

Aspartate aminotransferase (AST/GOT)-Liquizyme (4+1) E.C.2.6.1.1.

REF: 291 000	(2 x 20 ml)	40 test
REF: 291 001	(4 x 20 ml)	80 test
REF: 291 002	(10 x 10 ml)	100 test
REF: 291 003	(9 x 20 ml)	180 test
REF: 291 004	(4 x 60 ml)	240 test
REF: 291 005	(5 x 20 ml)	100 test
REF: 291 006	(4 x 50 ml)	200 test
REF: 291 007	(5 x100 ml)	500 test
REF: 291 008	(6 x100 ml)	600 test

Intended Use

Spectrum Diagnostics liquizyme AST reagent is intended for the in-vitro quantitative, diagnostic determination of AST in human serum on both automated and manual systems.

Background

The enzyme aspartate aminotransferase (AST) is widely distributed in erythrocytes and tissues, principally heart, liver, muscle, and kidney. Elevated serum levels are found in diseases involving these tissues such as myocardial infarction, viral hepatitis and muscular dystrophy. Following myocardial infarction, serum AST is elevated and reaches a peak two days after onset. Two isoenzymes of AST have been detected, cytoplasmic and mitochondrial. Only the cytoplasmic isoenzyme occurs in normal serum, while the mitochondrial, together with the cytoplasmic isoenzyme, has been detected in the sera of patients with coronary and hepatobiliary diseases.

Method

Kinetic method according to the International Federation of Clinical Chemistry (IFCC) (3).

Assay Principle

The series of the reaction involved in the assay system is as follows:

- The amino group is enzymatically transferred by AST present in the sample from L-aspartate to the carbon atom of 2-oxoglutarate yielding oxaloacetate and L-glutamate.



- Oxaloacetate in presence of NADH and malate dehydrogenase (MDH), is reduced to L-malate. In this reaction NADH is oxidized to NAD. The reaction is monitored by measuring the rate of decrease in absorbance at 340 nm due to oxidation of NADH to NAD.



- Addition of lactate dehydrogenase (LDH) to the reagent is necessary to achieve rapid and complete reduction of endogenous pyruvate so that it does not interfere with the assay.



Reagents

Reagent 1 (R1 Buffer / Enzymes)

Tris buffer (pH 7.7)	80 mmol/L
L- Aspartate	240 mmol/L
MDH	> 450 U/L
LDH	> 1200 U/L
Sodium Hydroxide	220 mmol/L
Sodium Azide	8 mmol/L

Irritant (Xi): R36/38: Irritating to eyes and skin. S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S37/39: Wear suitable gloves and eye/face protection.

Reagent 2 (R2 Coenzyme)

NADH	> 0.18 mmol/L
2 – Oxoglutarate	18 mmol/L
Sodium Azide	8 mmol/L

SYMBOLS IN PRODUCT LABELLING

	Authorised Representative		Use by/Expiration Date
	For in-vitro diagnostic use		CAUTION. Consult instructions
	Batch Code/Lot number		for use
	Catalogue Number		Manufactured by
	Consult instructions for use		(Xi) - Irritant
	Temperature Limitation		

For further information, refer to the Aspartate aminotransferase reagent material safety data sheet.

Precautions and Warnings

Do not ingest or inhale. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.

Both reagents (R1) and (R2) contains sodium azide which may react with copper or lead plumbing.

Reagent Preparation

Prepare working solution as following:

REF:291 000 : add 4 ml from R2 to one bottle of R1; mix gently.
 REF:291 001 : add 4 ml from R2 to one bottle of R1; mix gently.
 REF:291 002 : add 2 ml from R2 to one bottle of R1; mix gently.
 REF:291 003 : add 4 ml from R2 to one bottle of R1; mix gently.
 REF:291 004 : add 12 ml from R2 to one bottle of R1; mix gently.
 REF:291 005 : add 4 ml from R2 to one bottle of R1; mix gently.
 REF:291 006 : add 10 ml from R2 to one bottle of R1; mix gently.
 REF:291 007 : add one bottle of R2 to one bottle of R1; mix gently.
 REF:291 008 : add one bottle of R2 to one bottle of R1; mix gently.

Or prepare the working solution according to the number of tests required by mixing 4 volumes of reagent 1 (R1) and 1 volume of reagent 2 (R2), e.g. 400 µl R1 +100 µl R2.

Reagent Storage and Stability

All reagents are stable until expiration date stated on label when stored refrigerated at 2 - 8 °C. Working solution is stable for 4 weeks at 2 – 8 °C or 2 days at 15 - 25 °C.

Deterioration

Do not use liquizyme AST reagent if it is turbid or if the absorbance of the working reagent is less than 1.0 at 340 nm. Failure to recover control values within the assigned range may be an indication of reagent deterioration.

Specimen Collection and Preservation

Use nonhemolyzed serum. Heparin and EDTA are the only acceptable anticoagulants. The biological half-life of AST in serum is 17 hours.

Stability: 1 day at 15 – 25 °C; 7 days at 4 - 8 °C;
12 weeks at -20 °C

System Parameters

Wavelength	340 nm (334 – 365 nm)
Optical path	1 cm
Assay type	Kinetic
Direction	decrease
Sample : Reagent Ratio	1 : 10
e.g.: Reagent volume	1 ml
Sample volume	100 µl
Temperature	37 °C or 30 °C
Equilibration time	60 seconds.
Read time	1 to 3 minutes
Zero adjustment	Against air
Reagent Blank Limits	Low 1.00 AU High 2.5 AU
Sensitivity	5 U/L
Linearity	400 U/L

Procedure

	Macro	Semi-Micro
Working solution	1.0 ml	500 µl
Specimen	100 µl	50 µl

Mix, read initial absorbance after 60 seconds. and start timer simultaneously. Read again after 1, 2 and 3 minutes. Determine the mean absorbance change per minute (ΔA/min).

Calculation

To calculate the AST/GOT activity use the following formulae:

$$\begin{aligned}U/I &= 1780 \times \Delta A 334 \text{ nm /min} \\U/I &= 1746 \times \Delta A 340 \text{ nm /min} \\U/I &= 3235 \times \Delta A 365 \text{ nm /min}\end{aligned}$$

Quality Control

Normal & abnormal control serum of known concentrations should be analyzed with each run.

Performance Characteristics

Precision

Within run (Repeatability)

	Level 1	Level 2
n	20	20
Mean (U/L)	32.6	133
SD	1.3	1.3
CV%	4.08	0.97

Run to run (Reproducibility)

	Level 1	Level 2
n	20	20
Mean (U/L)	33.1	135.5
SD	1.5	1.42
CV%	4.25	1.13

Methods Comparison

A comparison between Spectrum Diagnostics AST (4+1) reagent and a commercial reagent of the same methodology was performed on 20 human serum. A correlation of 0.991 was obtained.

Sensitivity

When run as recommended, the minimum detection limit of this assay is 5.0 U/L.

Linearity

The reaction is linear up to AST concentration of 400 U/L; specimens showing higher concentration should be diluted 1+5 with physiological saline and repeat the assay (result×6).

Interfering Substances

Serum, plasma

Hemolysis

Erythrocyte contamination elevates results, since AST activities in erythrocytes are 15 times higher than those in normal sera.

Icterus

No significant interference.

Lipemia

Lipemic specimens may cause high absorbance flagging. Diluted sample is recommended.

Anticoagulants

Citrate and fluoride inhibit the enzyme activity.

Drugs

Calcium dobesilate and doxycycline HCL cause artificially low AST values at the tested drug level.

Expected values

37 °C Females up to 31 U/I (up to 0.52 µKat/L)
Males up to 37 U/I (up to 0.62 µKat/L)

30 °C Females up to 21 U/I (up to 0.35 µKat/L)
Males up to 25 U/I (up to 0.42 µKat/L)

Temperature conversion factor is 1.37 (25 → 30 °C) and 2.04 (25 → 37 °C).

Spectrum Diagnostics does not interpret the results of a clinical laboratory procedure; interpretation of the results is considered the responsibility of qualified medical personnel. All indications of clinical significance are supported by literature references.

Analytical Range

5 – 400 U/L.

Waste Disposal

This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment. refer to special instructions/safety data sheets.

References

1. Breuer J, Report on the symposium "drug effects in clinicalchemistry methods". Eur J Clin Chem Clin Biochem. 1996;34:385-386.
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3. IFCC expert panel on enzymes part 3. J Clin Chem Clin Biochem 1986;24:481-95.
4. Henry RJ, et al. Am j Clin Path 1960 :34:381
5. Sherwin JE. Liver function. In:kaplan LA, PESCE AJ, eds.Clinical chemistry, theory, analysis, and correlation. Stlouis:mosby;1984:420-438.
6. Young DS. Effects of drugs on clinical laboratory tests.Third edition. 1990 :3:6-12.
7. Zilva JF, pannall PR : Plasma enzymes in diagnosis inclinical chemistry in diagnosis and treatment lloyd- luke london 1979:chap 17: 338.

ORDERING INFORMATION

CATALOG NO.	QUANTITY
291 000	2 x 20 ml
291 001	4 x 20 ml
291 002	10 x 10 ml
291 003	9 x 20 ml
291 004	4 x 60 ml
291 005	5 x 20 ml
291 006	4 x 50 ml
291 007	5 x 100 ml
291 008	6 x 100 ml



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