ALP2

cobas®

Alkaline phosphatase acc. to IFCC Gen.2

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08056757190	Alkaline Phosphatase acc. to IFCC Gen.2 (1100 tests)	System-ID 2011 001	**cobas c** 303, **cobas c** 503
10759350360	Calibrator f.a.s. (12 x 3 mL)	Code 20401	
05947626160	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 20391	
05947774160	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 20392	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

English

For use in the USA only

System information

ALP2: ACN 20110

Intended use

In vitro test for the quantitative determination of alkaline phosphatase in human serum and plasma on Roche/Hitachi **cobas c** systems.

Summary[1,2,3,4,5,6]

Alkaline phosphatase in serum consists of four structural genotypes: the liver-bone-kidney type, the intestinal type, the placental type and the variant from the germ cells. It occurs in osteoblasts, hepatocytes, leukocytes, the kidneys, spleen, placenta, prostate and the small intestine. The liver-bone-kidney type is particularly important.

A rise in the alkaline phosphatase occurs with all forms of cholestasis, particularly with obstructive jaundice. It is also elevated in diseases of the skeletal system, such as Paget's disease, hyperparathyroidism, rickets and osteomalacia, as well as with fractures and malignant tumors. A considerable rise in the alkaline phosphatase activity is sometimes seen in children and juveniles. It is caused by increased osteoblast activity following accelerated bone growth.

The assay method was first described by King and Armstrong, modified by Ohmori, Bessey, Lowry and Brock and later improved by Hausamen et al. In 2011 the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Scientific Division, Committee on Reference Systems of Enzymes (C-RSE) recommended a reference procedure for the determination of alkaline phosphatase using an optimized substrate concentration and 2-amino-2-methyl-1-propanol as buffer plus the cations magnesium and zinc at 37 °C. This assay follows the recommendations of the IFCC, but was optimized for performance and stability.

Test principle

Colorimetric assay in accordance with a standardized method.
In the presence of magnesium and zinc ions, p-nitrophenyl phosphate is cleaved by phosphatases into phosphate and p-nitrophenol.

$$\text{p-nitrophenyl phosphate} + H_2O \xrightarrow{\text{ALP}} \text{phosphate} + \text{p-nitrophenol}$$

The p-nitrophenol released is directly proportional to the catalytic ALP activity. It is determined by measuring the increase in absorbance.

Reagents - working solutions

R1 2-amino-2-methyl-1-propanol: 1.724 mol/L, pH 10.44 (30 °C); magnesium acetate: 3.83 mmol/L; zinc sulfate: 0.766 mmol/L; N-(2-hydroxyethyl)-ethylenediamine triacetic acid: 3.83 mmol/L

R3 p-nitrophenyl phosphate: 132.8 mmol/L, pH 8.50 (25 °C); preservatives

R1 is in position B and R3 is in position C.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H315 Causes skin irritation.

H319 Causes serious eye irritation.

Prevention:

P264 Wash skin thoroughly after handling.

P280 Wear protective gloves/ eye protection/ face protection.

Response:

P302 + P352 IF ON SKIN: Wash with plenty of water.

P332 + P313 If skin irritation occurs: Get medical advice/attention.

P337 + P313 If eye irritation persists: Get medical advice/attention.

P362 + P364 Take off contaminated clothing and wash it before reuse.

Product safety labeling follows EU GHS guidance.
Contact phone: 1-800-428-2336

Reagent handling

Ready for use

Storage and stability

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	8 weeks

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.
Serum
Plasma: Li-heparin plasma

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

See the limitations and interferences section for details about possible sample interferences.

Stability:[7]	7 days at 20-25 °C
	7 days at 4-8 °C
	2 months at –20 °C

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the

responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

See "Order information" section

General laboratory equipment

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

Test definition

Reporting time	10 min		
Wavelength (sub/main)	480/450 nm		
Reagent pipetting		Diluent (H_2O)	
R1	56 µL	19 µL	
R3	13 µL	16 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.1 µL	–	–
Decreased	2.1 µL	20 µL	80 µL
Increased	2.1 µL	–	–

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

Calibration

Calibrators	S1: H_2O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	Full calibration - after reagent lot change - as required following quality control procedures

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against the IFCC procedure (2011).[6]

Quality control

For quality control, use control materials as listed in the "Order information" section.

In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. It is recommended to perform quality control always after lot calibration and subsequently at least every 8 weeks. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

cobas c systems automatically calculate the analyte activity of each sample in the unit U/L (µkat/L).

Conversion factor: U/L x 0.0167 = µkat/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial value at an alkaline phosphatase activity of 100 U/L.

Icterus:[8] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[8] No significant interference up to an H index of 200 (approximate hemoglobin concentration: 124 µmol/L or 200 mg/dL).

Lipemia (Intralipid):[8] No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[9,10]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[11]

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions refer to the operator's manual.

Limits and ranges

Measuring range

5-1200 U/L (0.084-20.0 µkat/L)

Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:5 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 5.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 5 U/L (0.084 µkat/L)
Limit of Detection	= 5 U/L (0.084 µkat/L)
Limit of Quantitation	= 5 U/L (0.084 µkat/L)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the activity below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low activity samples.

The Limit of Detection corresponds to the lowest analyte activity which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte activity that can be reproducibly measured with a total error of 20 %. It has been determined using low activity alkaline phosphatase samples.

Expected values

U/L

Adults[12]

Males (n = 221)	40-129 U/L
Females (n = 229)	35-104 U/L

Children[13]

Males

Age	0 – 14 days	83-248 U/L
	15 days – < 1 year	122-469 U/L
	1 – < 10 years	142-335 U/L

ALP2

Alkaline phosphatase acc. to IFCC Gen.2

	10 – < 13 years	129-417 U/L
	13 – < 15 years	116-468 U/L
	15 – < 17 years	82-331 U/L
	17 – < 19 years	55-149 U/L
Females		
Age	0 – 14 days	83-248 U/L
	15 days – < 1 year	122-469 U/L
	1 – < 10 years	142-335 U/L
	10 – < 13 years	129-417 U/L
	13 – < 15 years	57-254 U/L
	15 – < 17 years	50-117 U/L
	17 – < 19 years	45-87 U/L

(measured at 37 °C)

µkat/L*

Adults[12]

Males (n = 221)	0.67-2.15 µkat/L
Females (n = 229)	0.58-1.74 µkat/L

Children[13]

Males		
Age	0 – 14 days	1.39-4.14 µkat/L
	15 days – < 1 year	2.04-7.83 µkat/L
	1 – < 10 years	2.37-5.59 µkat/L
	10 – < 13 years	2.15-6.96 µkat/L
	13 – < 15 years	1.94-7.82 µkat/L
	15 – < 17 years	1.37-5.53 µkat/L
	17 – < 19 years	0.92-2.49 µkat/L
Females		
Age	0 – 14 days	1.39-4.14 µkat/L
	15 days – < 1 year	2.04-7.83 µkat/L
	1 – < 10 years	2.37-5.59 µkat/L
	10 – < 13 years	2.15-6.96 µkat/L
	13 – < 15 years	0.95-4.24 µkat/L
	15 – < 17 years	0.84-1.95 µkat/L
	17 – < 19 years	0.75-1.45 µkat/L

*calculated by unit conversion factor

Roche has not evaluated reference ranges in a pediatric population.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on a **cobas c** 503 analyzer.

Repeatability	*Mean* U/L	*SD* U/L	*CV* %
PCCC1[a)]	98.9	0.408	0.4
PCCC2[b)]	223	0.673	0.3
Human serum 1	10.2	0.319	3.1
Human serum 2	36.2	0.293	0.8
Human serum 3	144	0.645	0.4
Human serum 4	606	1.27	0.2
Human serum 5	1094	2.66	0.2

Intermediate precision	*Mean* U/L	*SD* U/L	*CV* %
PCCC1[a)]	98.4	1.42	1.4
PCCC2[b)]	223	2.83	1.3
Human serum 1	9.27	1.08	11.6
Human serum 2	35.3	1.21	3.4
Human serum 3	144	1.63	1.1
Human serum 4	607	3.30	0.5
Human serum 5	1095	5.21	0.5

a) PreciControl ClinChem Multi 1

b) PreciControl ClinChem Multi 2

Method comparison

Alkaline phosphatase values for human serum and plasma samples obtained on a **cobas c** 503 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 88

Passing/Bablok[14]	Linear regression
y = 0.987x – 1.24 U/L	y = 1.013x – 4.31 U/L
τ = 0.985	r = 1.000

The sample activities were between 15.0 and 1171 U/L.

Alkaline phosphatase values for human serum and plasma samples obtained on a **cobas c** 303 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas** c 501 analyzer (x).

Sample size (n) = 75

Passing/Bablok[14]	Linear regression
y = 0.985x – 0.691 U/L	y = 0.996x – 3.04 U/L
τ = 0.994	r = 1.000

The sample activities were between 15.8 and 1177 U/L.

References

1 Greiling H, Gressner AM, eds. Lehrbuch der Klinischen Chemie und Pathobiochemie, 3rd ed. Stuttgart/New York: Schattauer Verlag 1995.

2 King EJ, Armstrong AR. Can Med Assoc J 1934;31:376

3 Ohmori Y. Uber die Phosphomomesterase. Enzymologia 1937;4:217-231.

4 Bessey OA, Lowry OH, Brock MJ. A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum. J Biol Chem 1946;164:321-329.

5 Hausamen TU, Helger R, Rick W, et al. Optimal conditions for the determination of serum alkaline phosphatase by a new kinetic method. Clin Chim Acta 1967;15:241-245.

6 Schumann G, Klauke R, Canalias F, et al. 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 Sep;49 (9):1439-46.

7 Guder WG, Narayanan S, Wisser H, et al. List of Analytes; Preanalytical Variables. Brochure in: Samples: From the Patient to the Laboratory. Darmstadt: GIT-Verlag 1996.

8 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.

9 Breuer J. Report on the Symposium "Drug effects in Clinical Chemistry Methods". Eur J Clin Chem Clin Biochem 1996;34:385-386.

10 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;38:376-385.

11 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.

12 Abicht K, El-Samalouti V, Junge W, et al. Multicenter evaluation of new GGT and ALP reagents with new reference standardization and determination of 37 °C reference intervals. Clin Chem Lab Med 2001;39:Special Supplement pp S 346.

13 Estey MP, Cohen AH, Colantonio DA, et al. CLSI-based transference of the CALIPER database of pediatric reference intervals from Abbott to Beckman, Ortho, Roche and Siemens Clinical Chemistry Assays: Direct validation using reference samples from the CALIPER cohort. Clin Biochem 2013;46:1197-1219.

14 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
→	Volume for reconstitution
GTIN	Global Trade Item Number

FOR US CUSTOMERS ONLY: LIMITED WARRANTY

Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

Additions, deletions or changes are indicated by a change bar in the margin.

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com

Distribution in USA by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support 1-800-428-2336