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AST (GOT)

FREEZE DRIED

Cat. No.:	41031	41032	41033
	9x50 ml	90x50 ml	40x50 ml

Reagent kit for the determination of the aspartate aminotransferase (AST) activity in serum based upon IFCC recommendations.

AST originates in various tissues and is a dimer molecule containing one molecule of Pyridoxal phosphate (coenzyme) in each monomer, which is essential to its catalytic activity. Depending on the sites of origin inside the cell there are two isoenzymes with different ph optimum: the mitochondrial m-AST, and the soluble cytosolic S-AST. The two izoenzymes can be separated by electrophoresis. The enzyme catalyses the transfer of amino groups during the metabolism of Amino acids and, alpha-Ketoacids. The activity of AST in the serum is significantly increased during heart, liver, kidney and muscle diseases (tissue injuries, functional disorders).The activity of the enzyme is increased 4-8 hours following a myocardial infarction, reaching its' peak in 2-3 days and declining on the fifth and sixth days.

Principle

Two substrates participate in the reaction catalyzed by AST, L-aspartate and Oxoglutarate. With the help of NADH coenzyme, Malate dehydrogenase (MDH) contained in the reagent catalyses the transformation of Oxalacetate released in the first reaction. The oxido-reductive process of NADH/NAD^+ is indicated by a decrease in absorbance at 340 nm. The Lactate dehydrogenase (LDH) in the medium counteracts the disturbing effect of Pyruvate contained in the sample.

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

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

Reference values

AST activity: **<37 U/l (0,62 µkat/l)**

It is recommended that each laboratory should assign its own normal range.

Reagents

1.Reagent (R1)

Tris buffer, pH:7.80	88 mmol/l
L-Aspartate	260 mmol/l

2.Reagent (R2)

NADH	0.22 mmol/l
LDH	900 U/l
MDH	600 U/l
α-Ketoglutarate	12 mmol/l

Samples

Serum free of haemolysis.
Haemolysis, lipaemia interfere with the test.

PROCEDURE

Preparation and stability of working reagent

Dissolve one vial of reagent (R2) in an appropriate volume of reagent (R1).
Stability: at 20-25 °C : 5 days
at 2-8 °C: 28 days
If the absorbance of working reagent is lower than 1.2 at 334 nm the reagent can not be used.

Assay conditions

Wavelength :	340 (334-365) nm
Temperature :	37°C
Cuvette :	1 cm light path
Read against:	distilled water
Method:	kinetic (decreasing)

Pipette into cuvette

Working reagent	1 ml
Sample or Control	100 µl

Mix and after a 1-minute incubation, measure the change of absorbance per minute (ΔA/min) during 3 minutes.
Determine the change of absorbance per minute (ΔA/min).

Calibration:

(37°C, IFCC method without pyridoxal-phosphate)
S1: Distilled water
S2: Roche C.F.A.S. (Calibrator for automated system) or
Randox Calibration Serum Level I or
Randox Calibration Serum Level II

Calibration frequency

Two point calibration is recommended
- after reagent lot change,
- as required following quality control procedures.

Calculation using calibration

$$\frac{\Delta A_{sample}}{\Delta A_{standard}} x C_{standard} = C_{sample}$$

A = Absorbance, C = Concentration,

Calculation using factor

340 nm: Activity (U/l)= ΔA/min x 2000; (µkat/l) = ΔA/min x 33,33
334 nm: Activity (U/l)= ΔA/min x 1790; (µkat/l) = ΔA/min x 29,83

Quality control

A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

PERFORMANCES DATA

The following data were obtained using the Olympus 600 analyzer (37°C).

Linearity

The test is linear up to 300 U/l (5,0 µkat/l)GOT activity.

Sensitivity

It is recommended that each laboratory establishes its own range of sensitivity as this is limited by the sensitivity of the spectrophotometer used. Under manual conditions however, a change of 0.001 Abs units/min is equivalent to 1.79 U/l (0,03 µkat/l) GOT activity at 334 nm.

Precision

	Reproducibility		
	Average activity (U/l)	SD	CV%
Sample I.	37.1	1.07	2.88
Sample II.	126	1.51	1.20

	Repeatability		
	Average activity (U/l)	SD	CV%
Sample I.	23.1	0.31	1.33
Sample II.	183	1.23	0.67

Correlation

Comparative studies were done to compare our reagent with another commercial GOT assay.
The results from these studies are detailed below.
Correlation coefficient: r=0.9999
Linear regression: y (U/l)= 1.014x+1.382
(x= other commercial reagent, y= own reagent).

Specificity

Bilirubin 855 µmol/l (50mg/dl), glucose 55.5 mmol/l (1000mg/dl), and ascorbic acid 0.57 mmol/l (10mg/dl) don't interfere with the assay up to the given levels.

Note

The test doesn't contain pyridoxal-phosphate.
Do not use reagents after the expiry date stated on each reagent container label. Do not use products, test solutions and reagents described above for any purpose other than described herein.

For *in vitro* diagnostic use only.

The following symbols are used on labels

IVD For in vitro diagnostic use

Use by (last day of the month)

Temperature limitation

LOT Batch Code

REF Code

Bibliography

Bergmeyer et.al. Clin.Chim.Acta 70, F19-42(1976), F21-22 (1977)

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