

# Aspartate aminotransferase (AST/GOT) - *Ultimate Single Reagent* E.C.2.6.1.1. t

REF: 261 001	(2 x 20 ml)	40	test
REF: 261 002	2 x 50 ml)	100	test
REF: 261 003	(6x 20 ml)	120	test
REF: 261 004	(4x 50 ml)	200	test
	(2 x 100 ml)	200	test
REF: 261 006	(4 x 100 ml)	400	test
REF: 261 007	(2 x 250 ml)	500	test

### Intended Use

Spectrum Diagnostics Ultimate AST reagent is intended for the invitro 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 ClinicalChemistry (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.
- L-Aspartate + 2-Oxoglutarate <u>AST</u>Oxaloacetate + 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>

# Reagent (R)

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
NADH	≥ 0.18 mmol/L
2 - Oxoglutarate	18 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. The reagent also contains additives required to maintain NADH in its reduced form.

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



### 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.

The Reagent (R) contain sodium azide which may react with copper or lead plumbing.

### **Reagent Preparation, Storage and Stability**

Spectrum Ultimate AST reagent is supplied ready-to-use and stable up to the expiry date labeled on the bottles at 2-8  $^{\circ}$ C. Once opened, the reagent is stable for 1 months at the specified temperature.

### Deterioration

Do not use Spectrum Ultimate AST reagent if it is turbid or if the absorbance of the working reagent is less than 0.9 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 <sup>o</sup>C; 7 days at 4 - 8<sup>o</sup>C; 12 weeks at -20 <sup>o</sup>C

# **System Parameters**

Wavelength Optical path Assay type Direction Sample : Reagent Ratio e.g.: Reagent volume Sample volume Temperature Equilibration time Read time Zero adjustment Reagent Blank Limits Sensitivity Linearity	340 nm (334 – 365 nm) 1 cm Kinetic decrease 1 : 10 1 ml 100 µl 37 °C or 30 °C 60 seconds. 180 seconds. Against air Low 0.9 AU High 2.5 AU 5 U/L 400 U/L
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#### Procedure

	Macro	Semi-Micro	
Reagent (R)	1.0 ml	500 μl	
Specimen	100 μl	50 μl	

Mix, read initial absorbance after 60 seconds and start timer simultaneously. Read again after 60, 120 and 180 seconds. Determine the mean absorbance change per minute ( $\Delta$ A/min).

### Calculation

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

U/I = 1780	х	∆A 334	nm /min
U/I = 1746	Х	∆A 340	nm /min
U/I = 3235	Х	∆A 365	nm /min

# **Quality Control**

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

# **Performance Characterstics**

# 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 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

### 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 <sup>0</sup> 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 <sup>o</sup>C ) and 2.04 (25 → 37 °C).



REP FC Schiffgraben 41

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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.

- **\$57:** use appropriate container to avoid environmental contamination.
- S61: avoid release in environment. refer to special instructions/safety data sheets.

### References

- Breuer J, Report on the symposium "drug effects in clinicalchemistry methods". Eur J Clin Chem Clin Biochem. 1996;34:385-386.
  ECCLS. Determination of the catalytic activity concentration in account on the catalytic activity concentration in
- serum on L- aspartate aminotransferase (EC 2.6.1.1,AST) Clin Chem. 1989;20:204-211.
- 3. IFCC expert panel on enzymes part 3. J Clin Chem Clin Biochem 1986;24:481-95.

 Henry RJ, et al. Am j Clin Path 1960 :34:381
 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 lioyd- luke london 1979:chap 17: 338.

ORDERING INFORMATION		
CATALOG NO.	QUANTITY	
261 001 261 002 261 003 261 004 261 005 261 006 261 007	2 x 20 ml 2 x 50 ml 6 x 20 ml 4 x 50 ml 2 x 100 ml 4 x 100 ml 2 x 250 ml	