ALP2

cobas®

Alkaline Phosphatase acc. to IFCC Gen.2

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
03333752 190	Alkaline Phosphatase acc. to IFCC Gen.2 ALP2S 200 tests	System-ID 07 6761 1	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
03333701 190	Alkaline Phosphatase acc. to IFCC Gen.2 ALP2L 400 tests	System-ID 07 6760 3	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
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	
10171743 122	Precinorm U (20 x 5 mL)	Code 300	
10171735 122	Precinorm U (4 x 5 mL)	Code 300	
10171778 122	Precipath U (20 x 5 mL)	Code 301	
10171760 122	Precipath U (4 x 5 mL)	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	
04489357 190	Diluent NaCl 9 % (50 mL)	System-ID 07 6869 3	

English

System information

For **cobas c** 311/501 analyzers:

ALP2S: ACN 158

ALP2L: ACN 683

For **cobas c** 502 analyzer:

ALP2S: ACN 8158

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[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 1983 the International Federation of Clinical Chemistry (IFCC) recommended a standardized method 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. The assay described here is based on this recommendation, but was optimized for performance and stability. The assay was standardized against the IFCC reference formulation proposed above.

Test principle[6]

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

R2 p-nitrophenyl phosphate: 132.8 mmol/L, pH 8.50 (25 °C); preservatives

R1 is in position B and R2 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: For prescription use only.

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.
H412	Harmful to aquatic life with long lasting effects.

Prevention:

P264	Wash skin thoroughly after handling.
P273	Avoid release to the environment.

P280	Wear protective gloves/ eye protection/ face protection.
Response:	
P332 + P313	If skin irritation occurs: Get medical advice/attention.
P337 + P313	If eye irritation persists: Get medical advice/attention.
Disposal:	
P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling primarily follows EU GHS guidance.
Contact phone: all countries: +49-621-7590, USA: 1-800-428-2336

Reagent handling
Ready for use

Storage and stability

ALP2S, ALP2L

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

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:	12 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.

Stability:[7]	7 days at 15-25 °C
	7 days at 2-8 °C
	2 months at (-15)-(-25) °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.

Applications for serum and plasma

cobas c 311 **test definition**

Assay type	Rate A
Reaction time / Assay points	10 / 13-31
Wavelength (sub/main)	480/450 nm
Reaction direction	Increase
Units	U/L (µkat/L)
Reagent pipetting	Diluent (H_2O)
R1	75 µL 25 µL
R2	17 µL 21 µL

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.8 µL	–	–
Decreased	2.8 µL	20 µL	80 µL
Increased	2.8 µL	–	–

cobas c 501 **test definition**

Assay type	Rate A
Reaction time / Assay points	10 / 19-48
Wavelength (sub/main)	480/450 nm
Reaction direction	Increase
Units	U/L (µkat/L)
Reagent pipetting	Diluent (H_2O)
R1	75 µL 25 µL
R2	17 µL 21 µL

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.8 µL	–	–
Decreased	2.8 µL	20 µL	80 µL
Increased	2.8 µL	–	–

cobas c 502 **test definition**

Assay type	Rate A
Reaction time / Assay points	10 / 19-48
Wavelength (sub/main)	480/450 nm
Reaction direction	Increase
Units	U/L (µkat/L)
Reagent pipetting	Diluent (H_2O)
R1	75 µL 25 µL
R2	17 µL 21 µL

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.8 µL	–	–
Decreased	2.8 µL	20 µL	80 µL
Increased	5.6 µL	–	–

Calibration

Calibrators	S1: H_2O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration • after reagent lot change • as required following quality control procedures

Traceability: This method has been standardized against the proposed IFCC formulation[6] using calibrated pipettes together with a manual photometer providing absolute values and the substrate-specific absorptivity, ε.

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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 phophatase activity of 100 U/L (1.67 µkat/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 Roche/Hitachi **cobas c** systems. The latest version of the carry-over evasion list can be found with the NaOHD-SMS-SmpCln1+2-SCCS Method Sheets. For further instructions refer to the operator's manual. **cobas c** 502 analyzer: All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is not required.

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).

Expected values

(measured at 37 °C)

Adults[12]		
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)
Consensus values[13]		
Males	40-130 U/L	(0.67-2.17 µkat/L)
Females	35-105 U/L	(0.58-1.75 µkat/L)
Children[14]		
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*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	99.2 (1.65)	0.7 (0.01)	0.7
Precipath U	241 (4.02)	1 (0.02)	0.6
Human serum 1	54.6 (0.912)	0.5 (0.008)	0.9
Human serum 2	648 (10.8)	4 (0.1)	0.7
Intermediate precision	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
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

Method comparison

Alkaline phosphatase values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 203

Passing/Bablok[15]	Linear regression
y = 0.988x + 1.31 U/L	y = 0.991x + 0.799 U/L
τ = 0.961	r = 0.997

The sample activities were between 50.0 and 1002 U/L (0.835 and 16.7 µkat/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

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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.

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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.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

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

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Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
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