CE
IVD

# GOT-LQ

NADH. Kinetic UV. IFCC rec.Liquid

Quantitative determination of aspartate aminotransferase GOT (AST)

***PACKAGING***

| | |
|---|---|
| Ref.: 101-0438 | Cont.: 4 x 240 / 4 x 60 mL |
| Ref.: 101-0574 | Cont.: 4 x 50 / 2 x 25 mL |

Store at 2-8º C

***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.

***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].

***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 |

**Optional (not included in the kit)**

| | | | |
|---|---|---|---|
| Contro-N | Ref.: 101-0252 | 4 x 5 mL | Lyophilized human control serum |
| | Ref.: 101-0083 | 20 x 5 mL | |
| Contro-P | Ref.: 101-0253 | 4 x 5 mL | Lyophilized human control serum |
| | Ref.: 101-0084 | 20 x 5 mL | |

***PREPARATION***

Working reagent (WR):

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

Stability: 21 days at 2-8º C 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 2-8º 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 25º C, 30° C or 37° C (± 0.1º C)
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

***SAMPLES***

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

***PROCEDURE***

**Notes:** CHRONOLAB SYSTEMS has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

1. Assay conditions:
   Wavelength: . . . . . 340 nm
   Cuvette: . . . . . .1 cm. light path
   Constant temperature . . . . . .25º C /30º C / 37º C
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 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 | | |
|---|---|---|---|
| | 25º C | 30º C | 37º C |
| 25º C | 1.00 | 1.37 | 2.08 |
| 30º C | 0.73 | 1.00 | 1.54 |
| 37º C | 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]***

| | 25º C | 30º C | 37º 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.



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***PERFORMANCE CHARACTERISTICS***

**Measuring range:** From detection limit of 0.00 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 ΔA/min.

**Accuracy:** Results obtained using CHRONOLAB 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 assay[1]
A list of drugs and other interfering substances with AST determination has been reported[2,3].

***BIBLIOGRAPHY***

1. Murray R. Aspartate aminotransferase. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1112-116.
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