BECKMAN COULTER

AU/DxC AU US

Instructions For Use

ALP

ALP

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 only.

For Rx use only

PRINCIPLE

INTENDED USE

System reagent for the quantitative determination of Alkaline Phosphatase activity in human serum and plasma on Beckman Coulter AU/DxC AU analyzers.

SUMMARY AND EXPLANATION

Measurements of serum alkaline phosphatase (ALP) (EC 3.1.3.1) are used in the diagnosis of hepatobiliary disorders and bone disease associated with increased osteoblastic activity. Certain conditions such as Hodgkin's Disease, congestive heart failure and ulcerative colitis will produce moderate elevation in alkaline phosphatase levels. Non-pathologic elevations can be observed in third trimester of pregnancy[1].

METHODOLOGY

This ALP procedure is based on the method developed by Bowers and McComb[2] and has been formulated as recommended by the AACC and IFCC[3].

Alkaline phosphatase activity is determined by measuring the rate of conversion of p-nitro-phenylphosphate (pNPP) in the presence of 2-amino-2-methyl-1-propanol (AMP) at pH 10.4.

$$\text{pNPP} + \text{AMP} \xrightarrow[Mg^{2+}]{\text{ALP}} \text{pNP} + \text{AMP-PO}_4$$

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.

SPECIMEN

SPECIMEN STORAGE AND STABILITY

Alkaline Phosphatase in serum and plasma is stable for 7 days when stored at 4 - 25°C and 2 months at -20°C.[4]

Specimen storage and stability information provides guidance to the laboratory. Based on specific needs, each laboratory may establish alternative storage and stability information according to good laboratory practice or from alternative reference documentation.

Additional handling conditions as designated by this laboratory:

SPECIMEN COLLECTION AND PREPARATION

Serum or Li/Na heparinized plasma, free from hemolysis, is the recommended specimen. Serum should be separated from the cells within two hours after collection. Avoid use of plasma with EDTA or oxalate.

Additional instructions for patient sample preparation as designated by this laboratory:

Additional type conditions as designated by this laboratory:

REAGENTS

CONTENTS

Alkaline Phosphatase (ALP) Reagent.

Reagent storage location in this laboratory:

WARNING AND PRECAUTIONS

1. Exercise the normal precautions required for handling all laboratory reagents.
2. 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	16.0 mmol/L
HEDTA	2.0 mmol/L
Zinc Sulfate	1.0 mmol/L
Magnesium Acetate	2.0 mmol/L
Also contains preservative	

⚠ 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

EQUIPMENT AND MATERIALS

For use on the AU480, AU680, AU5800, DxC 500 AU, DxC 500i and DxC 700 AU Beckman Coulter Analyzers.

OSR6604 for use on the AU5800 systems only.

Storage location of test tubes or sample cups in this laboratory:

REAGENT PREPARATION

The ALP Reagents are ready for use. No preparation is required.

STORAGE AND STABILITY

1. The unopened reagents are stable until the expiration date printed on the label when stored at 2 – 8°C.
2. Opened reagents are stable for 14 days when stored in the refrigerated compartment of the analyzer. Replace the reagent vial once QC values have drifted by more than 10%.
3. Opened reagents are stable for 7 days when stored in the refrigerated compartment of the AU5800 analyzer. Replace the reagent vial once QC values have drifted by more than 10%.

INDICATIONS OF DETERIORATION

Visible signs of microbial growth, turbidity or precipitate, or any change in reagent color may indicate degradation and warrant discontinuance of use.

Additional storage requirements as designated by this laboratory:

STABILITY OF FINAL REACTION MIXTURE

The Beckman Coulter AU/DxC AU analyzer automatically computes every determination at the same time interval.

CALIBRATION

CALIBRATION INFORMATION

Calibration of this alkaline phosphatase procedure is based upon the bichromatic extinction coefficient for p-Nitrophenol, which has a molar absorptivity of 17,900 at 410/480 nm.

The theoretical extinction coefficient for alkaline phosphatase is 4525.

DxC 500 AU and DxC 500i CALIBRATION INFORMATION ONLY

Ensure that Manufacturer Factor A is 1.0.

Prepare 3 fresh vials of System Calibrator Cat No. 66300X and run as samples in duplicate. Examine the data for outliers which should be repeated and replaced and calculate the **Overall Mean** Value. Calculate the analyzer specific Manufacturer Factor A:

$$\textbf{Calculated Factor} = \frac{\textbf{System Calibrator REF 66300X Set Point per product Insert}}{\textbf{Overall Mean Value}}$$

Enter the **Calculated Factor** in the Manufacturer Factor A field in the General Parameters section of the Chemistry Details Screen. Quality control procedures should be undertaken immediately following calibration in accordance with good laboratory practice. Re-establishment of the analyzer specific Manufacturer Factor A is recommended when QC results are not within the laboratories' established ranges following replacement of a critical part of the analyzer.

Reagent blank measurement is recommended when changing to a new lot of reagent.

AU480 / AU680 / DxC 700 AU / AU5800 CALIBRATION INFORMATION ONLY

Ensure that the MB Factor in the MB Type Factor field is set to the assay specific **Theoretical Extinction Coefficient**. Prepare 3 fresh vials of System Calibrator Cat No. 66300X and run as samples in duplicate. Examine the data for outliers which should be repeated and replaced and calculate the **Overall Mean** Value. Calculate the **Derived Multiplier:**

$$\textbf{Derived Multiplier} = \frac{\textbf{System Calibrator REF 66300X Set Point per product Insert}}{\textbf{Overall Mean Value}}$$

Multiply the **Theoretical Extinction Coefficient for the assay** by the **Derived Multiplier** to get the **analyzer specific MB type factor**. Enter the **analyzer specific MB type Factor** in the MB Type Factor section of the calibration specific menu. For the AU5800, ensure the correct unit and cuvette wheel is selected. Quality control procedures should be undertaken immediately following calibration in accordance with good laboratory practice.

Re-establishment of the analyzer specific MB factor is recommended when QC results are not within the laboratories' established ranges following replacement of a critical part of the analyzer.

Reagent blank measurement is recommended when changing to a new lot of reagent.

Note for the AU5800 analyzer: the analyzer specific MB Factor is generated for each analyzer unit and cuvette wheel that the enzyme assay is used.

QUALITY CONTROL

During operation of the Beckman Coulter AU/DxC AU analyzer, at least two levels of an appropriate quality control material should be tested a minimum of once a day. In addition, controls should be performed after calibration, with each new lot of reagent, and after specific maintenance or troubleshooting steps described in the appropriate Beckman Coulter AU/DxC AU analyzer Instructions For Use (IFU) and Reference Manual. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

Location of controls used at this laboratory.

CONTROL NAME	SAMPLE TYPE	STORAGE

TESTING PROCEDURE(S)

A complete list of test parameters and operational procedures are provided in the relevant AU/DxC AU analyzer IFU and Reference Manual.

RESULTS INTERPRETATION

The default unit of measure is U/L, for conversion to SI units µkat/L the result is divided by 60.

REPORTING RESULTS

EXPECTED RESULTS

Reference[5]

Male: 43 - 115 U/L

Female: 33 - 98 U/L

Reference Intervals shown above were taken from the literature. 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.

Expected reference ranges in this laboratory:

INTERVALS	SAMPLE TYPE	UNITS

Additional reporting information as designated by this laboratory:

PROCEDURAL NOTES

INTERFERENCES

Results of laboratory studies[6] show that the following substances interfere with alkaline phosphatase determination: citrate, oxalate, fluoride, EDTA, glycine, monoethanolamine and high concentrations of phosphate and chloride.

Results of studies[6] show that the following substances interfere with this alkaline phosphatase assay:

The criteria for no significant interference is recovery within 10% of the initial value.

Bilirubin:	No significant interference up to 32 mg/dL Bilirubin
Hemolysis:	No significant interference up to 450 mg/dL Hemolysate
Lipemia:	No significant interference up to 1,000 mg/dL Intralipid*

The information presented is based on results from Beckman Coulter studies and is current at the date of publication. Beckman Coulter Inc. makes no representation about the completeness or accuracy of results generated by future studies. Further information on interfering substances, are available.[7]

*Intralipid, manufactured by KabiVitrium Inc., is a 20% IV fat emulsion used to emulate extremely turbid samples.

Laboratory specific procedure notes:

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.

DYNAMIC RANGE / ANALYTICAL MEASURING RANGE

The ALP procedure is linear from 5 to 1,500 U/L. Samples exceeding the upper limit of linearity should be diluted and repeated. The sample may be diluted, repeated and multiplied by the dilution factor automatically utilizing the AUTO REPEAT RUN.

SENSITIVITY

Typical change in absorbance per minute for 1 U/L of ALP is 0.22 mAbsorbance.

METHODS COMPARISON

Reference[8]

Patient serum samples were evaluated in method comparison studies.

Results of Deming regression analysis were as follows:

Y Method	DxC 700 AU
X Method	AU5800
Slope	1.000
Intercept	0.167
Correlation Coeff. (r)	1.000
No. of Samples (n)	120
Range (U/L)	25 - 1411

PRECISION

Reference[8]

Estimates of precision, based on CLSI recommendations,[9] are consistent with typical performance. The within run precision is less than 5% CV and total precision is less than 10% CV. Assays of control sera were carried out and data reduced following CLSI guidelines.

N = 80	**Within-run**		**Total**	
Mean, U/L	**SD**	**CV%**	**SD**	**CV%**
28.6	0.28	1.0	0.75	2.6
330.5	1.92	0.6	4.49	1.4
1198.3	5.99	0.5	15.87	1.3

ADDITIONAL INFORMATION

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

Test Name	**Description**
ALP1U	ALP (Serum)

Refer to the Beckman Coulter Chemistry Systems Reagent Guide (BAGUIDE) for specific chemistry information for the AU/DxC AU clinical chemistry systems and guidance on symbols used on all AU/DxC AU product labelling.

Setting Sheet Footnotes

User defined

* Values set for working in U/L. To work in SI units (µkat/L) divide by 60

The theoretical extinction coefficient is 4525

Ψ Analyzer Specific MB Factor = Theoretical Extinction Coefficient*Derived Multiplier.

AU5800 only:

MB Factor adjustment must be completed separately for each ring.

REVISION HISTORY

Add DxC 500i instrument to IFU

Preceding version revision history

Updated Specimen Section

Updated REPORTING RESULTS section

Updated PROCEDURAL NOTES section

Updated Performance Characteristics section

Updated References section

REFERENCES

1. Friedman, R.B., and Young, D.S., Effects of Disease on Clinical Laboratory Tests, 3rd Edition, AACC Press, 1997.
2. Bowers, G.N., and McComb, R.B., Clin Chem. 21: 1988 -1995, 1975.
3. Tietz, N.W.(ed), Textbook of Clinical Chemistry, W.B. Saunders,1986.
4. 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.
5. 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.
6. CLSI. Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition. CLSI document EP07-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.
7. AACC Effects on Clinical Laboratory Tests: Drugs, Disease, Herbs and Natural Products https://clinfx.wiley.com/aaccweb/aacc/
8. Data is on file for specific AU/DxC AU analyzers.
9. CLSI. Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition. CLSI document EP05-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.

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