BECKMAN COULTER CE

AU

Instructions For Use

ALP
ALKALINE PHOSPHATASE

REF OSR6004 4 x 12 mL R1, 4 x 12 mL R2
OSR6104 4 x 30 mL R1, 4 x 30 mL R2
OSR6204 4 x 53 mL R1, 4 x 53 mL R2
OSR6604 4 x 173 mL R1, 4 x 173 mL R2

For *in vitro* diagnostic use.

PRINCIPLE

INTENDED USE

Kinetic colour test for the quantitative determination of alkaline phosphatase, EC 3.1.3.1 (ALP), in human serum and plasma on Beckman Coulter AU analysers.

OSR6604 for use on the AU5800, AU2700 and AU5400 systems only.

SUMMARY AND EXPLANATION

Reference[1,2]

Alkaline phosphatase (ALP) is present in almost all body tissues, located at or in cell membranes. It occurs at particularly high levels in interstitial epithelium, kidney tubules, bone (osteoblasts), liver and placenta. The precise metabolic function of ALP has not yet been fully elucidated, however the enzyme is associated with intestinal lipid transport and bone calcification. ALP originates in approximately equal proportions from the liver and the skeletal system. Approximately 25% of healthy individuals also have intestinal ALP which accounts for approximately 10% of the total ALP in a fasting sample. Increases in total ALP are either due to physiological causes, or are caused by diseases of the liver or bone. Physiological increases in ALP are found in pregnancy from the 2nd trimester onwards due to placental ALP, in growing children due to bone ALP and postprandially in individuals with blood groups B and O, who are secretors of blood group substance H (intestinal ALP).

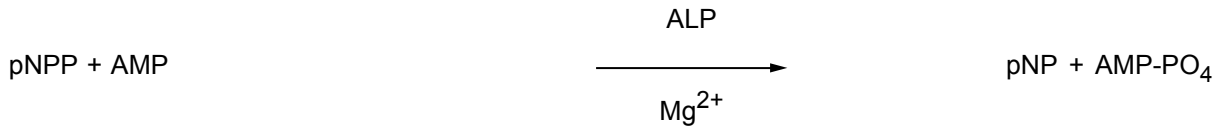
The most common cause of elevated ALP is hepatobiliary disease, with pathological ALP levels found in approximately 60% of patients with disease of the liver or biliary tract. ALP levels may also be elevated in primary bone diseases, such as osteomalacia, osteogenesis imperfecta, vitamin D intoxication and primary bone tumours. ALP levels may also be increased in secondary bone diseases, such as skeletal metastases, and in diseases such as multiple myeloma, acromegaly, renal insufficiency, hyperthyroidism, ectopic ossification, sarcoidosis, bone tuberculosis and healing fractures. In bone diseases such as Paget's disease, vitamin D deficiency rickets and metastatic bone disease, ALP activity is a good indicator of bone activity, in the absence of co-existing chronic liver disease. Total ALP is only occasionally elevated in some metabolic bone diseases such as hyperparathyroidism, osteopenia or osteoporosis. Reduced levels of ALP are found in familial hypophosphatasia, hypoparathyroidism, achondroplasia, adynamic bone disease in dialysis patients, pituitary dwarfism, chronic radiation sickness and malnutrition.

METHODOLOGY

Reference[3]

Method based on the recommendations of the "International Federation for Clinical Chemistry" (IFCC).
Alkaline phosphatase activity is determined by measuring the rate of conversion of p-nitro-phenylphosphate (pNPP) to p-nitrophenol (pNP) in the presence of magnesium and zinc ions and of 2-amino-2-methyl-1-propanol (AMP) as phosphate acceptor at pH 10.4. The rate of change in absorbance due to the formation of pNP is measured bichromatically at 410/480 nm and is directly proportional to the ALP activity in the sample.

CHEMICAL REACTION SCHEME



SPECIMEN

TYPE OF SPECIMEN

Serum and heparinised plasma. Complexing anticoagulants such as citrate, oxalate and EDTA should be avoided.[4]
Strongly haemolysed samples should be avoided.
Stable in serum and plasma for 7 days when stored at 2…25°C.[5]

REAGENTS

WARNING AND PRECAUTIONS

Exercise the normal precautions required for handling all laboratory reagents.
During the reaction p-nitrophenol is produced. This is harmful when inhaled, swallowed or absorbed through skin.
Dispose of all waste material in accordance with local guidelines.

REACTIVE INGREDIENTS

Final concentration of reactive ingredients:

2-Amino-2-Methyl-1-Propanol (AMP) pH 10.4	0.35 mol/L
p-Nitrophenyl phosphate	16 mmol/L
HEDTA	2 mmol/L
Zinc Sulphate	1 mmol/L
Magnesium Acetate	2 mmol/L
Preservative	

The concentrations of the reactive components of the reagents shown on the kit label are the actual concentrations in the individual R1/R2 vials. The reagent composition which is shown in the Instructions For Use is the final concentration of these components in the reaction cuvette after addition of R1, Sample, and R2.

⚠ CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).
To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.

GHS HAZARD CLASSIFICATION

ALP R1	WARNING	
		
	H315	Causes skin irritation.
	H319	Causes serious eye irritation.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P337+P313	If eye irritation persists: Get medical advice/attention.
		2-Amino-2-methyl-1-propanol 10 - 15%
ALP R2	WARNING	
		
	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long lasting effects.
	P273	Avoid release to the environment.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%

SDS	Safety Data Sheet is available at beckmancoulter.com/techdocs

REAGENT PREPARATION

The reagents are ready for use and can be placed directly on board the instrument.

STORAGE AND STABILITY

The reagents are stable, unopened, up to the stated expiry date when stored at 2…8°C.

Absorption of atmospheric CO_2 by the reagent on board the analyser can impair its stability. This effect will vary depending upon the rate of use.

	Bottle replacement is recommended when one of the following conditions are encountered:
	14 days have elapsed on board the analyser
	Significant shift in control values (>7%) following local QC procedures.
AU5800:	Bottle replacement is recommended when one of the following conditions are encountered:

7 days have elapsed on board the analyser

Significant shift in control values (>7%) following local QC procedures.

CALIBRATION

CALIBRATION INFORMATION

The test is run in MB-mode. To provide a robust approach to generate the analyser specific MB factor, it is recommended that 5 separate calibration events should be used. A fresh vial of calibrator, utilising System Calibrator Cat No. 66300 in the AB calibration mode, should be used for each of these runs. When calculating the mean factor from the separate runs the data should be examined for obvious outliers which should be repeated and replaced. For the AU2700/AU5400 this procedure needs to be performed for each ring. Quality control procedures should be undertaken immediately following calibration in accordance with good laboratory practice.

The calibrator value is traceable to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) 37°C reference method for Alkaline Phosphatase.
Re-establishment of the analyser specific MB factor is recommended when a critical part of the analyser is replaced;
Reagent blank measurement is recommended when changing to a new lot of reagent.

QUALITY CONTROL

Controls Cat. No. ODC0003 and ODC0004 or other control materials with values determined by this Beckman Coulter system may be used.
Each laboratory should establish its own control frequency.
Good laboratory practice suggests that controls be tested each day patient samples are tested and each time calibration is performed. Values obtained for the controls should fall within specified limits as defined by the user. If any trends or sudden shifts in values are detected, review all operating parameters.
Each laboratory should establish guidelines for corrective action to be taken if controls do not recover within the specified limits.

TESTING PROCEDURE(S)

Refer to the appropriate Beckman Coulter AU analyser User Guide/Instructions For Use (IFU) for analyser-specific assay instructions for the sample type as listed in the Intended Use statement. The paediatric application is suitable for use with small volume serum/plasma samples.

CALCULATIONS

The Beckman Coulter analysers automatically compute the alkaline phosphatase activity of each sample.

REPORTING RESULTS

REFERENCE INTERVALS

Reference[6]

Females (18 - 49y)	33 - 98 U/L
Males (≥ 20y)	43 - 115 U/L

Expected values may vary with age, sex, sample type, diet and geographical location. Each laboratory should verify the transferability of the expected values to its own population, and if necessary determine its own reference interval according to good laboratory practice. For diagnostic purposes, results should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

PROCEDURAL NOTES

INTERFERENCES

Results of studies conducted to evaluate the susceptibility of the method to interference were as follows:

Icterus:	Interference less than 10% up to 28 mg/dL or 479 µmol/L bilirubin
Haemolysis:	Interference less than 10% up to 4.5 g/L haemoglobin
Lipemia:	Interference less than 3% up to 1,000 mg/dL Intralipid

Refer to Young[7] for further information on interfering substances.

PERFORMANCE CHARACTERISTICS

PERFORMANCE CHARACTERISTICS

Data contained within this section is representative of performance on Beckman Coulter systems. Data obtained in your laboratory may differ from these values.

Linearity

The test is linear within an enzyme activity range of 5 – 1,500 U/L (0.1 – 25.0 µkat/L).

Precision

The following data was obtained on an AU5800 using 3 serum pools analysed over 20 days.

n = 80	Within-run		Total	
Mean U/L	SD	CV%	SD	CV%
38	0.81	2.1	1.60	4.2
360	4.69	1.3	8.21	2.3
1254	16.27	1.3	22.99	1.8

Sensitivity

The lowest detectable level in serum on an AU5800 analyzer was estimated at 2 U/L.
The lowest detectable level represents the lowest measurable level of ALP that can be distinguished from zero. It is calculated as the absolute mean plus three standard deviations of 20 replicates of an analyte free sample.

Method Comparison

Patient serum samples were used to compare this assay on the AU5800 against the AU2700. Results of linear regression analysis were as follows:

y = 1.009x + 2.756	r = 1.000	n = 104	Sample range = 39 - 1478 U/L

ADDITIONAL INFORMATION

DxC 700 AU requires that each reagent application has a standard format of abbreviated Closed Test Name. This Closed Test Name is required to allow automated loading of the calibrator information for each application as part of the DxC 700 AU Closed System. Refer to the table below for the Closed Test Name assigned to each application for this assay.

Test Name	Description
ALP1N	ALP (Serum)
ALP1NP	ALP (Serum Paediatric)

Setting Sheet Footnotes
User defined
* Values set for working in U/L. To work in SI units (µkat/L) divide by 60.
§ For use in AB mode only, refer to IFU for further instruction.
‡ Depends on usage pattern in the laboratory.

REVISION HISTORY

Added new languages

Preceding version revision history

Revised GHS section

REFERENCES

1. Moss DW, Henderson RA. Clinical Enzymology. In: Burtis CA, Ashwood ER, eds. Tietz textbook of clinical chemistry. Philadelphia:WB Saunders Company, 1999; 676-684.

2. Thomas L. Alkaline phosphatase (ALP). In: Thomas L, ed. Clinical laboratory diagnostics. Use and assessment of clinical laboratory results. Frankfurt/Main: TH-Books Verlagsgesellschaft, 1998:36-46.

3. Tietz NW, Rinker D, Shaw LM. IFCC methods for the measurement of catalytic concentration of enzymes Part 5. IFCC method for alkaline phosphatase. J Clin Chem Clin Biochem 1983;21:731-48.

4. Moss DW, Henderson RA, Kachmar JF. Enzymes In: Tietz NW, ed. Fundamentals of clinical chemistry. Philadelphia:WB Saunders Company, 1987:387pp.

5. Ehret W, Heil W, Schmitt Y, Töpfer G, Wisser H, Zawta B, et al. Use of Anticoagulants in Diagnostic Laboratory Investigations and Stability of Blood, Plasma and Serum Samples. WHO/DIL/LAB/99.1 Rev.2:21pp.

6. Schumann G,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;49(9):1439–1446

7. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.

EC REP Beckman Coulter Ireland Inc., Lismeehan, O'Callaghan's Mills, Co. Clare, Ireland +(353) (0) 65 683 1100

Beckman Coulter, Inc., 250 S. Kraemer Blvd., Brea, CA 92821 U.S.A.
www.beckmancoulter.com