# Aspartate aminotransferase (AST/GOT) UV-Kinetic (4+1)

REF.	Pack size	
173 02 020 173 01 050 173 10 010 173 04 050	(2 x 20 ml) 40 tests (1 x 50 ml) 50 tests (10 x 10 ml) 100 tests (4 x 50 ml) 200 tests	

#### Intended Use

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

## Introduction

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).

#### **Principle**

The series of the reactions 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.

L-Aspartate + 2-Oxoglutarate AST Dxaloacetate + L-Glutamate

 Oxaloacetate in presence of NADH and malate dehyrogenase (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.

Oxaloacetate + NADH + H<sup>+</sup> MDH L-Malate + NAD<sup>+</sup>

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.

Sample pyruvate + NADH + H<sup>+</sup> LDH L-Lactate + NAD<sup>+</sup>

## Reagents

 Reagent 1 (Buffer)

 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 (Coenzyme)

 NADH
 > 0.18 mmol/L

 2 - Oxoglutarate
 18 mmol/L

 Sodium Azide
 8 mmol/L

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

IVD

## Reagents preparation, storage and stability

#### Prepare working solution as following:

Prepare working solution by adding 4 volumes from R1 and 1 volume of R2, e.g. 400  $\mu$ l R1 + 100  $\mu$ l R2. Working solution is stable for4 weeks at 2 - 8  $^{\rm O}C$  or 2 days at 15 - 25  $^{\rm O}C$ .

All reagents are stable until expiration date stated on label when stored refrigerated at 2 - 8  $^{
m O}$ C.Once opened, the reagent is stable for 1 months at the specified temperature.

#### **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.

## **Precautions and Warnings**

Do not ingest or inhalate. 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.

#### Specimen collection and preservation

Use non-hemolyzed 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

# **Procedure**

Wavelength 340 nm Optical path 1 cm Kinetic Assay type Direction decrease Sample: Reagent Ratio 1 : 10 37 °C or 30 °C Temperature 60 seconds. Delay time 1 to 3 minutes Read time Zero adjustment Against Dist. water Low 1.00 AU High 2.5 AU Reagent Blank Limits

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

Mix, read initial absorbance after 60 seconds and start timersimultaneously. Read again after 1, 2 and 3 minutes. Determine the mean absorbance change per minute ( $\Delta A/min$ ).

## Calculation

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

 $U/L = 1746 \times \Delta A 340 \text{ nm /min}$ 

## **Quality control**

Normal and abnormal control serum of known concentration should be analyzed with each run.

#### 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).

## Interference

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

No significant interference.

#### Lipemia

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

#### **Anticoagulants**

Citrate and fluoride inhibit the enzyme activity.

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

# **Expected values**

37 °C	Females	up to 31 U/L	(up to 0.52 μKat/L)	
	Males	up to 37 U/L	(up to 0.62 μKat/L)	
	Females	up to 21 U/L	(up to 0.35 μKat/L)	
	Males	up to 25 U/L	(up to 0.42 μKat/L)	
Temperature conversion factor is $1.37(25 \longrightarrow 30^{\circ}C)$ and $2.04 (25 \longrightarrow 37^{\circ}C)$ .				

# **Performance characteristics**

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

#### 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

# **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. ECCLS. Determination of the catalytic activity concentration in serum on L- aspartate aminotransferase (EC 2.6.1.1,AST) Clin

Chem. 1989;20:204-211.
2. Henry RJ, et al. Am j Clin Path 1960 :34:381
3. Young DS. Effects of drugs on clinical laboratory tests. Third edition.

# SYMBOLS IN PRODUCT LABELLING

IVD For in-vitro diagnostic use LOT Batch Code/Lot number REF Catalogue Number Consult instructions for use ď Temperature Limitation Use by/Expiration Date CAUTION. Consult instructions for use Manufactured by



Spectrum For Diagnostics Industries - Free Zone Ismailia Free Zone Industrial Area, Block 5.

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