FOSFATASE ALCALINA (IFCC)

ALKALINE PHOSPHATASE (IFCC)

Anvisa 80115310054

ORDER INFORMATION

Cat. No.	Kit Size
2030075K	R1: 3 x 20 mL + R2: 1 x 15 mL
2030250K	R1: 5 x 40 mL + R2: 1 x 50 mL
2030075M	R1: 3 x 20 mL + R2: 1 x 15 mL
2030179.2R	R1: 4 x 34,5 mL + R2: 4 x 10,3 mL
2030050MK	R1: 1 x 40 mL + R2: 1 x 10 mL

INTENDED USE

Diagnostic reagent for quantitative *in vitro* determination of Alkaline phosphatase in serum or plasma on photometric systems.

SUMMARY

Alkaline phosphatase (AP) is a cell membrane bound enzyme, expressed by all tissues [1]. AP, with its cofactors zinc and magnesium, catalyzes hydrolysis of organic phosphate esters in the extracellular space [2]. AP exists in blood in numerous distinct forms which originate mainly from bone and liver, but also from other tissues like kidney, placenta, testes, thymus, lung and tumors. An increase in AP activity can be physiologically induced, e.g. during the 2nd trimester of pregnancy and in childhood during growth. Pathologic conditions, that lead to increased AP activities, are hepatobiliary diseases, diseases of skeletal system, malignant tumors and systemic diseases without primary liver and bone involvement. Decreased AP activities in serum are very rare and are found e.g. in hereditary hypophosphemia, Wilson's disease and in corticoid induced osteoporosis [1].

METHOD

Kinetic photometric test, according to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) [modified] [3].

PRINCIPLE

$$\text{p-Nitrophenylphosphate} + H_2O \xrightarrow{AP} \text{Phosphate} + \text{p-Nitrophenol}$$

One unit of alkaline phosphatase is the amount of enzyme that will convert 1.0 µmol of p-nitrophenylphosphate in presence of H_2O to phosphate and p-nitrophenol per minute at the enzyme specific conditions.

REAGENTS

Components and Concentrations

R1	2-Amino-2-methyl-1-propanol	< 5 mol/L
	Magnesium acetate	2 mmol/L
	Zinc sulphate	0.5 mmol/L
	HEDTA	2.5 mmol/L
R2	p-Nitrophenylphosphate	80 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 the reagents!
The stability of the opened reagent vial is 12 months, as long as it does not exceed the expiration date.

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. During the reaction, p-nitrophenol is produced which is poisonous when inhaled, swallowed or absorbed through skin. If the reaction mixture comes in contact with skin or mucous membranes wash copiously with water!
3. In very rare cases, samples of patients with gammopathy might give falsified results [4].
4. In case of product malfunction or altered appearance that could affect the performance, contact the manufacturer.
5. Any serious incident related to the product must be reported to the manufacturer and the competent authority where the user and/or patient is located.
6. Please refer to the safety data sheets (SDS) 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.
7. 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.

Starting with Sample

Mix 4 parts of R1 with 1 part of R2
(e.g. 20 mL R1 + 5 mL R2) = monoreagent

Stability:	5 days	at	15 – 25 ºC
	4 weeks	at	2 - 8 ºC

Protect the monoreagent from light!

MATERIALS REQUIRED BUT NOT PROVIDED

1. NaCl solution 9 g/L.
2. General laboratory equipment.

SPECIMEN

Human serum or heparin plasma.
Do not use hemolytic samples!
Only use suitable tubes or collection containers for specimen collection and preparation.
When using primary tubes, follow the manufacturer's instructions.

Stability [5]:	7 days	at	20 - 25 ºC
	7 days	at	4 - 8 ºC
	2 months	at	-20 ºC

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	Hg 405 nm (400 - 420 nm)
Optical path	1 cm
Temperature	37 ºC
Measurement	Against reagent blank

Starting with Substrate

	Blank	**Sample or calibrator**
Sample or calibrator	**-**	20 µL
Distilled water	20 µL	**-**
Reagent 1	1000 µL	1000 µL
Mix, incubate for approximately 1 min, then add:		
Reagent 2	250 µL	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

	Blank	**Sample or calibrator**
Sample or calibrator	**-**	20 µL
Distilled water	20 µL	**-**
Monoreagent	1000 µL	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 = Activity AP [U/L]

Starting with Substrate	405 nm	3433
Starting with Sample	405 nm	2757

With calibrator

$$AP\ [U/L] = \frac{\Delta A/min_{Sample}}{\Delta A/min_{Cal.}} \times Conc.\ Cal.\ [U/L]$$

Conversion factor

AP [U/L] x 0.0167 = AP [µkat/L]

CALIBRATORS ANS CONTROLS

For calibration in automated photometric systems, Kovalent Topkal U calibrator is recommended. 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 AP activities within a range of 2 - 1400 U/L.
In the case of manual procedure, the test is suitable for detect AP activity, which correspond to a maximum ΔA/min of 0.25.
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 up to 60 mg/dL, bilirubin unconjugated up to 25 mg/dL, hemoglobin up to 100 mg/dL, and lipemia up to 2000 mg/dL of triglycerides. For more information on interfering substances, see the literature [6-8].

Sensitivity / Limit of Detection

The lowest detection limit is 2 U/L.

Precision

Within run n = 10	Mean [U/L]	SD [U/L]	CV [%]
Normal Control	70.1 68.1	0.70 0.56	1.00 0.82
Pathological Control	180.5 155.3	0.87 0.94	0.48 0.60

Between day n = 15	Mean [U/L]	SD [U/L]	CV [%]
Normal Control	68.54 69.16	0.95 1.22	1.38 1.76
Pathological Control	177.0 153.5	1.91 1.02	1.08 0.67

Method comparison

Method comparison between Kovalent Fosfatase Alcalina (y) and a commercial test available (x) using 30 samples demonstrated the following results: y = 0.993x + 0.3028; R^2 = 0.9984.

REFERENCE VALUES [1]

	Female		**Male**	
Children	**[U/L]**	**[µkat/L]**	**[U/L]**	**[µkat/L]**
0 – 1 year	89 – 370	1.49 – 6.3	89 – 370	1.49 – 6.3
1 – 3 years	91 – 334	1.52 – 5.6	91 – 334	1.52 – 5.6
4 – 6 years	97 – 316	1.61 – 5.3	97 – 316	1.61 – 5.3
7 – 11 years	120 – 340	2.00 – 5.7	110 – 316	1.83 – 5.3
13 – 17 years	49 – 328	0.82 – 5.5	75 – 363	1.25 – 6.1
Adults	33 – 98	0.55 – 1.64	43 – 115	0.72 – 1.92

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

LITERATURE

1. Thomas L. Clinical Laboratory Diagnostics [Internet]. Prof. Lothar Thomas; 2024 [cited 2024 Jun 10]. Available from: https://www.clinical-laboratory-diagnostics.com/
2. Lowe D, Sanvictores T, Zubair M, et al. Alkaline Phosphatase. [Updated 2023 Oct 29]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. [cited 2023 Dec 29]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK459201/
3. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37°C. Part 9: Reference procedure for the measurement of catalytic concentration of alkaline phosphatase; Clin Chem Lab Med 2011;49(9).
4. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 2007;45(9):1240-1243.
5. Guder WG, da Fonseca-Wollheim F, Heil W, Schmitt Y, Töpfer G, Wisser H, Zawta B. Quality of Diagnostic Samples. 3rd edition; 2010. p. 32-3.
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 [Internet]. AACC Press and John Wiley and Sons, Inc; 2020 [cited 2024 June]. Available from: https://clinfx.wiley.com/ aaccweb/aacc/
8. Sonntag O, Scholer A. Drug interference in clinical chemistry: recommendation of drugs and their concentrations to be used in drug interference studies. Ann Clin Biochem. 2001 Jul;38:376-85.

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.
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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
80115310054	R1: 2 x 50 mL + R2: 2 x 12,5 mL
80115310054	R1: 2 x 40 mL + R2: 2 x 10 mL
80115310054	R1: 3 x 40 mL + R2: 3 x 10 mL
80115310054	R1: 4 x 40 mL + R2: 4 x 10 mL

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Expiration date and Lot no.: See label