

Store at +2 to +8°C

PRINCIPLE

α_2 -macroglobulin is a quantitative turbidimetric test for the measurement of α_2 -macroglobulin in human serum or plasma.

Anti human α_2 -macroglobulin antibodies when mixed with samples containing α_2 -macroglobulin, form insoluble complexes. These complexes cause an absorbance change, dependent upon the α_2 -macroglobulin concentration of the patient sample, that can be quantified by comparison from a calibrator of known α_2 -macroglobulin concentration.

CLINICAL SIGNIFICANCE

α_2 -macroglobulin is the major plasma proteinase inhibitor. It is very large molecule (725 kD), that doesn't diffuse out of the plasma space in significant amount, acts as a substrate for proteases and inhibits many different classes of proteinases. Primarily hepatic cells synthesize α_2 -macroglobulin and the normal half-life of the intact protein in 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 α_2 -macroglobulin is believed to modulate immunologic and inflammation reactions.

Levels of α_2 -macroglobulin are decreased in plasma as a consequence of acute phase response, pancreatitis and prostatic carcinoma.

Levels of α_2 -macroglobulin are increases in plasma as a result of hormonal effects (estrogens may contribute to increase the level of α_2 -macroglobulin), the age (infants and children have 2-3 times adult levels), and nephritic syndrome.

REAGENTS

- Diluent (R1)** Tris buffer 20 mmol/l, PEG 8000, pH 8.2
Sodium azide 0.95 g/l
- Antibody (R2)** Goat serum, anti-human α_2 -macroglobulin, pH 7.5
Sodium azide 0.95 g/l
- Optional:** 101-0485 General proteins calibrator
101-0509 General proteins control

REAGENTS

The assay is calibrated to the reference Material CRM 470/RPPHS (Institute for Reference Materials and Measurements, IRMM). It is recommended the use of the General Protein Calibrator for calibration.

PREPARATION

Reagents: Ready to use.

Calibration Curve: Prepare the following General Protein Calibrator dilutions in NaCl 9 g/l as diluent. Multiply the concentration of the α_2 -macroglobulin calibrator by the corresponding factor stated in table below to obtain the α_2 -macroglobulin concentration of each dilution.

Calibrator dilution	1	2	3	4	5	6
Calibrator (μ l)	-	10	25	50	75	100
NaCl 9 g/l (μ l)	100	90	75	50	25	-
Factor	0	0.1	0.25	0.5	0.75	1.0

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed 2-8 °C and contaminations are 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

- thermostatic bath at 37 °C.
- spectrophotometer or photometer thermostatable at 37 °C with a 340 nm filter (320 – 360 nm).

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 before testing.

Do not use highly hemolyzed or lipemic samples.

PROCEDURE

1. Bring the reagents and the photometer (cuvette holder) to 37°C.
2. Assay conditions:

Wavelength: 340 nm
 Temperature: 37°C
 Cuvette light path: 1 cm

3. Adjust the instrument to zero with distilled water.
4. Pipette into a cuvette:

Reagent R1 (μ l)	950
Sample or Calibrator (μ l)	10
5. Mix and read the absorbance (A_1) after the sample addition.
6. Immediately, pipette into cuvette:

Reagent R2 (μ l)	50
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7. Mix and read the absorbance (A_2) of calibrators and sample exactly 2 minutes after the R2 addition.

Chronolab has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

CALCULATIONS

Calculate the absorbance difference (A_2-A_1) of each point of the calibration curve and plot the values obtained against α_2 -macroglobulin concentration of each calibrator dilution. α_2 -macroglobulin concentration in the sample is calculated by interpolation of its (A_2-A_1) in the calibration curve.

QUALITY CONTROL

Control sera are recommended to monitor the performance of manual and automated assay procedures. Chronolab General protein control is available. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES

Adults: 130-300 mg/dl.

Childrens (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 (note 1), under the described assay conditions. Samples with higher concentrations, should be diluted 1/5 in NaCl 9 g/l and retested again. The linearity limit and measurement range depends on the sample / reagent ration. 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 none-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 (mg/dl)	Intra-assay (n=10)			Inter-assay (n=10)		
	62.6	115.3	239.8	62.6	115.3	239.8
SD	1.21	2.56	9.98	2.16	3.54	11.39
CV	1.94	2.24	4.16	3.45	3.09	4.75

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

The results of the performance characteristics depend on the used analyzer.

INTERFERENCES

Hemoglobin (19 g/l), bilirubin (40 mg/dl), rheumatoid factors (800 IU/ml) do not interfere. Lipemia (5 g/l), interferes. Other substances may interfere.

NOTES

1. Linearity depends on the calibration concentration.
2. Clinical diagnosis should not be made on findings of a single test result, but should be integrated both clinical and laboratory data.

REFERENCES

1. Clinical Guide to Laboratory Tests, Edited by NW Tietz W B Saunders Co., Philadelphia 483, 1983
2. Muck- Petersen C et al. Eur L Clin Invest 1988; 18: 596-603.
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.
6. Young DS. Effects of drugs on clinical laboratory tests, 4th ed. AACC Pres, 1995.
7. Freidman and Young. Effects of diseases on clin. Laboratory tests, 3th ed. AACC Pres, 1997.

PACKAGING

Ref: 101-0484 Cont.: 1x50 ml / 1x2 ml