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AST (GOT)

CONCENTRATED LIQUID REAGENT

CE

Order information:

Catalogue number	Size
9511C	10 x 65 + 10 x 33 ml
9512C	4 x 65 + 4 x 33 ml

Reagent kit for quantitative in vitro determination of aspartate-aminotransferase (AST/GOT) in serum and plasma.

Summary

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.

Method

Optimized IFCC method without pyridoxal-5-phosphate

$$\text{L-aspartate} + \alpha\text{-ketoglutarate} \xrightleftharpoons{\text{GOT}} \text{L-glutamate} + \text{oxalacetate}$$

$$\text{oxalacetate} + \text{NADH} + \text{H}^+ \xrightleftharpoons{\text{MDH}} \text{L-malate} + \text{NAD}^+$$

Reagents

Composition and concentrations

Reagent 1	
Tris buffer, pH=7.80	88 mmol/l
L-aspartate	850 mmol/l
LDH	4500 U/l
MDH	2700 U/l
NADH	860 µmol/l
Reagent 2	
α-ketoglutarate	36 mmol/l

Storage and stability

The reagent is stable up to the end of the indicated month of expiry without opening, if stored at 2 – 8°C, protected from light and contamination is avoided. Do not freeze!
Onboard stability after opening and the frequeny of calibration is 72 days.
The absorbance at 340 nm should not be lower than 1,2

Warnings and precautions

Do not use reagents after the expiry date stated on each reagent container label.

Chemical safety

This product is not classified as dangerous. Safety data sheet is available upon request. The product contains sodium azide. Sodium azide can react with copper and lead plumbing to form explosive metal azides. If disposal into a drain is in compliance with federal, state, and local requirements, flush reagents with a large amount of water to prevent the buildup of azides.

Preparation

The reagent is ready for use.

Sample

Serum, EDTA, heparin, citrate plasma.
3 days loss of activity in serum
<8% at 2 – 8°C
<10% at 15 – 25°C
Stability in serum: 3 months at -20°C

Expected values and reference range

Serum: <35 U/l
It is recommended that each laboratory should assign its own normal range.

Assay procedure

Wavelength:	340 nm / 410 nm (primary/secondary)
Optical path:	1 cm
Temperature:	37°C
Measurement:	against water blank
Reaction:	kinetic, decreasing

	blank	sample or standard
reagent 1	200 µl	200 µl
dist. water (diluent)	400 µl	400 µl
dist. water (blank)	90 µl	-
sample or standard	-	90 µl
Mix and incubate for 1 minute		
reagent 2	100 µl	100 µl
dist. water (diluent)	200 µl	200 µl
Mix and incubate for 1 minute then continuously read the absorbances for 3 minutes		

Calculation

GOT[U/l]=ΔA sample/ΔA standard × standard concentration[U/l]

Conversion factor

[U/l]=[µkat/l]×60

Calibration and quality control

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 is recommended:

- after opening new reagent batch
- after system maitenance or troubleshooting

For internal quality control, two levels controls are recommended (normal and pathological) at least once a day. The measured values must in the range which was given by the control's manufacturer. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

Preformance characteristics

Measuring range

The method is linear in the range 5 – 260 U/l

Interferences

No significant intereference was observed by bilirubin up to 1000 µmol/l bilirubin, triglycerides up to 12 mmol/l, hemoglobin up to 0,2 g/l and ascorbate up to 4 g/l. Significant interference: >10%.

Limit of detection

The limit of detection is 0,67 U/l

Precision

Repeatability	mean	SD	CV
n = 20	[U/l]	[U/l]	[%]
normal sample	36,2	1,06	2,92
pathological sample	156	1,32	0,84
Reproduceability	mean	SD	CV
n = 10	[U/l]	[U/l]	[%]
normal sample	35,2	1,05	2,97
pathological sample	154	1,67	1,08

Method comparison

Comparison with the non-concentrated reagent.
analyser: Advia 2400
number of samples: 133
range: 7 – 602 U/l
correlation coefficient: 0,9996
regression line equation: y = 0,967x – 0,592
(x= normal reagent, y= concentrated reagent)

For in vitro diagnostic use only!

The following symbols can be used on the labels

IVD — In vitro diagnostic device
Manufacturer
CE — CE-marking
Temperature limitations
Use by (year/month)
LOT — Batch code
REF — Catalogue number
This way up
Biological risk

Literature

Expert Panel on enzyme of the IFCC, Clin. Chim. Acta, 1976. 70:F19
Tietz Clinical Guide To Laboratory Tests, 4th edition, Elsevier, 2006