GOT (AST). NADH. Kinetic UV. IFCC rec.Liquid	Ref.:GOT-008
Quantitative Determination of aspartate aminotransferase	
Only for *in vitro* use in clinical laboratory	R1 5 x 40 mL
Store at 2-8ºC	R2 1 x 50 mL

GOT (AST)

CE

PRINCIPLE OF THE METHOD

Aspartate aminotransferase (AST) formerly called glutamate oxaloacetate (GOT) catalyses the reversible transfer of an amino group from aspartate to α-ketoglutarate forming glutamate and oxalacetate. The oxalacetate produced is reduced to malate by malate dehydrogenase (MDH) and NADH:

$$\text{L-Aspartate} + \alpha\text{-Ketoglutarate} \xrightarrow{\text{AST}} \text{Glutamate} + \text{Oxalacetate}$$

$$\text{Oxalacetate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{MDH}} \text{Malate} + \text{NAD}^+$$

The rate of decrease in concentration of NADH, measured photometrically, is proportional to the catalytic concentration of AST present in the sample[1].

CLINICAL SIGNIFICANCE

The AST is a cellular enzyme, is found in highest concentration in heart muscle, the cells of the liver, the cells of the skeletal muscle and in smaller amounts in other weaves.
Although an elevated level of AST in the serum is not specific of the hepatic disease, is used mainly to diagnostic and to verify the course of this disease with other enzymes like ALT and ALP.
Also it is used to control the patients after myocardial infarction, in skeletal muscle disease and other[1,4,5].
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

R 1 Buffer	TRIS pH 7.8	80 mmol/L
	Lactate dehydrogenase (LDH)	800 U/L
	Malate dehydrogenase (MDH)	600 U/L
	L-Aspartate	200 mmol/L
R 2 Substrate	NADH	0.18 mmol/L
	α-Ketoglutarate	12 mmol/L

PREPARATION

Working reagent (WR):
Mix: 4 vol. (R1) Buffer + 1 vol. (R2) Substrate

Stability: 21 days at 2-8ºC or 72 hours at room temperature (15-25ºC).

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC, protected from light and contaminations prevented during their use.
Do not use reagents over the expiration date.
Signs of reagent deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 340 nm < 1.00.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 340 nm.
- Thermostatic bath at 25ºC, 30ºC o 37ºC (± 0.1ºC)
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

SAMPLES

Serum or plasma[1]: Stability 7 days at 2-8ºC.

PROCEDURE

1. Assay conditions:
   Wavelength: . . . . . 340 nm
   Cuvette: . . . . . .1 cm. light path
   Constant temperature . . . . . .25ºC /30ºC / 37ºC
2. Adjust the instrument to zero with distilled water or air.
3. Pipette into a cuvette:

WR (mL)	1.0
Sample (µL)	100

4. Mix, incubate for 1 minute.
5. Read initial absorbance (A) of the sample, start the stopwatch and read absorbances at 1 minute intervals thereafter for 3 minutes.
6. Calculate the difference between absorbances and the average absorbance differences per minute (ΔA/min).

CALCULATIONS

ΔA/min x 1750 = U/L of AST

Units: One international unit (IU) is the amount of enzyme that transforms 1 µmol of substrate per minute, in standard conditions. The concentration is expressed in units per litre of sample (U/L).

Temperature conversion factors
To correct results to other temperatures multiply by:

Assay temperature	Conversion factor to		
	25ºC	30ºC	37ºC
25ºC	1.00	1.37	2.08
30ºC	0.73	1.00	1.54
37ºC	0.48	0.65	1.00

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures.
If control values are found outside the defined range, check the instrument, reagents and technique for problems.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[1]

	25ºC	30ºC	37ºC
Men	up to 19 U/L	26 U/L	38 U/L
Women	up to 16 U/L	22 U/L	31 U/L

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: From detection limit of 1 U/L to linearity limit of 260 U/L.
If the results obtained were greater than linearity limit, dilute the sample 1/10 with NaCl 9 g/L and multiply the result by 10.
Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (U/L)	17.0	135	17.3	131
SD	0.72	1.05	0.81	2.25
CV (%)	4.27	0.77	4.68	1.72

Sensitivity: 1 U/L = 0,0048 ΔA/min.
Accuracy: Results obtained using BSM reagents (y) did not show systematic differences when compared with other commercial reagents (x).
The results obtained using 100 samples were the following:
Correlation coefficient (r): 0.9839.
Regression equation: y= 0.9866x + 0.588.
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Anticoagulants currently in use like heparin, EDTA, oxalate and fluoride do not affect the results. Haemolysis interferes with the assay[1]
A list of drugs and other interfering substances with AST determination has been reported by Young et. al[2,3].

NOTES

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

BIBLIOGRAPHY

1. Murray R. Aspartate aminotransferase. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1112-116.
2. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
3. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.
4. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
5. Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995.