Instructions for Use
For *in vitro* diagnostic use

TGO (IFCC)

ASAT (GOT) (IFCC)

Anvisa 80115310047

ORDER INFORMATION

Cat. No.	Kit size
2040075K	R1: 3 x 20 mL + R2: 1 x 15 mL
2040250K	R1: 5 x 40 mL + R2: 1 x 50 mL
2040075M	R1: 3 x 20 mL + R2: 1 x 15 mL
2040179.2R	R1: 4 x 34,5 mL + R2: 4 x 10,3 mL
2040050MK	R1: 1 x 40 mL + R2: 1 x 10 mL

INTENDED USE
Diagnostic reagent for quantitative in vitro determination of ASAT (GOT) in human serum or heparin plasma on automated photometric systems.

SUMMARY
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. [1,2]

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

PRINCIPLE
L-Aspartate + 2-Oxoglutarate ⟷ (ASAT) ⟷ L-Glutamate + Oxalacetate

Oxalacetate + NADH + H^+ ⟷ (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		< 500 mmol/L
	MDH (malate dehydrogenase)		< 2 KU/L
	LDH (lactate dehydrogenase)		< 5 KU/L
R2	2-Oxoglutarate		< 100 mmol/L
	NADH		1,09 mmol/L

STORAGE AND STABILITY
Reagents are stable up to the date of expiry indicated on the kit, if stored at 2 – 8°C and contamination is avoided. Do not freeze and protect from light.

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 and biological material. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practice.
3. Reagent 2 contains biological material. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practice.
4. In very rare cases, samples of patients with gammopathy might give falsified results [4].
5. 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.
6. For professional use only.

WASTE MANAGEMENT
Follow the requirements of the current guidelines about technical regulation for the management of healthcare service waste, as well as other equivalent biosafety practices.

REAGENT PREPARATION

Starting with Substrate

The reagents are ready to use.
For the determination with pyridoxal-5-phosphate (P-5-P), mix 1 part of P-5-P with 100 parts of Reagent 1 (R1)
(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

Starting with Sample

Without pyridoxal-5-phosphate (P-5-P)
Mix 4 parts of R1 with 1 part of R2
(e.g. 20 mL R1 + 5 mL R2) = monoreagent

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

Protect the monoreagent from light!

MATERIALS REQUIRED, BUT NOT PROVIDED
1. NaCl solution 9 g/L.
2. General laboratory equipment.
3. Pyridoxal-5-phosphate solution for determination with P-5-P: Good's Buffer pH 9.6 (100 mmol/L) + Pyridoxal-5-Phosphate (13 mmol/L).

SPECIMEN
Human serum or heparin plasma.

Stability [5]:

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

Only freeze once. Discard contaminated specimens.

ASSAY PROCEDURE
Applications for automatic systems are available upon request or on our website: www.kovalent.com.br

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

Starting with Substrate

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

Starting with Sample
Do not use Starting with Sample with pyridoxal-5-phospate (P-5-P)!

Sample or calibrator	100 µL
Monoreagent	1000 µL
Mix, read the absorbance after 1 min and start the stopwatch. Read the absorbance again after 1, 2 and 3 min.	

CALCULATION

With factor

From the absorbance readings, calculate the ΔA/min and multiply by the corresponding factor from the table below:
ΔA/min x factor = ASAT Activity [U/L]

	Starting with Substrate	Starting with Sample
340nm	2143	1745
334nm	2184	1780
365nm	3971	3235

With calibrator

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

Conversion factor

ASAT [U/L] x 0.0167 = ASAT [µkat/L]

CALIBRATORS AND CONTROLS
For calibration in automated photometric systems, Kovalent Topkal U calibrator is recommended. This method has been standardized against the original IFCC formulation. Use Kovalent Topkon N and P for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

WARRANTY
These instructions for use should be read carefully before using the product and the information contained therein should be strictly adhered to. The reliability of the test results cannot be guaranteed if the instructions are not followed.

PERFORMANCE CHARACTERISTICS

Measuring range
In automated systems the test is suitable for the determination of ASAT activities within a range of 1.2 - 600 U/L.
In the case of manual procedure, the test is suitable for ASAT activities, which correspond to a maximum ΔA/min of 0.16 at 340 and 334 nm or 0.08 at 365nm. If these values are exceeded, the samples should be diluted 1 + 9 with NaCl solution (9 g/L) and the results multiplied by 10.

Specificity / Interferences
No interference was observed by ascorbic acid up to 30 mg/dL, bilirubin (conjugated and unconjugated) up to 60 mg/dL, hemoglobin up to 100 mg/dL, and lipemia up to 200 mg/dL of triglycerides. For more information on interfering substances, see Young DS [6,7].

Sensitivity / Limit of Detection
The lowest detection limit is 1.2 U/L.

Precision

Within run n = 10	Mean [U/L]	SD [U/L]	CV [%]
Normal Control	40.6 37.7	1.09 0.64	2.69 1.70
Pathological Control	155.2 156.0	1.15 1.02	0.74 0.65

Between day n = 15	Mean [U/L]	SD [U/L]	CV [%]
Normal Control	41.51 38.57	1.18 0.98	2.85 2.55
Pathological Control	157.3 155.9	2.09 2.95	1.33 1.89

Method comparison
Method comparison between Kovalent TGO (y) and a commercial test of the same methodology (x) using 30 samples demonstrated the following results:
y = 0.9601x + 0.3596; R2 = 0.9982

REFERENCE VALUES

With P-5-P activation

		[U/L]	[µkat/L]
Women [8]		<31	<0.52
Men [8]		<35	<0.58
Children [1]	1 - 3 years	<50	<0.83
	4 - 6 years	<45	<0.75
	7 - 9 years	<40	<0.67
	10 - 12 years	<40	<0.67
	13 - 15 years	<35	<0.58
	16 - 18 years	<35	<0.58

Without P-5-P activation

	[U/L]	[µkat/L]
Women [9,10]	<31	<0.52
Men [9,10]	<35	<0.58

Each laboratory should verify that reference values can be used in its own patient population and determine its own reference values, if necessary.

LITERATURE

1. Thomas L. Alanine aminotransferase (ALT), Aspartate aminotransferase (AST). In: Thomas L, editor. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998. p. 55-65.
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3. Bergmeyer HU, Horder M, Rej R. Approved Recommendation (1985) on IFCC Methods for the Measurement of Catalytic Concentration of Enzymes. L. Clin. Chem. Clin. Biochem. 1986; 24: 497-510.
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8. Schumann G, Bonora R, Ceriotti F, Férard G et al. IFCC primary reference procedure for the measurement of catalytic activity concentrations of enzymes at 37 °C. Part 5: Reference procedure for the measurement of catalytic concentration of aspartate aminotransferase. Clin Chem Lab Med 2002;40:725-33.
9. Lorentz K, Rohle G, Siekmann L. Einfuhrung der neuen Standardmethoden 1994 zur Bestimmung der katalytischen Enzymkonzentrationen bei 37o.C DG Klinische Chemie Mitteilungen 26; 1995; Heft 4.
10. Zawta B, Klein G, Bablok W.. Temperature Conversion in Clinical Enzymology? Klin. Lab. 1994; 33-42.

CONSUMER INFORMATION

Symbols used:

	Manufacturer
	Temperature limit
	In vitro diagnostic device
	Caution
	Operating instructions
	Recycling material
	Do not discard directly into the environment
	Batch code
	Date of manufacture
	Use by date
	Biological hazards
	Highly toxic
	Corrosive
	Harmful

Manufacturer:
Kovalent do Brasil Ltda.
Rua Cristóvão Sardinha, 110 – Jd. Bom Retiro
São Gonçalo – RJ – CEP 24722-414 – Brasil
www.kovalent.com.br
CNPJ: 04.842.199/0001-56

Kit sizes variations on demand:

Anvisa No.	Kit size
80115310047	R1: 2 x 50 mL + R2: 2 x 12,5 mL
80115310047	R1: 3 x 26,67 mL + R2: 1 x 20 mL
80115310047	R1: 2 x 40 mL + R2: 2 x 10 mL
80115310047	R1: 3 x 40 mL + R2: 3 x 10 mL
80115310047	R1: 4 x 40 mL + R2: 4 x 10 mL

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Expiration date and Lot no.: See label