (Institute for Reference Materials and Measurements, IRMM).

PREPARATION

Reagents: Ready to use.

Samples and Controls Pre-Dilution: Before the use, samples and/or controls must be diluted in this way:

- 25 µL sample or control + 200 µL NaCl 9 g/L(1:9)

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C and contamination is prevented during their use. Do not use reagents over the expiration date.

Reagent deterioration: The presence of particles and turbidity. Do not use.

Do not freeze; frozen Antibody or Diluent could change the functionality of the test.

ADDITIONAL EQUIPMENT

- Pipette 100 - 1000 µL
- Pipette 10 - 100 µL
- Cuvettes and microstirrers (ref. code ACC16-037)

SAMPLES

Fresh serum or plasma. EDTA or heparin should be used as anticoagulant.

Stable 7 days at 2-8°C or 3 months at -20°C.

The samples with presence of fibrin should be centrifuged.

Do not use highly hemolyzed or lipemic samples.

TEST PARAMETER

Filter: set filter on position B

Reading time: 120 seconds

PROCEDURE

1. Pipette into an appropriate number of cuvettes:

kind of reagent	µL
R1	500
Pre-diluted sample or control	50

2. Incubate the cuvettes in positions 1-4 for at least 1 minute
3. Transfer each cuvette in the reading channel and, when requested on the display, add 25 µL of R2
4. Read the result which will appear automatically on the reader's display after 120 sec.

QUALITY CONTROL

Control sera are recommended to monitor the performance of manual and automated assay procedures. Vital Diagnostics SERUM PROT CONTROL BILEVEL (Cod.: ACC16-038) is available. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES¹

Adults: 130-300 mg/dL.

Children (2-4 years): approximately twice the adult concentration.

Women > 40 years: 20-30% higher than men.

Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

1. Linearity: Up to 550 mg/dL (Note1), under the described assay conditions. Samples with higher concentrations, should be diluted 1/5 in NaCl 9 g/L and re-tested again. The linearity limit and measurement range depends on the sample to reagent / ratio. It will be higher by decreasing the sample volume, although the sensitivity of the test will be proportionally decreased.
2. Detection Limit: Values less than 1 mg/dL give non-reproducible results.
3. Prozone effect: No prozone effect was detected upon 4100 mg/dL.
4. Sensitivity: Δ 5.6 mA. mg/dL (33 mg/dL), Δ 2.5 mA. mg/dL (262 mg/dL).
5. Precision:

	Mean	SD	CV%
Intra-assay (n=10)	62.6	1.21	1.94
Inter-assay (n=10)	62.6	2.16	3.45

6. Accuracy: Results obtained using this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.

INTERFERENCES

Hemoglobin (19 g/L), bilirubin (40 mg/dL) and rheumatoid factors (800 IU/mL), do not interfere. Lipemia (5 g/L), interferes. Other substances may interfere.⁶⁻⁷

NOTES

1. The linearity depends on the calibrator concentration.
2. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

BIBLIOGRAPHY

1. Clinical Guide to Laboratory Tests, Edited by NW Tietz W B Saunders Co., Philadelphia, 483, 1983.
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3. Viedma JA et al. Gut 1994; 35: 822-827.
4. Pesce AJ and Kaplan, LA. Methods in Clinical Chemistry. The CV Mosby Company, St. Louis MO, 1987.
5. Dati F et al. Eur J Clin Chem Clin Biochem 1996; 34: 517-520.
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PACKAGING

Ref: ACC16-004	Cont.	: 1 x 50 mL R1 : 1 x 2 mL R2 : 1 x 50 mL Saline Solution
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