

Aspartate aminotransferase (AST/GOT)-Colorimetric

REF: 260 001 (2 x 50 ml) 100 test
REF: 260 002 (2 x 100 ml) 200 test

Intended Use

Spectrum Diagnostics colorimetric AST reagent is intended for the in-vitro quantitative, diagnostic determination of AST in human serum.

Background

The enzyme aspartate aminotransferase (AST) is widely distributed in erythrocytes and tissues, principally heart, liver, muscles, and kidneys. 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

AST – (Colorimetric method).

Assay Principle

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.



AST activity is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine.

Reagents

Reagent 1 (R1 Buffer)

Phosphate buffer 100 mmol/L
L- aspartate 100 mmol/L
2-Oxoglutarate 5 mmol/L
Sodium Hydroxide 140 mmol/L
Sodium Azide 12 mmol/L

Harmful (Xn): R20/22: Harmful by inhalation and if swallowed.
S24/25: Avoid contact with skin and eyes.

Reagent 2 (R2)

2,4-dinitrophenyl-hydrazine 2 mmol/L
HCl 8.4 %

(C)-Corrosive contains caustic materials.

R35 Causes severe burns.

R41 Risk of serious damage to eyes.

S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

S28 After contact with skin, wash immediately with plenty of soap and water.

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

Additional Reagent

Sodium hydroxide 0.4 mol/L.

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.

Reagent (R1) contains sodium azide which may react with copper or lead plumbing.

SYMBOLS IN PRODUCT LABELLING

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

Reagent preparation, Storage and Stability

The reagents are supplied ready-to-use and stable up to the expiry date labeled on the bottles when stored at 2 – 8 °C.

Deterioration

Do not use The AST regents if precipitate forms. Failure to recover control values within the assigned range may be an indication of reagent deterioration.

Specimen Collection and Preservation

Use only non haemolyzed serum. The only acceptable anticoagulants are heparin and EDTA. 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	546 nm (530-550 nm)
Optical path	1 cm
Assay type	Endpoint
Direction	Increase
Sample : Reagent Ratio	1 : 60
Temperature	37 °C and 20 – 25 °C
Zero adjustment	Reagent or Sample blank
Sensitivity	7 U/L
Linearity	89 U/L

Procedure

1. Measurement against Reagent Blank

Pipette into test tubes

	Reagent blank	Sample
R1(buffer)	0.5 ml	0.5 ml
Sample	-----	100 µl
Distilled water	100 µl	-----

Mix and incubate for exactly 30 minutes at 37 °C

R2	0.5 ml	0.5 ml
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Mix and incubate for exactly 20 minutes at 20 – 25 °C

Sodium hydroxide	5.0 ml	5.0 ml
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Mix, measure absorbance of specimen against reagent blank at 546 nm after 5 minutes.

2. Measurement against Sample Blank

	Sample blank	Sample
R1(buffer)	0.5 ml	0.5 ml
Sample	-----	100 µl

Mix and incubate for exactly 30 minutes at 37 °C

R2	0.5 ml	0.5 ml
Sample	100 µl	-----

Mix and incubate for exactly 20 minutes. at 20 – 25 °C

Sodium hydroxide	5.0 ml	5.0 ml
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Mix, measure absorbance of specimen against sample blank at 546 nm after 5 minutes.

Calculation

Obtain the AST activity from the following table

Absorbance	U/L	Absorbance	U/L
0.020	7	0.100	36
0.030	10	0.110	41
0.040	13	0.120	47
0.050	16	0.130	52
0.060	19	0.140	59
0.070	23	0.150	67
0.080	27	0.160	76
0.090	31	0.170	89

Quality Control

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

Sensitivity

If run as recommended, the minimum detection level is 7 U/L.

Linearity

The assay is linear up to 89 U/L. If the absorbance exceeds 0.170 at 546 nm (89 U/L), samples should be diluted 1 + 9 using sodium chloride and repeat the assay (result × 10).

Interfering Substances

Serum, plasma

Haemolysis

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.

Note

High concentration of aldehydes, ketones, or oxo-acids in some sera may cause false high transaminases levels. Measurement against a serum blank instead of a reagent blank avoids the risk of finding such artifacts.

Expected values

Up to 12 U/L.

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

7 – 89 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. Henry RJ et al. Am J Clin Path 1960 :34:381.
2. Reitman S and Frankel S.Am .J.Clin.Path, 1975 ;28;65.
3. Sherwin JE. Liver function. In:kaplan LA, PESCE AJ, eds.Clinical chemistry, theory,analysis, and correlation. St louis:Mosby;1984:420-438.
4. Young DS. Effects of drugs on clinical laboratory tests. Third edition. 1990 :3:6-12.

ORDERING INFORMATION	
CATALOG NO.	QUANTITY
260 001	2 x 50 ml
260 002	2 x 100 ml



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