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 COBAS INTEGRA 800
03333701 190	ALP IFCC Gen.2 Large (400 tests)	System-ID 07 6760 3	COBAS INTEGRA 400 plus COBAS INTEGRA 800
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	
10171743 122	Precinorm U (20 × 5 mL)	System-ID 07 7997 0	
10171735 122	Precinorm U (4 × 5 mL)	System-ID 07 7997 0	
10171778 122	Precipath U (20 × 5 mL)	System-ID 07 7998 9	
10171760 122	Precipath U (4 × 5 mL)	System-ID 07 7998 9	
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 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 meets the recommendations of the IFCC.

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

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

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

Shelf life at 2-8 °C	See expiration date on **cobas c** pack label
COBAS INTEGRA 400 plus system	
On-board in use at 10-15 °C	4 weeks
COBAS INTEGRA 800 system	
On-board in use at 8 °C	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: 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.

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.

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

COBAS INTEGRA 400 plus 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	

COBAS INTEGRA 800 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	58/98
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

Traceability: This method has been standardized manually against the original IFCC formulation.

Quality control

Reference range	Precinorm U, Precinorm U plus or PreciControl ClinChem Multi 1
Pathological range	Precipath U, 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

COBAS INTEGRA analyzers automatically calculate the analyte activity of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400 plus/800 analyzers).

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 and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 718 µmol/L or 42 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)

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]	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 and plasma samples obtained on a COBAS INTEGRA 700 analyzer with the COBAS INTEGRA ALP IFCC Gen.2 (ALP2L) reagent (y) were compared to those determined using the same reagent on a Roche/Hitachi 917 analyzer (x), and to those determined using the previous reagent (ALP6) on a COBAS INTEGRA 700 analyzer (x).

Roche/Hitachi 917 analyzer	Sample size (n) = 97
Passing/Bablok[15]	Linear regression
$y = 1.021x - 2.95$ U/L	$y = 1.036x - 5.78$ U/L
$\tau = 0.978$	r = 0.999
SD (md 95) = 10.9	Sy.x = 4.20

The sample activities were between 37 and 866 U/L (0.618 and 14.5 µkat/L).

COBAS INTEGRA 700 analyzer	Sample size (n) = 93
Passing/Bablok[15]	Linear regression
$y = 1.006x + 0.034$ U/L	$y = 1.004x + 0.351$ U/L
$\tau = 0.982$	r = 1.00
SD (md 95) = 7.00	Sy.x = 3.17

The sample activities were between 38 and 924 U/L (0.635 and 15.4 µkat/L).

References

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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.

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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 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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