

ASAT (GOT) FS* (IFCC mod.)

with/without pyridoxal-5-phosphate

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

Order Information

Cat. No.	Kit size					
1 2601 99 10 021	R1	5 x	20 mL	+	R2	1 x 25 mL
1 2601 99 10 026	R1	5 x	80 mL	+	R2	1 x 100 mL
1 2601 99 10 023	R1	1 x	800 mL	+	R2	1 x 200 mL
1 2601 99 10 704	R1	8 x	50 mL	+	R2	8 x 12.5 mL
1 2601 99 10 917	R1	8 x	60 mL	+	R2	8 x 15 mL
1 2601 99 90 314	R1	10 x	20 mL	+	R2	2 x 30 mL
For determination with pyridoxal-5-phosphate activation additionally required:						
2 5010 99 10 030		6 x				3 mL

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 $\xrightarrow{\text{ASAT}}$ L-Glutamate + Oxalacetate

Oxalacetate + NADH + H⁺ $\xrightarrow{\text{MDH}}$ 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 FS			
	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

- The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
- Reagent 1 contains animal material. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practices.
- In very rare cases, samples of patients with gammopathy might give falsified results [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.
- 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 pyridoxal-5-phosphate mix 1 part of P-5-P with 100 parts of reagent 1,

e.g. 100 μ L P-5-P + 10 mL R1

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

Sample Start

without pyridoxal-5-phosphate

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

DiaSys Pyridoxal-5-Phosphate FS in case of determination with P-5-P activation (Cat.-No. 2 5010 99 10 030)

NaCl solution 9 g/L

General laboratory equipment

Specimen

Serum or heparin plasma

Stability [5]:

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 μ L
Reagent 1	1000 μ L
Mix, incubate for 5 min., then add:	
Reagent 2	250 μ L
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 μ L
Mono reagent	1000 μ L
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 $\Delta A/\text{min}$ and multiply by the corresponding factor from table below:

$\Delta A/\text{min} \times \text{factor} = \text{ASAT activity [U/L]}$

Substrate Start

340 nm	2143
334 nm	2184
365 nm	3971

Sample Start

340 nm	1745
334 nm	1780
365 nm	3235

With calibrator

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

Conversion factor

ASAT [U/L] x 0.0167 = ASAT [µkat/L]

Calibrators and Controls

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

	Cat. No.	Kit size
TruCal U	5 9100 99 10 063	20 x 3 mL
	5 9100 99 10 064	6 x 3 mL
TruLab N	5 9000 99 10 062	20 x 5 mL
	5 9000 99 10 061	6 x 5 mL
TruLab P	5 9050 99 10 062	20 x 5 mL
	5 9050 99 10 061	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 $\Delta A/\text{min}$ 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 [6].

Sensitivity/Limit of Detection

The lower limit of detection is 2 U/L.

Precision**Without P-5-P**

Intra-assay precision n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	25.1	0.82	3.25
Sample 2	51.3	1.57	3.06
Sample 3	116	0.90	0.77

Inter-assay precision n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	25.7	1.13	4.40
Sample 2	48.6	0.67	1.38
Sample 3	115	0.80	0.69

With P-5-P

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 P-5-P**

A comparison of DiaSys ASAT (GOT) FS with P-5-P (y) with the IFCC reference reagent (x) using 51 samples gave following results:
 $y = 1.000 x - 0.800 \text{ U/L}$; $r = 0.999$

A comparison of DiaSys ASAT (GOT) FS (y) with P-5-P and a commercially available test (x) using 51 samples gave following results:
 $y = 0.970 x + 0.350 \text{ U/L}$; $r = 0.999$

Without P-5-P

A comparison of DiaSys ASAT (GOT) FS without P-5-P (y) and a commercially available test (x) using 51 samples gave following results:
 $y = 0.997 x + 0.621 \text{ U/L}$; $r = 1.000$

Reference Range**With pyridoxal-5-phosphate activation**

Women [7]		< 31 U/L	< 0.52 µkat/L
Men [7]		< 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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**Manufacturer**

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