

REF 10214-4 4 x 20 mL / 4 mL

ALKALINE PHOSPHATASE (ALP)

Each wedge contains a usable volume of 20 mL of R1 reagent and 4 mL of R2 reagent.

INTENDED USE

The EasyRA ALP reagent is intended for the quantitative determination of alkaline phosphatase in human serum and plasma (with lithium heparin as anticoagulant), using the MEDICA EasyRA[®] clinical chemistry analyzer. Measurement of alkaline phosphatase or its isoenzymes are used in the diagnosis and treatment of liver, bone, parathyroid and intestinal diseases. For *in vitro* diagnostic use. For professional use only.

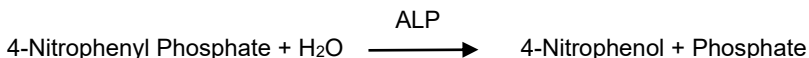
SUMMARY AND EXPLANATION

Alkaline phosphatase refers to a group of phosphatases with an optimum pH of approximately 10. Alkaline phosphatase is found throughout the body's tissues.¹ Most of the ALP in normal adult serum is from the liver or biliary tract.² Normal ALP levels are age-dependent and are elevated in young children and adolescents who exhibit periods of active bone growth. Adult males have higher values than adult females, with the exception of pregnant females, who have increased levels due to placental secretion of ALP.

Elevated ALP values occur in hepatobiliary disorders³ such as hepatitis, cirrhosis, malignancy, and in bone diseases in adults associated with increased osteoblastic activity, which include metastatic carcinoma, rickets, Paget's disease and osteomalacia.³ High ALP serum values are also observed in Hodgkin's disease, congestive heart failure, ulcerative colitis, regional enteritis and intra-abdominal bacterial infections.⁴

PRINCIPLE OF THE PROCEDURE

This test measures the activity of serum ALP by the kinetic method similar to that of Bowers and McComb.⁵ ALP hydrolyses the 4-nitrophenyl phosphate substrate to form 4-nitrophenol and phosphates.



The 4-nitrophenol component is yellow in color at pH 10.4 with an absorbance peak at 405 nm. The rate of the formation of 4-nitrophenol is directly proportional to the alkaline phosphatase activity in the sample.

REAGENTS

ALP Buffer Reagent (R1):

2-Amino-2-methyl-1-propanol, pH 10.4	1.1 mol/L
Magnesium Acetate	2.0 mmol/L
Zinc Sulfate	0.5 mmol/L
HEDTA	2.5 mmol/L

ALP Substrate Reagent (R2):

4-Nitrophenyl Phosphate	80 mmol/L
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PRECAUTIONS

1. Good laboratory safety practices should be followed when handling any laboratory reagent. (CLSI, GP17-A3).
2. The reagents contain less than 0.1% sodium azide, which may react with lead and copper plumbing to form highly explosive metal azides. Refer to the Safety Data Sheet for risk, hazard and safety information.
3. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
4. Do not use washed cuvettes.

INSTRUCTIONS FOR REAGENT HANDLING, STORAGE AND STABILITY

The reagent is ready to use as supplied. Unopened reagent is stable until the expiration date listed on the label if stored at 2-8°C. Uncap the reagent only during the performance of a test. **Keep the reagent tightly closed when not in use.** When used in this way, the reagent is stable on-board the EasyRA clinical chemistry analyzer for the number of days programmed on the RFID chip on the reagent wedge. Do not use the reagent if it is turbid or cloudy or if it fails to recover known serum control values. Protect from light.

SPECIMEN COLLECTION AND STORAGE / STABILITY

Lithium heparin coated tubes may be used for plasma collection. Clear non-hemolyzed serum or plasma should be used. ALP is relatively stable in serum or plasma for 7 days at 2 – 8°C.⁶ However, on storage the enzyme activity increases slightly. An increase in ALP activity is also observed with some reconstituted control sera, stored both at room temperature and in the refrigerator.⁷

PROCEDURE

Materials Provided

Medica ALP Reagent Wedge, REF 10214-4

Additional Materials Required

Medica EasyQC[®] Chemistry/Electrolytes – Level A, REF 10793

Medica EasyQC[®] Chemistry/Electrolytes – Level B, REF 10794

Medica Precision Test Dye Wedge, REF 10764

Medica Cleaner Wedge – Chemistry & ISE, REF 10660 *or*

Medica Cleaner Wedge – Chemistry, REF 10661

Instructions for Use

The reagent is ready to use as supplied. Remove the cap and place the reagent in the EasyRA clinical chemistry analyzer reagent tray located in the reagent area. The on-board stability (8 days maximum) is programmed on the RFID chip on the reagent wedge.

Note: Check inside the necks of the wedge for foam after removing the caps and placing the wedge on the EasyRA clinical chemistry analyzer. If there is foam, remove it with a swab or a disposable pipette before performing the test. Use separate swabs or disposable pipettes for R1 and R2.

Calibration

Not applicable.

Quality Control

It is recommended that two levels of human serum-based controls (normal and abnormal) be run with the assay daily whenever patient testing is performed and with each reagent lot change. Failure to obtain the proper range of values in the assay of control material may indicate reagent deterioration, instrument malfunction or procedural errors. The laboratory should follow local, state and federal quality control guidelines when using quality control materials.

Results

After completion of the assay, the EasyRA clinical chemistry analyzer calculates the ALP concentration from the change in absorbance per minute, sample volume, total reaction volume, pathlength (cm) of 0.6 and molar absorptivity of 18.75.

$$\text{ALP (U/L)} = (\Delta A/\text{Min}) \times \frac{(\text{Total Volume}(\mu\text{L}) \times 1000)}{(\text{Molar absorptivity} \times \text{Pathlength}(\text{cm}) \times \text{Sample Volume}(\mu\text{L}))}$$

EXPECTED VALUES¹

The reference range for ALP in serum is as follows:

Adult: 34 –114 U/L (37°C)

These values are guidelines. It is recommended that each laboratory establish its own range of expected values since differences exist among instruments, laboratories and local populations.

Procedural Limitations (e.g., if sample is above assay range)

The ALP test is linear to 800 U/L. If the Absorbance change per Minute ($\Delta A/\text{Min}$) is greater than 0.1915, it will be flagged with “SD” (substrate depletion) by the analyzer. Absorbance changes per minute above this are above the linear range of the test. If scheduled by the operator, the sample will be re-tested using a smaller sample volume. The retest results are calculated to reflect the use of the smaller sample volume. This will extend the reportable range of the ALP test to 1600 U/L.

PERFORMANCE CHARACTERISTICS⁸

Reportable Range

The reportable range is 10 to 800 U/L. Extended range is 10 to 1600 U/L when half of the sample is used (1:1 dilution).

Inaccuracy/ Correlation (CLSI, EP09-A2)

The following table lists the data obtained in a comparison of the Medica reagent for ALP (y) on the EasyRA clinical chemistry analyzer utilizing a primary wavelength of 405 nm only to the performance of the same ALP reagent (x) on the EasyRA clinical chemistry analyzer utilizing a primary wavelength of 405 nm and a secondary wavelength of 700 nm. The data shown below represents single determinations obtained utilizing a primary wavelength and a secondary wavelength on the EasyRA clinical chemistry analyzer vs. the average of two replicate values obtained utilizing a primary wavelength on the EasyRA clinical chemistry analyzer.

Number of Samples	47	Range of Samples	17 to 744 U/L
Slope	0.9938	y Intercept	3.6906
Correlation Coefficient	0.9998	Regression Equation	$Y = 0.9938 * X + 3.6906$

The following table lists the data obtained in a comparison of matched serum (x) and the lithium heparinized plasma (y) samples using the Medica reagent for Alkaline Phosphatase on the EasyRA clinical chemistry analyzer. The data below represents a single plasma determination vs. the average of two replicate serum values.

Number of Samples	74	Range of Samples	13 to 764 U/L
Slope	1.0284	y Intercept	-6.4285
Correlation Coefficient	0.9902	Regression Equation	$Y = 1.0284 * X - 6.4285$

NOTE: Plasma ALP results may be about 5% to 6% lower than serum ALP results.⁹ Medica recommends that clinical laboratories establish their own reference ranges for the ALP test depending on sample type.

Imprecision (CLSI, EP05-A2)

Duplicate measurements of each of three levels of QC material were tested twice a day for 20 days. Both within-run precision and total precision were determined from these data.

Within run imprecision:

QC Level U/L	Within Run SD U/L	Within Run CV %
295	2.8	1.0
184	2.1	1.2
73	1.3	1.7

Total Imprecision:

QC Level U/L	Total Imprecision SD U/L	Total Imprecision CV %
295	6.2	2.1
184	4.4	2.4
73	2.1	2.9

Linearity (CLSI, EP06)

Linear from 10 to 800 U/L, based on the linear regression $Y = 0.9845 * X + 3.3253$.

Limit of Blank (LOB):	3.0 U/L	(CLSI, EP17-A)
Limit of Detection (LOD):	4.5 U/L	(CLSI, EP17-A)
Limit of Quantitation (LOQ):	9.1 U/L	(CLSI, EP17-A)

Interfering Substances (CLSI, EP07)

Less than 10% interference was classified as "no significant interference."

There is significant interference to hemolysis. Use samples free of hemolysis.

No significant interference was found in levels up to 20 mg/dL of bilirubin

No significant interference was found in levels of up to 500 mg/dL of triglycerides (using Intralipid*).

No significant interference was found in levels of up to 30 mg/dL of ascorbic acid.

*Intralipid is a registered trademark of Pharmacia AB, Clayton, NC.

Young provides a list of drugs and other substances that interfere with clinical chemistry tests.^{10, 11}

REFERENCES

- 1 Tietz NW. Textbook of Clinical Chemistry, 2nd ed. WB Saunders and Co., Philadelphia, PA, p. 831-832 (1994).
- 2 Kaplan MM, Righetti A. *J Clin Inv.* 1955; 34:126.
- 3 Searcy, RL. *Diagnostic Biochemistry.* New York, NY: McGraw-Hill; 1969.
- 4 Kaplan MM. *New Engl J Med.* 1972;286:200.
- 5 Bowers, G.N., Jr., McComb, R.B.: A continuous spectrophotometric method for measuring the activity of serum alk. phos. *Clin Chem* 12:70-89, 1966.
- 6 Gunder WG, Zawata B et al. *The Quality of Diagnostic Samples.* 3rd Edition: 2010. p. 32-3
- 7 Massion, C.G., Granckenfeld, J.K.: Alkaline phosphatase: Liability in fresh and frozen human serum and in lyophilized control material. *Clin Chem.* 18: 366, 1972.
- 8 Data on file at Medica.

- 9 Ciuti R., Rinaldi G. *Serum and Plasma Compared for Use in 19 Common Chemical Tests Performed in the Hitachi 737 analyzer*, Clin. Chem. 35:1562(1989).
- 10 Young, DS., Pestaner, L.C., Gibberman, V.; Effects of drugs on clinical laboratory tests. *Clin Chem* 21: 246D, 1975.
- 11 Young DS. Young's Effects on-line. *Effects of Drugs, Physiology, Preanalytical variables and herbs on Clinical Laboratory Tests*. AACC www.fxol.org

EASYRA ASSAY PARAMETERS (ALP)

Primary wavelength (nm)	405
Secondary wavelength (nm)	700
Reaction type	Enzyme (0)
Reaction direction	Increase
Reagent blank	No
Sample blank	No
Max. first interval Abs. change	0.075
Reaction time	7.6 min
Reagent on-board stability	8 days

Serum/Plasma

Sample volume (µl)	6.0
Diluent 1 volume (µl)	20
Diluent 2 volume (µl)	20
Reagent volume R1 (µl)	200
Reagent volume R2 (µl)	36
Decimal places (default)	0
Units (default values)	U/L
Dilution factor	1:1 (to extend measuring range)
Linearity	10 to 800 U/L

