Reactivos GPL

Barcelona, España

CE

- GOT(AST)-

GOT/AST
MDH-NADH. Kinetic UV

Store at: +2+8ºC.

Presentation:
Cod. EZ012 CONT: R 20 x 2 mL .
EZ013 CONT: R 15 x 15 mL.
EZ014 CONT: R 9 x 50 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:

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

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

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

REAGENTS COMPOSITION

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

REAGENT PREPARATION AND STABILITY
Working reagent (WR):
Ref.: EZ012 Dissolve (→) 1 tablet of R.2 in 2 mL. of R.1 (Buffer).
Ref.: EZ013 Dissolve (→) 1 tablet of R.2 in 15 mL of R.1 (Buffer).
Ref.: EZ014 Dissolve (→) 1 tablet of R.2 in 50 mL of R.1 (Buffer).

Cap and mix gently to dissolve contents.
Stability: 21 days at 2-8ºC or 72 hours at room temperature.
All the components of the kit are stable until the expiration date on the label when stored at 2-8ºC, protected from light and contamination prevented during their use. Do not use reagents over the expiration date. Do not use tablets if appears broken.
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 2-8ºC. Do not use reagents over the expiration date.

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

MATERIAL REQUIRED BUT NOT PROVIDED
- 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.

TEST PROCEDURE
1. Assay Conditions
   - Wavelenght : ..... 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(note 1):

WR (mL.)	1.0
Sample (µL.)	100

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

CALCULATIONS
GOT/AST U/L. = ΔA/min. x 1750 (note 2)

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 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 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).

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 360 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*:

	Intra-assay n= 20		Inter-assay n= 20	
Mean (U/L)	55.5	165	55.0	162
SD	1.30	3.44	0.92	2.52
CV	2.35	2.07	1.68	1.55

- Sensitivity: 1 U/L = 0.00051 ΔA/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): 0.98277
  Regression Equation: y=0.9259x – 5.1685
  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 assay[1]
- A list of drugs and other interfering substances with AST determination has been reported by Young et. al[2,3].

NOTES
1. Use clean disposable pipette tips for its dispensation.
2. Formulation to reach constant:

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

$$^* \frac{Tv \times 1000}{\varepsilon \times LP \times Sv}$$

Tv= Total volume in mL
ε NAD= 6.22 at 340 nm
LP= Light path
Sv= Sample volume in mL

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