

## Order information

REF	CONTENT	Analyzer(s) on which <b>cobas c</b> pack(s) can be used
0333752 190	Alkaline Phosphatase acc. to IFCC Gen.2 ALP2S 200 tests	System-ID 07 6761 1 Roche/Hitachi <b>cobas c</b> 311, <b>cobas c</b> 501/502
0333701 190	Alkaline Phosphatase acc. to IFCC Gen.2 ALP2L 400 tests	System-ID 07 6760 3 Roche/Hitachi <b>cobas c</b> 311, <b>cobas c</b> 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<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 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<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

**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:

P273 Avoid release to the environment.

P280 Wear eye protection/ face protection.

P280 Wear protective gloves.

**Response:**

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

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:<sup>7</sup> 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 <sub>2</sub> O)
R1	75 μL 25 μL

R2	17 μL	21 μL
<i>Sample volumes</i>	<i>Sample</i>	<i>Sample dilution</i>
		<i>Sample Diluent (NaCl)</i>
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 <sub>2</sub> O)	
R1	75 μL 25 μL	
R2	17 μL 21 μL	
<i>Sample volumes</i>	<i>Sample</i>	<i>Sample dilution</i>
		<i>Sample Diluent (NaCl)</i>
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 <sub>2</sub> O)	
R1	75 μL 25 μL	
R2	17 μL 21 μL	
<i>Sample volumes</i>	<i>Sample</i>	<i>Sample dilution</i>
		<i>Sample 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

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

**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 =  $\mu$ kat/L

#### Limitations - interference

Criterion: Recovery within  $\pm 10\%$  of initial value at an alkaline phosphatase activity of 100 U/L (1.67  $\mu$ kat/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  $\mu$ mol/L or 60 mg/dL).

Hemolysis:<sup>8</sup> No significant interference up to an H index of 200 (approximate hemoglobin concentration: 124  $\mu$ mol/L or 200 mg/dL).

Lipemia (Intralipid):<sup>8</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. 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  $\mu$ kat/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  $\mu$ kat/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<sup>12</sup>

Males (n = 221) 40-129 U/L (0.67-2.15  $\mu$ kat/L)

Females (n = 229) 35-104 U/L (0.58-1.74  $\mu$ kat/L)

##### Consensus values<sup>13</sup>

Males 40-130 U/L (0.67-2.17  $\mu$ kat/L)

Females 35-105 U/L (0.58-1.75  $\mu$ kat/L)

##### Children<sup>a)</sup>

aged 1 day < 250 U/L (< 4.17  $\mu$ kat/L)

aged 2-5 days < 231 U/L (< 3.84  $\mu$ kat/L)

aged 6 days-6 months < 449 U/L (< 7.49  $\mu$ kat/L)

aged 7 months-1 year < 462 U/L (< 7.69  $\mu$ kat/L)

aged 1-3 years < 281 U/L (< 4.67  $\mu$ kat/L)

aged 4-6 years < 269 U/L (< 4.48  $\mu$ kat/L)

aged 7-12 years < 300 U/L (< 5.00  $\mu$ kat/L)

aged 13-17 years (f) < 187 U/L (< 3.11  $\mu$ kat/L)

aged 13-17 years (m) < 390 U/L (< 6.51  $\mu$ kat/L)

a) Calculated from published reference ranges for the ALP opt. method (DGKC)<sup>14</sup> using a factor of 0.417 derived from a method comparison.

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 ( $\mu$ kat/L)	U/L ( $\mu$ kat/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 ( $\mu$ kat/L)	U/L ( $\mu$ kat/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 <sup>15</sup>	Linear regression
y = 0.988x + 1.31 U/L	y = 0.991x + 0.799 U/L
r = 0.961	r = 0.997

The sample activities were between 50.0 and 1002 U/L (0.835 and 16.7  $\mu$ kat/L).

#### References




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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.

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

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