AST (IFCC)

DiaSystem Scandinavia AB

with/without Pyridoxal-5-phosphate

Diagnostic reagent for quantitative in vitro determination of ASAT (GOT) in serum or plasma on photometric systems

Order Information

01 00006 70 04 0125	R1	5 x	20 mL	+	R2	1 x	25 mL
01 00006 70 04 0500	R1	5 x	80 mL	+	R2	1 x	100 mL
01 00006 70 10 0180	R1	4 x	36 mL	+	R2	4 x	9 mL
01 00006 70 02 0180	R1	4 x	36 mL	+	R2	4 x	9 mL
CDT-AST	R1	3 x	30 mL	+	R2	2 x	11,3 mL

For determination with Pyridoxal-5-phosphate activation additionally required:

Summary [1,2]

Alanine Aminotransferase (ALAT/ALT), formerly called Glutamic Pyruvic Transaminase (GPT) and Aspartate Aminotransferase (ASAT/AST), formerly called Glutamic Oxalacetic Transaminase (GOT) are the most important representatives of a group of enzymes, the aminotransferases or transaminases, which catalyze the conversion of α-keto acids into amino acids by transfer of amino groups. As a liver specific enzyme ALAT is only significantly elevated in hepatobiliary diseases. Increased ASAT levels, however, can occur in connection with damages of heart or skeletal muscle as well as of liver parenchyma. Parallel measurement of ALAT and ASAT is, therefore, applied to distinguish liver from heart or skeletal muscle damages. The ASAT/ALAT ratio is used for differential diagnosis in liver diseases. While ratios < 1 indicate mild liver damage, ratios >1 are associated with severe, often chronic liver diseases.

Method

Optimized UV-test according to IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) [modified]

Principle

L-Aspartate + 2-Oxoglutarate $\xleftarrow{ASAT}$ L-Glutamate + Oxalacetate

Oxalacetate + NADH + H^+ $\xleftarrow{MDH}$ L-Malate + NAD^+

Addition of pyridoxal-5-phosphate (P-5-P), recommended by IFCC stabilizes the activity of transaminases and avoids falsely low values in samples containing insufficient endogenous P-5-P, e.g. from patients with myocardial infarction, liver disease and intensive care patients [1,3].

Reagents

Components and Concentrations

R1:	TRIS	pH 7.65	110 mmol/L
	L-Aspartate		320 mmol/L
	MDH (malate dehydrogenase)		≥ 800 U/L
	LDH (lactate dehydrogenase)		≥ 1200 U/L
R2:	2-Oxoglutarate		85 mmol/L
	NADH		1 mmol/L
Pyridoxal-5-phosphate			
	Good's buffer	pH 9.6	100 mmol/L
	Pyridoxal-5-phosphate		13 mmol/L

Storage Instructions and Reagent Stability

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 – 8°C, protected from light and contamination is avoided. Do not freeze the reagents!

Warnings and Precautions

1. The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. Reagent 1 contains animal material. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practices.
3. In very rare cases, samples of patients with gammopathy might give falsified results [4].
4. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
5. For professional use only!

Waste Management

Please refer to local legal requirements.

Reagent Preparation

Substrate Start

The reagents are ready to use.
For the determination with Pyridoxalphosphate (P-5-P) mix 1 part of P-5-P with 100 parts of reagent 1,
e.g. 100 µL P-5-P + 10 mL R1

Stability after mixing:	6 days	at	2 - 8 °C
	24 hours	at	15 - 25 °C

Sample Start

without Pyridoxalphosphate
Mix 4 parts of R1 + 1 part of R2
(e.g. 20 mL R1 + 5 mL R2) = mono reagent

Stability:	4 weeks	at	2 – 8 °C
	5 days	at	15 – 25 °C

The mono reagent must be protected from light!

Materials required but not provided

DiaSystem Pyridoxal-5-Phosphate in case of determination with P-5-P activation (Cat.-no. 01 00120 70 04 0018)
NaCl solution 9 g/L
General laboratory equipment

Specimen

Serum or heparin plasma
Stability [3]:

4 days	at	20 – 25 °C
7 days	at	4 – 8 °C
3 months	at	-20 °C

Discard contaminated specimens. Only freeze once!

Assay Procedure

Application sheets for automated systems are available on request.

Wavelength	340 nm, Hg 365 nm, Hg 334 nm
Optical path	1 cm
Temperature	37 °C
Measurement	Against air

Substrate Start

Sample/Calibrator	100 µL
Reagent 1	1000 µL
Mix, incubate for 5 min., then add:	
Reagent 2	250 µL
Mix, read absorbance after 1 min and start stopwatch. Read absorbance again 1, 2 and 3 min thereafter.	

Sample Start

Don't use sample start with Pyridoxal-5-phosphate!

Sample/Calibrator	100 µL
Mono reagent	1000 µL
Mix, read absorbance after 1 min and start stopwatch. Read absorbance again 1, 2 and 3 min thereafter.	

Calculation

With factor

From absorbance readings calculate ΔA/min and multiply by the corresponding factor from table below:

ΔA/min x factor = ASAT activity [U/L]

Substrate Start	
340 nm	2143
334 nm	2184
365 nm	3971
Sample Start	
340 nm	1745
334 nm	1780

With calibrator

$$\text{ASAT [U/L]} = \frac{\Delta A/\text{min Sample}}{\Delta A/\text{min Calibrator}} \text{ x Conc. Calibrator [U/L]}$$

Conversion factor

ASAT [U/L] x 0.0167 = ASAT [µkat/L]

Calibrators and Controls

For the calibration of automated photometric systems, DiaSystem UniCal CC calibrator is recommended. This method has been standardized against the original IFCC formulation. For internal quality control, DiaSystem UniLab N and DiaSystem UniLab controls should be assayed. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat. No.	Kit size
UniCal CC	06 00122 70 04 0018	6 x 3 mL
UniLab N	07 00123 70 05 0030	6 x 5 mL
UniLab P	07 00124 70 05 0030	6 x 5 mL

Performance Characteristics

Measuring range

On automated systems the test is suitable for the determination of ASAT activities up to 700 U/L. In case of a manual procedure, the test is suitable for ASAT activities which correspond to a maximum of ΔA/min of 0.16 at 340 and 334 nm or 0.08 at 365 nm. If such values are exceeded the samples should be diluted 1 + 9 with NaCl solution (9 g/L) and results multiplied by 10.

Specificity/Interferences

No interference was observed by ascorbic acid up to 30 mg/dL, bilirubin up to 40 mg/dL and lipemia up to 2000 mg/dL triglycerides. The presence of hemoglobin in serum indicates destruction of erythrocytes with release of ASAT, thus producing high interference. For further information on interfering substances refer to Young DS [8].

Sensitivity/Limit of Detection

The lower limit of detection is 2 U/L.

Precision

Without Pyridoxal-5-phosphate

Intra-assay precision n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	25.10	0.82	3.25
Sample 2	51.30	1.57	3.06
Sample 3	116.00	0.90	0.77

Inter-assay precision n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	25.70	1.13	4.40
Sample 2	48.60	0.67	1.38
Sample 3	115.00	0.80	0.69

With Pyridoxalphosphate

Intra-assay precision n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	43.6	1.10	2.51
Sample 2	74.5	1.79	2.41
Sample 3	174	3.18	1.83

Inter-assay precision n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	44.0	1.59	3.61
Sample 2	77.0	3.05	3.97
Sample 3	187	3.37	1.80

Method Comparison

With Pyridoxal-5-phosphate

A comparison of DiaSystem AST (IFCC) with Pyridoxal-5-phosphate (y) with the IFCC reference reagent (x) using 51 samples gave following results: y = 1.000 x – 0.800 U/L; r = 0.999.

A comparison of DiaSystem AST (IFCC) (y) with Pyridoxal-5-phosphate and a commercially available test (x) using 51 samples gave following results: y = 0.970 x + 0.350 U/L; r= 0.999.

Without Pyridoxal-5-phosphate

A comparison of DiaSystem AST (IFCC) without Pyridoxal-5-phosphate (y) and a commercially available test (x) using 51 samples gave following results:
y = 0.997 x + 0.621 U/L; r= 1.000.

Reference Range

With pyridoxal-5-phosphate activation

Women [4]		< 31 U/L	< 0.52 µkat/L
Men [4]		< 35 U/L	< 0.58 µkat/L
Children [1]	1 – 3 years	< 50 U/L	< 0.83 µkat/L
	4 – 6 years	< 45 U/L	< 0.75 µkat/L
	7 – 9 years	< 40 U/L	< 0.67 µkat/L
	10 – 12 years	< 40 U/L	< 0.67 µkat/L
	13 – 15 years	< 35 U/L	< 0.58 µkat/L
	16 – 18 years	< 35 U/L	< 0.58 µkat/L

Without pyridoxal-5-phosphate activation

Women [8,9]	< 31 U/L	< 0.52 µkat/L
Men [8,9]	< 35 U/L	< 0.58 µkat/L

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Literature

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