

TGP (IFCC)

ALAT (GTP)

Anvisa 80115310051

ORDER INFORMATION

Cat. No.	Kit Size
2050075K	R1: 3 x 20 mL + R2: 1 x 15 mL
2050250K	R1: 5 x 40 mL + R2: 1 x 50 mL
2050075M	R1: 3 x 20 mL + R2: 1 x 15 mL
2050179.2R	R1: 4 x 34,5 mL + R2: 4 x 10,3 mL
2050050MK	R1: 1 x 40 mL + R2: 1 x 10 mL

INTENDED USE

Diagnostic reagent for quantitative *in vitro* determination of ALAT (GPT) in serum or plasma on 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-Alanine + 2-Oxoglutarate $\xrightarrow{\text{ALAT}}$ L-Glutamate + Pyruvate

Pyruvate + NADH + H⁺ $\xrightarrow{\text{LDH}}$ D-Lactate + 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 7,15	137,5 mmol/L
	L-Alanine		<1 mol/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, protected from light and if contamination is avoided. Do not freeze!

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 and biological material. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practice.
- Reagent 2 contains biological material. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practice.
- In very rare cases, samples of patients with gammopathy might give falsified results [4].
- Sulfasalazine and sulfapyridine medication may cause false results in patient samples. Blood collection must be performed prior to drug administration.

- 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

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 μ L P-5-P + 10 mL R1)

Stability after	6 days	at	2 - 8 °C
mixing:	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

- NaCl solution 9 g/L.
- General laboratory equipment.
- 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

Serum or heparin plasma.

Stability [5]:	3 days	a	20 - 25 °C
	7 days	a	4 - 8 °C
	7 days	a	-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 μ L
Reagent 1	1000 μ L
Mix, incubate for 5 min, then add:	
Reagent 2	250 μ L
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-phosphate (P-5-P)!

Sample or calibrator	100 μ L
Monoreagent	1000 μ L
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 $\Delta A/\text{min}$ and multiply by the corresponding factor from the table below:

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

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

Instructions for Use

For *in vitro* diagnostic use



With calibrator

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

Conversion factor

$$\text{ALAT [U/L]} \times 0,0167 = \text{ALAT [\mu\text{kat/L}]}$$

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 ALAT activities within a range of 6 - 1000 U/L.

In the case of manual procedure, the test is suitable for ALAT activities, which correspond to a maximum $\Delta A/\text{min}$ of 0.16 at 340 and 334 nm or 0.08 at 365 nm. 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 (ALAT = 40 U/L) and up to 60 mg/dL (ALAT = 84.8 U/L), conjugated bilirubin up to 60 mg/dL (ALAT = 40 U/L) and up to 60 mg/dL (ALAT = 97.1 U/L), unconjugated bilirubin up to 55 mg/dL (ALAT = 40 U/L) and up to 60 mg/dL (ALAT = 81.3 U/L), hemoglobin up to 500 mg/dL (ALAT = 40 U/L) and up to 1000 mg/dL (ALAT = 98.6 U/L), and lipemia up to 400 mg/dL of triglycerides (ALAT = 40 U/L) and up to 1000 mg/dL (ALAT = 76.3 U/L). For more information on interfering substances, see Young DS [6,7].

Sensitivity / Limit of Detection

The lowest detection limit is 6 U/L.

Precision

Within run n = 10	Mean [U/L]	SD [U/L]	CV [%]
Normal Control	53.2 51.9	1.01 0.78	1.90 1.50
Pathological Control	118.7 127.7	0.63 0.88	0.61 0.69

Between Day n = 15	Mean [U/L]	SD [U/L]	CV [%]
Normal Control	51.9 50.5	0.96 0.88	1.84 1.75
Pathological Control	118.0 123.0	1.31 1.02	1.11 0.83

Method Comparison

Method comparison between Kovalent TGP (y) and a commercial test of the same methodology (x) using 30 samples demonstrated the following results:
 $y = 0.984x + 0.0819$; $R^2 = 0.9985$

REFERENCE VALUES

With P-5-P activation		[U/L]	[μkat/L]
Women [8]		< 34	< 0.57
Homens [8]		< 45	< 0.75
Children [1]	1 - 30 days	< 25	< 0.42
	2 - 12 months	< 35	< 0.58
	1 - 3 years	< 30	< 0.50
	4 - 6 years	< 25	< 0.42
	7 - 9 years	< 25	< 0.42
	10 - 18 years	< 30	< 0.50
Without P-5-P activation		[U/L]	[μkat/L]
Women [9,10]		< 31	< 0.52
Men [9,10]		< 41	< 0.68

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.
2. Moss DW, Henderson AR. Clinical enzymology. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999. p. 617-721.
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: 481-495.
4. Bakker AJ, Mucke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007; 45(9):1240-1243.
5. Guber WG, Zawta B et al. The Quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001; p. 14-5.
6. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
7. Young DS. Effects on Clinical Laboratory Tests - Drugs Disease, Herbs & Natural Products, <https://clinf.wiley.com/aaccweb/aacc/>, accessed in September 2021. Published by AACC Press and John Wiley and Sons, Inc.
8. Schumann G, Bonora R, Ceriotti F, Féraud 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. Einführung der neuen Standardmethoden 1994 zur Bestimmung der katalytischen Enzymkonzentrationen bei 37°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

Instructions for Use
For *in vitro* diagnostic use



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
80115310051	R1: 2 x 50 mL + R2: 2 x 12,5 mL
80115310051	R1: 3 x 26,67 mL + R2: 1 x 20 mL
80115310051	R1: 2 x 40 mL + R2: 2 x 10 mL
80115310051	R1: 3 x 40 mL + R2: 3 x 10 mL
80115310051	R1: 4 x 40 mL + R2: 4 x 10 mL

SAC: sac@kovalent.com.br - (21) 3907-2534 / 0800 015 1414

Expiration date and Lot no.: See label