ALKALINE PHOSPHATASE (ALP)

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**
OSR6504*	**4 x 103 mL**	**R1**
	4 x 103 mL	**R2**

Intended Use
System reagent for the quantitative determination of Alkaline Phosphatase activity in human serum and plasma on Beckman Coulter AU analyzers.
*Alkaline Phosphatase (ALP) reagent OSR6504 for use on the AU2700/5400/680 system only.

Summary
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.

$$pNPP + AMP \xrightarrow[Mg^{2+}]{ALP} pNP + AMP\text{-}PO_4$$

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.

System Information
For AU400/400^e/480, AU600/640/640^e/680 and AU2700/5400 Beckman Coulter Analyzers.

Reagents
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

Precautions
1. For *in vitro* diagnostic use.
2. **IRRITANT:** Irritating to eyes and skin. Do not ingest, avoid contact with eyes, skin and mucous membranes. External Contact: Rinse affected area with large amounts of water. Internal Contact: Seek medical attention immediately.
3. Contains sodium azide as a preservative which may react with lead joints in copper plumbing to form explosive compounds. Even though the reagent contains minute quantities of sodium azide, drains should be well flushed with water when discarding the reagent.

Preparation of Reagents
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%.

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.

Specimen Collection and Preparation
Serum or 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.

Sample Storage and Stability
Alkaline Phosphatase in serum is stable for 4 days when stored refrigerated (2 – 8°C). It should be stored frozen (-20°C) after this time.[4]

Alkaline Phosphatase (ALP)

Interfering Substances
Results of laboratory studies[5] 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[5] 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 1000 mg/dL Intralipid*

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

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. For further information on interfering substances, refer to Young[6] for a compilation of reported interferences with this test.

Procedure
A complete list of test parameters and operational procedure can be found in the User's Guide appropriate to the analyzer.

Materials Provided
Alkaline Phosphatase (ALP) Reagent.

Stability of Final Reaction Mixture
The Beckman Coulter AU analyzer automatically computes every determination at the same time interval.

Calibration
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.

Quality Control
During operation of the Beckman Coulter AU analyzer at least two levels of appropriate quality control material should be tested a minimum of once a day. In addition, controls should be performed with each new lot of reagent, and after specific maintenance or troubleshooting steps described in the appropriate User's Guide. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

Results
Automatically printed out for each sample in U/L at 37°C.

Dynamic Range
The ALP procedure is linear from 5 to 1500 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.

Expected Values
Adult[7]: 34 – 104 U/L
Expected values may vary with age, sex, diet and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

Specific Performance Characteristics
The following data was obtained using the ALP Reagent on Beckman Coulter AU analyzers according to established procedures. Results obtained in individual laboratories may differ.

Precision[9]
Estimates of precision, based on CLSI recommendations,[8] 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 = 100	Within run		Total	
Mean, U/L	SD	CV%	SD	CV%
53.2	0.6	1.1	0.8	1.5
238.5	1.8	0.7	3.6	1.5

Method Comparison[9]
Patient samples were used to compare this ALP Reagent. The table below demonstrates representative performance on the Beckman Coulter AU analyzers.

Y Method	AU640
X Method	AU600
Slope	0.977
Intercept	- 0.3
Correlation Coeff. (r)	1.000
No. of Samples (n)	183
Range (U/L)	11-1102

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

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. College of American Pathologists: Patient Preparation & Specimen Handling. Fascicle VI. Chemistry/Clinical Microscopy 1992.
5. CLSI/NCCLS, Interference Testing in Clinical Chemistry, EP7-P, 1986.
6. Young, D.S., Effects of Drugs on Clinical Laboratory Tests, 5th Edition, AACC Press, 2000.
7. Beckman Coulter Inc. data on samples collected from 200 blood donors in North Texas.
8. CLSI/NCCLS, Guideline EP5-T2, 1992.
9. Data is on file for specific AU analyzers.

Manufactured by: Beckman Coulter, Inc., 250 S. Kraemer Blvd. Brea, CA 92821, USA

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