



Store at: +2+8°C.

Presentation:

Cod. EZ012LQ CONT: R1 1 x 100 + R2 1 x 25 mL .
 EZ012LQ-SP CONT: R1 1 x 40 + R2 1 x 10 mL .
 EZ013LQ CONT: R1 2 x 100 + R2 2 x 25 mL .

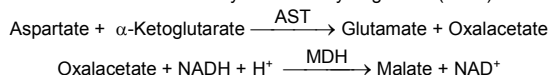
Procedure

Quantitative determination of GOT/AST.

Only for in vitro use in clinical laboratory (IVD)

TEST SUMMARY

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.

REAGENTS COMPOSITION

R 1 Buffer	TRIS PH 7.8	80mmol/L.
	L-Aspartate	200 mmol/L.
	Lactate dehydrogenase (LDH)	800 U/L.
R 2 Substrate	Malate dehydrogenase (MDH)	600 U/L.
	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.

REAGENT PREPARATION AND STABILITY

Working reagent (WR):
 Mix 1 volume of R2 with 4 volumes of R1.
 Stability: 21 days at 2-8° C or 72 hours at room temperature (15-25° C).
 All the components of the kit are stable until the expiration date on the label when stored at 2-8° C, protected from light and contamination 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

All the reagents of the kit are stable up to the end of the indicated month and year of expiry. Store at tightly closed at 2-8°C. Do not use reagents over the expiration date.

SPECIMEN

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

MATERIAL REQUIRED BUT NOT PROVIDED

- Spectrophotometer or colorimeter measuring at 340 nm.
- Thermostatic bath at 25°C, 30°C o 37°C (\pm 0.1°C).
- Matched cuvettes 1.0 cm. light path.

General laboratory equipment.

TEST 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^(note 1):

WR (mL.)	1.0
Sample (μ L.)	100

- Mix and incubate for 1 minute.
- Read the absorbance (A) of the sample, start the stopwatch and read absorbance at 1 min. interval thereafter for 3 min.
- Calculate the difference of absorbance and the average absorbance difference per minute (Δ A/min.)

CALCULATIONS

GOT/AST U/L. = Δ A/min. x 1750 (note 2)

Units: One international unit (IU) is the amount of enzyme that transforms 1 μ mol 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 the procedure, Control H Normal Ref. QC003 and Control H Pathological Ref. QC004. If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

Serum controls are recommended for internal quality control. Each laboratory should establish its own Quality Control scheme and corrective actions

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).

It is suggested that each laboratory establish its own reference range

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.

REAGENT PERFORMANCE

- Measuring Range:

From detection limit of 0.000 U/L. to linearity limit of 467 U/L., under the described assay conditions.
 If results obtained were greater than linearity limit, dilute the sample 1/10 with NaCl 9 g/L. and multiply result by 10.

- Precision:

Mean (U/L)	Intra-assay n= 20		Inter-assay n= 20	
	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 Δ A/min

- **Accuracy:** Results obtained GPL reagents did not show systematic differences when compared with other commercial reagents.

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.

INTERFERING SUBSTANCES

- 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 by Young et. al^{2,3}.

NOTES

- Use clean disposable pipette tips for its dispensation.
- Formulation to reach constant:

$$\Delta\text{A/min} \times 1750^* = \frac{\text{U/L of AST} \times \text{LP} \times \text{Sv}}{\text{Tv} \times 1000}$$

Tv= Total volume in mL
 ϵ NAD= 6,22 at 340 nm
 LP= Light path
 Sv= Sample volume in mL

BIBLIOGRAPHY

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