

**Alkaline Phosphatase acc. to IFCC Gen.2****Order information**

REF	CONTENT	Analyzer(s) on which <b>cobas c</b> pack(s) can be used
05166888 190*	Alkaline Phosphatase acc. to IFCC Gen.2 (1050 tests)	System-ID 05 6760 3 Roche/Hitachi <b>cobas c</b> 701/702
05166888 214*	Alkaline Phosphatase acc. to IFCC Gen.2 (1050 tests)	System-ID 05 6760 3 Roche/Hitachi <b>cobas c</b> 701/702

Materials required (but not provided):

10759350 190	Calibrator f.a.s. (12 x 3 mL)	Code 401	
10759350 360	Calibrator f.a.s. (12 x 3 mL, for USA)	Code 401	
12149435 122	Precinorm U plus (10 x 3 mL)	Code 300	
12149435 160	Precinorm U plus (10 x 3 mL, for USA)	Code 300	
12149443 122	Precipath U plus (10 x 3 mL)	Code 301	
12149443 160	Precipath U plus (10 x 3 mL, for USA)	Code 301	
05117003 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391	
05947626 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391	
05947626 160	PreciControl ClinChem Multi 1 (4 x 5 mL, for USA)	Code 391	
05117216 190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 392	
05947774 190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 392	
05947774 160	PreciControl ClinChem Multi 2 (4 x 5 mL, for USA)	Code 392	
05172152 190	Diluent NaCl 9 % (119 mL)	System-ID 08 6869 3	

\* Some kits shown may not be available in all countries.

**English****System information****ALP2L:** ACN 8683**Intended use**

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

**Summary**<sup>1,2,3,4,5,6</sup>

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**<sup>6</sup>

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.



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 for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:

Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:

Apply all relevant local disposal regulations to determine the safe disposal.

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.

**Alkaline Phosphatase acc. to IFCC Gen.2**

Contact phone: all countries: +49-621-7590, USA: 1-800-428-2336

**Reagent handling**

Ready for use

**Storage and stability***ALP2*Shelf life at 2-8 °C: See expiration date on **cobas c** pack label.

On-board in use and refrigerated on the analyzer: 4 days

On-board on the Reagent Manager: 24 hours

*Diluent NaCl 9 %*Shelf life at 2-8 °C: See expiration date on **cobas c** pack label.

On-board in use and refrigerated on the analyzer: 4 weeks

On-board on the Reagent Manager: 24 hours

**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.

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.

Stability:<sup>7</sup>

7 days at 20-25 °C
7 days at 4-8 °C
2 months at -20 °C

**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****cobas c 701/702 test definition**

Assay type	Rate A
Reaction time / Assay points	10 / 24-38
Wavelength (sub/main)	480/450 nm

Reaction direction	Increase	
Units	U/L (µkat/L)	
Reagent pipetting	Diluent (H <sub>2</sub> O)	
R1	75 µL 25 µL	
R3	17 µL 21 µL	
<i>Sample volumes</i>	<i>Sample</i>	<i>Sample dilution</i>
	<i>Sample</i>	<i>Diluent (NaCl)</i>
Normal	2.8 µL – –	
Decreased	2.8 µL 20 µL 80 µL	
Increased	5.6 µL – –	

**Calibration**Calibrators S1: H<sub>2</sub>O  
S2: C.f.a.s.

Calibration mode Linear

Calibration frequency 2-point 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).<sup>6</sup>

**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. 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**

Roche/Hitachi **cobas c** systems automatically calculate the analyte activity of each sample.

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 (1.67 µkat/L).

Icterus:<sup>8</sup> 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:<sup>8</sup> No significant interference up to an H index of 200 (approximate hemoglobin concentration: 124 µmol/L or 200 mg/dL).

Lipemia (Intralipid):<sup>9</sup> 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.<sup>9,10</sup>

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.<sup>11</sup>

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 Roche/Hitachi **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is required in certain cases. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SmpCln1+2/SCCS Method Sheet and for further instructions refer to the operator's manual.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

### 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

Lower detection limit of the test:

5 U/L (0.084 µkat/L)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 3 standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Values below the lower detection limit (< 5 U/L) will not be flagged by the instrument.

#### Expected values

(measured at 37 °C)

##### Adults<sup>12</sup>

Males (n = 221)	40-129 U/L	(0.67-2.15 µkat/L)
Females (n = 229)	35-104 U/L	(0.58-1.74 µkat/L)

##### Children<sup>13</sup>

###### Males

###### Age

0 – 14 days	83-248 U/L	(1.39-4.14 µkat/L)
15 days – < 1 year	122-469 U/L	(2.04-7.83 µkat/L)
1 – < 10 years	142-335 U/L	(2.37-5.59 µkat/L)
10 – < 13 years	129-417 U/L	(2.15-6.96 µkat/L)
13 – < 15 years	116-468 U/L	(1.94-7.82 µkat/L)
15 – < 17 years	82-331 U/L	(1.37-5.53 µkat/L)
17 – < 19 years	55-149 U/L	(0.92-2.49 µkat/L)

###### Females

###### Age

0 – 14 days	83-248 U/L	(1.39-4.14 µkat/L)
15 days – < 1 year	122-469 U/L	(2.04-7.83 µkat/L)
1 – < 10 years	142-335 U/L	(2.37-5.59 µkat/L)
10 – < 13 years	129-417 U/L	(2.15-6.96 µkat/L)
13 – < 15 years	57-254 U/L	(0.95-4.24 µkat/L)
15 – < 17 years	50-117 U/L	(0.84-1.95 µkat/L)
17 – < 19 years	45-87 U/L	(0.75-1.45 µkat/L)

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. Results obtained in individual laboratories may differ.

#### Precision

Precision was determined using human samples and controls in an internal protocol with repeatability (n = 21) and intermediate precision (3 aliquots per run, 1 run per day, 21 days). The following results were obtained:

Repeatability	Mean U/L (µkat/L)	SD U/L (µkat/L)	CV %
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Precinorm U	84.3 (1.41)	0.6 (0.01)	0.7
Precipath U	222 (3.70)	1 (0.02)	0.5
Human serum A	52.6 (0.88)	0.5 (0.01)	1.0
Human serum B	160 (2.66)	1 (0.02)	0.6
Human serum C	966 (16.1)	3 (0.1)	0.3

#### Intermediate precision

	Mean U/L (µkat/L)	SD U/L (µkat/L)	CV %
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Precinorm U	92.8 (1.56)	2.2 (0.04)	2.4
Precipath U	224 (3.74)	4 (0.06)	1.7
Human serum 3	82.2 (1.37)	1.8 (0.03)	2.1
Human serum 4	1025 (17.1)	9 (0.2)	0.9

Results for intermediate precision were obtained on the master system **cobas c 501** analyzer.

#### Method comparison

Alkaline phosphatase values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c 701** analyzer (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi **cobas c 501** analyzer (x).

Sample size (n) = 73

Passing/Bablok <sup>14</sup>	Linear regression
y = 1.0x - 1.0 U/L	y = 0.999x - 1.6 U/L
τ = 0.991	r = 1.0

The sample activities were between 52 and 1089 U/L (0.87 and 18.2 µkat/L).

#### References

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- 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.
- 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.
- 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.
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- Breuer J. Report on the Symposium "Drug effects in Clinical Chemistry Methods". Eur J Clin Chem Clin Biochem 1996;34:385-386.
- 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.
- Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.
- 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.

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- 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.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

**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):

	Contents of kit
	Volume after reconstitution or mixing
	Global Trade Item Number

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 Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim  
www.roche.com  
 +800 5505 6606



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