ALP2

ALP IFCC Gen.2

cobas®

Enzymes

Order information

REF	CONTENT		Analyzers on which **cobas c** pack can be used
03333752 190	ALP IFCC Gen.2 Small (200 tests)	System-ID 07 6761 1	COBAS INTEGRA 400 plus
03333701 190	ALP IFCC Gen.2 Large (400 tests)	System-ID 07 6760 3	COBAS INTEGRA 400 plus

Materials required (but not provided):

10759350 190	Calibrator f.a.s. (12 × 3 mL)	System-ID 07 3718 6	
10759350 360	Calibrator f.a.s. (12 × 3 mL, for USA)	System-ID 07 3718 6	
12149435 122	Precinorm U plus (10 × 3 mL)	System-ID 07 7999 7	
12149435 160	Precinorm U plus (10 × 3 mL, for USA)	System-ID 07 7999 7	
12149443 122	Precipath U plus (10 × 3 mL)	System-ID 07 8000 6	
12149443 160	Precipath U plus (10 × 3 mL, for USA)	System-ID 07 8000 6	
05117003 190	PreciControl ClinChem Multi 1 (20 × 5 mL)	System-ID 07 7469 3	
05947626 190	PreciControl ClinChem Multi 1 (4 × 5 mL)	System-ID 07 7469 3	
05947626 160	PreciControl ClinChem Multi 1 (4 × 5 mL, for USA)	System-ID 07 7469 3	
05117216 190	PreciControl ClinChem Multi 2 (20 × 5 mL)	System-ID 07 7470 7	
05947774 190	PreciControl ClinChem Multi 2 (4 × 5 mL)	System-ID 07 7470 7	
05947774 160	PreciControl ClinChem Multi 2 (4 × 5 mL, for USA)	System-ID 07 7470 7	

English

System information

cobas c pack ALP2S, Cat. No. 03333752190:
Test ALP2S, test-ID 0-551

cobas c pack ALP2L, Cat. No. 03333701190:
Test ALP2L, test-ID 0-550

Intended use

In vitro test for the quantitative determination of the catalytic activity of alkaline phosphatase (EC 3.1.3.1; ortho-phosphoric monoester phosphohydrolase, alkaline optimum) in human serum and plasma on COBAS INTEGRA 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[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 at 409 nm.

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
SR	p-nitrophenyl phosphate: 132.8 mmol/L, pH 8.5 (25 °C); preservatives

R1 is in position B and SR 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.

Contact phone: all countries: +49-621-7590, USA: 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 at 10-15 °C	4 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: Collect serum using standard sampling tubes.
Plasma: Heparin (Li-, Na-, NH_4^+-) 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.

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.

Application for serum and plasma

Test definition

Measuring mode	Absorbance
Abs. calculation mode	Kinetic
Reaction mode	R1-S-SR
Reaction direction	Increase
Wavelength A/B	409/659 nm
Calc. first/last	41/64
Unit	U/L

Pipetting parameters

		Diluent (H_2O)
R1	75 µL	16 µL
Sample	2.75 µL	20 µL
SR	17 µL	10 µL
Total volume	140.75 µL	

Calibration

Calibrator	Calibrator f.a.s.
	Use deionized water as zero calibrator.
Calibration mode	Linear regression
Calibration replicate	Duplicate recommended
Calibration interval	Each lot

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

Reference range	Precinorm U plus or PreciControl ClinChem Multi 1
Pathological range	Precipath U plus or PreciControl ClinChem Multi 2
Control interval	24 hours recommended
Control sequence	User defined
Control after calibration	Recommended

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

The COBAS INTEGRA 400 plus analyzer automatically calculates the analyte activity of each sample. For more details, please refer to Data Analysis in the Online Help.

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

Limitations - interference

Criterion: Recovery within ± 10 % of initial value.

Icterus:[8] No significant interference up to an I index of 42 for conjugated bilirubin and 60 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 718 µmol/L or 42 mg/dL; approximate unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[8] No significant interference up to an H index of 250 (approximate hemoglobin concentration: 155 µmol/L or 250 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 INTEGRA analyzers. Refer to the CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.
Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

3.0-1200 U/L (0.05-20 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:
3.0 U/L (0.05 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 a zero sample (zero sample + 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)

Children[13]	Age		
Male	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)
Female	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 (1 aliquot 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 %*
Precinorm U	80.1 (1.34)	1.3 (0.02)	1.6
Pecipath U	228 (3.81)	4 (0.07)	1.8
Human serum 1	72.7 (1.21)	1.5 (0.03)	2.0
Human serum 2	225 (3.76)	4 (0.07)	1.8

Intermediate precision	*Mean U/L (µkat/L)*	*SD U/L (µkat/L)*	*CV %*
Precinorm U	81.8 (1.37)	2.3 (0.04)	2.8
Pecipath U	230 (3.84)	6 (0.10)	2.8
Human serum 1	70.0 (1.17)	1.9 (0.03)	2.7
Human serum 2	220 (3.67)	6 (0.10)	2.7

Method comparison

ALP values for human serum patient samples obtained on a COBAS INTEGRA 400 plus analyzer with the COBAS INTEGRA ALP IFCC Gen.2 (ALP2) traceable to IFCC[6] method (y), were compared with those determined on the same analyzer with the same ALP2 reagent traceable to IFCC[14] method (x).

COBAS INTEGRA 400 plus analyzer	Sample size (n) = 104
Passing/Bablok[15]	Linear regression
y = 1.04x – 0.078 U/L	y = 1.04x – 0.039 U/L
r = 0.997	r = 1.00

The sample activities were between 15.0 and 1036 U/L (0.251 and 17.3 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. A convenient method for determining serum and bile phosphatase activity. Can Med Assoc J 1934;31(4):376-381.

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

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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.

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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.

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):

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

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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.

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