MEDICA

IVD CE

**REF 10214-4 4 x 20 mL / 4 mL**

## ALKALINE PHOSPHATASE (ALP)

Wedges each contain usable volumes 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 only. 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 disorders[3] such as hepatitis, cirrhosis, malignancy, and in bone diseases in adults associated with increased ostoblastic 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.

4-Nitrophenyl Phosphate + $H_2O$ (ALP)$\rightarrow$ 4-Nitrophenol + Phosphate

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 | 0.35 mol/L |
| Magnesium Chloride | 2.0 mmol/L |
| Zinc Sulfate | 1.0 mmol/L |
| HEDTA | 2.0 mmol/L |

**ALP Substrate Reagent (R2):**

| | |
|---|---|
| 4-Nitrophenyl Phosphate | 16 mmol/L |

### PRECAUTIONS

1. Good laboratory safety practices should be followed when handling any laboratory reagent. (CLSI, GP17-A2).
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 2º-8ºC. 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 in the refrigerated reagent area of the Medica EasyRA Chemistry Analyzer for the number of days programmed on the RFID chip on the reagent wedge. If the analyzer does not have the refrigeration option, the reagents need to be recapped and stored at 2º-8º C after use. Do not use the reagent if it is turbid or cloudy or if it fails to recover known serum control values.

**SPECIMEN COLLECTION AND STORAGE / STABILITY**
Lithium heparin coated tubes may be used for plasma collection. Clear unhemolyzed serum or plasma should be used. Serum ALP is relatively stable for 7 days at 2 – 8°C. 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.[6]

**PROCEDURE**

**MATERIALS PROVIDED**
Medica ALP Reagent Wedge, REF 10214

**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 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 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 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.

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

**Expected Values[1]**
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 (ΔA/Min) is greater than 0.1875, 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 CHARACTERISTICS[7]**

**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, EP9-A2)**
The following table lists the data obtained in a comparison of the Medica Reagent for ALP (y) on the EasyRA Analyzer utilizing a primary wavelength of 405 nm only to the performance of the same ALP reagent (x) on the EasyRA 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 Analyzer vs. the average of two replicate values obtained utilizing a primary wavelength on the EasyRA Analyzer.

| | | | |
|---|---|---|---|
| Number of Samples | 47 | Range of Samples | 17 – 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 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 – 764 U/L |
| Slope | 1.0284 | y Intercept | -6.4285 |
| Correlation Coefficient | 0.9902 | Regression Equation | Y = 1.0284*X – 6.4285 |

**Cobas Mira is a registered trademark of Roche Diagnostics, INC., Indianapolis, IN.*

NOTE: Plasma ALP results may be about 5% to 6% lower than serum ALP results.[8] Medica recommends that clinical laboratories establish their own reference ranges for the ALP test depending on sample type.

**Imprecision (CLSI, EP5-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, EP6-A)**
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 EP7-A)**
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*).

**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.[9,10]

**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 Massion, C.G., Grankenfeld, J.K.: Alkaline phosphatase: Liability in fresh and frozen human serum and in lyophilized control material. *Clin Chem*. 18: 366, 1972.
7 Data on file at Medica.
8 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).
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10 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 |

**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 |
| Molar Absorptivity | 18.75 |
| Reagent on-board stability | 8 days |

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