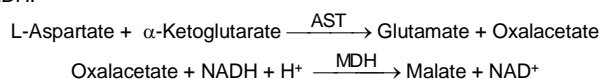


**Quantitative determination of aspartate aminotransferase
GOT (AST)
IVD**

Store at 2-8°C

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:



The rate of decrease in concentration of NADH, measured photometrically, is proportional to the catalytic concentration of AST present in the sample¹.

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 tissues.

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

PRECAUTIONS

R1: H290-May be corrosive to metals.

Follow the precautionary statements given in MSDS and label of the product.

PREPARATION

Working reagent (WR):

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

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

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, 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 25°C, 30°C or 37°C ($\pm 0,1^\circ\text{C}$)
- Matched cuvettes 1,0 cm light path.
- General laboratory equipment.

SAMPLES

Serum or plasma¹: Stability 7 days at 2-8°C.

PROCEDURE

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

WR (mL)	1,0
Sample (μL)	100

- Mix, incubate for 1 minute.

- Read initial absorbance (A) of the sample, start the stopwatch and read absorbances at 1 minute intervals thereafter for 3 minutes.
- Calculate the difference between absorbances and the average absorbance differences per minute ($\Delta A/\text{min}$).

CALCULATIONS

$$\Delta A/\text{min} \times 1750 = \text{U/L of AST}$$

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		
	25°C	30°C	37°C
25°C	1,00	1,37	2,08
30°C	0,73	1,00	1,54
37°C	0,48	0,65	1,00

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures: CONTROL Normal and Pathologic.

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 VALUES¹

	25°C	30°C	37°C
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 0 U/L to linearity limit of 467 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)	48,1	159	47,4	156
SD	0,56	0,57	1,42	4,35
CV (%)	1,16	0,36	3,00	2,79

Sensitivity: 1 U/L = 0,00053 $\Delta A/\text{min}$.

Accuracy: Results obtained using Audit Diagnostics reagents (y) did not show systematic differences when compared with other commercial reagents (x).

The results obtained using 50 samples were the following:

Correlation coefficient (r): 0,99956.

Regression equation: $y = 1,042x - 0,342$.

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 assay¹

A list of drugs and other interfering substances with AST determination has been reported^{2,3}.

NOTES

Audit Diagnostics has instruction sheets for several automatic analyzers.

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