BIOLABO
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ALKALINE PHOSPHATASE (DEA)

Reagent for quantitative determination of alkaline phosphatase activity [EC 3.1.3.1] in human serum and plasma

REF 92214	**R1** 8 x 30 mL	**R2** 8 x 30 mL
REF 92314	**R1** 10 x 100 mL	**R2** 10 x 100 mL

TECHNICAL SUPPORT AND ORDERS
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CE

IVD IN VITRO DIAGNOSTIC USE

CLINICAL SIGNIFICANCE (1)

Alkaline phosphatase (ALP) is found in many tissues, including bone, liver, intestine, kidney, and placenta. Serum ALP measurements are of particular interest in the investigation of two groups of conditions: hepatobiliary diseases (hepatitis, cirrhosis or malignacy) and bone diseases associated with increased osteoblastic activity (child's rickets with D vitamin deficiency, Paget's disease, hyperparathyroidism in the skeleton, metastasic carcinoma).
ALP determined by usual biochemical methods reflects total serum levels and does not distinguish the source of the isoenzyme. Clinicians must thererefore rely on other parameters of liver or other organ function or a more specific determination of ALP to assess its source.

PRINCIPLE (1) (4) (5)

Optimized method based on DGKC (German Society of Clinical Chemistry, 1972) and SCE (Scandinavian Society of Clinical Chemistry) recommendations.
In alkaline solution, ALP catalyses the hydrolysis of p-nitrophenyl phosphate in p-nitrophenol and phosphate.
The rate of formation of p-nitrophenol, proportional to the ALP activity, is measured at 405 nm.

REAGENTS COMPOSITION

Vial R1 **BUFFER**

D.E.A. (Diethanolamine) buffer pH 10 (25°C)	1	mol/L
Magnesium Chloride	0.5	mmol/L
Preservative		

Vial R2 **SUBSTRATE**

p-nitrophenylphosphate	10	mmol/L

SAFETY CAUTIONS

BIOLABO reagents are designated for professional, in vitro diagnostic use.
- Verify the integrity of the contents before use.
- Use adequate protections (overall, gloves, glasses).
- Do not pipette by mouth.
- In case of contact with skin or eyes, thoroughly wash affected areas with plenty of water and seek medical advice.
- Reagents contain sodium azide (concentration < 0.1%) which may react with copper and lead plumbing. Flush with plenty water when disposing.
- Material Safety Data Sheet is available upon request.
- Waste disposal: Respect legislation in force in the country.

All specimens should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

REAGENTS PREPARATION

Vial R2: Use a non-sharp instrument to remove aluminium cap.
Add promptly the contents of vial R2 (Substrate) into vial R1 (Buffer)
Mix gently and wait for complete dissolution before using reagent (approximately 2 minutes).

STABILITY AND STORAGE

Store at 2-8°C, well recap in the original vial and away from light.
- Unopened, reagents are stable until expiry date stated on the label of the kit when stored and used as described in the insert.
- Once reconstituted, working reagent is stable at least for 30 days when free from contamination.
- Discard reagent if cloudy or if absorbance at 405 nm is > 0.800.
- Don't use working reagent after expiry date stated on the label of the Kit.

SPECIMEN COLLECTION AND HANDLING (2)

Unhemolysed serum or heparinised plasma immediately refrigerated.

ALP activity is stable in the specimen for:
- 2-3 days at 2-8°C.
- 1 month at –25°C.

INTERFERENCES (3)

Avoid hemolysed serum.

Triglycerides:	No significant interference up to 1000 mg/dl
Hemoglobin:	No significant interference up to 1.6 g/dl
Total bilirubin:	No significant interference up to 15 mg/dl

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. Normal and pathological control sera.

CALIBRATION

1. Results will depend on the accuracy of the instrument calibration, the time counting, the respect of reagent/specimen ratio and the temperature control.
- Use the theoretical calibration factor (§ **CALCULATION**)
- Or REF 95015 BIOLABO Multicalibrator (calibration value determined with validated statistical technics and metrologically controlled instrument)
- or a multiparametric calibrator traceable to a reference method or material

QUALITY CONTROL

- BIOLABO EXATROL-N Level I REF 95010.
- BIOLABO EXATROL-P Level II REF 95011.
- Other assayed control sera referring to the same method.
- External quality control program.

It is recommended to control in the following cases:

- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If control is out of range, apply following actions:

1. Repeat the test with the same control.
2. If control is still out of range, prepare a fresh control serum and repeat the test.
3. If control is still out of range, verify analysis parameters: wavelength, temperature, specimen/reagent ratio, time counting, calibration factor.
4. If control is still out of range, use a new vial of reagent and reassay.
5. If control is still out of range, please contact BIOLABO technical support or your local Agent.

EXPECTED VALUES (2)

IU/L at 37°C	Men	Women
20-29 years	100-320	70-260
30-39 years	90-320	70-260
40-49 years	100-360	80-290
50-59 years	110-390	110-380
60-69 years	120-450	110-380
> 69 years	120-460	90-430

Children: Values may be increased (up to threefold during puberty)

Example of values given for information: 245-768 IUI/L à 37°C

Each laboratory should establish its own normal ranges for the population that it serves.

PERFORMANCE CHARACTERISTICS

Values were determined with Specimen/Reagent Ratio 15/1000 at 37°C.

Within-run N = 33	*Normal level*	*High level*
Mean IU/L	133	306
S.D. IU/L	2.26	3.95
C.V. %	1.7	1.29

Beetwen run N = 33	*Normal level*	*High level*
Mean IU/L	147	762
S.D. IU/L	2.52	11.42
C.V. %	1.71	1.5

Detection limit: approximately 30 IU/L

Sensitivity for 10 IU/L: approximately 0.002 ΔAbs/min at 405 nm.

Comparison with commercially available reagent:

y = 0.9793 x + 3.1261 r = 0.9962

LINEARITY

The assay is linear up to 1200 IU/L (20 µKat/L).

If ΔAbs/min > 0.225, dilute specimen with saline solution and re-assay taking into account the dilution factor to calculate the result. Linearity limit depends on the specimen/reagent ratio.

MANUAL PROCEDURE

Let stand reagent and specimens at room temperature.

Pipette into 1 cm path length thermostated cuvette:	
Reagent	1 mL
Bring to 37°C then add:	
Specimen	10 µL
Mix. Start a timer. Record initial absorbance after 1 minute at 405 nm. Record the absorbance again every minute during 3 minutes.	
Calculate absorbance change per minute (ΔAbs/min).	

Note:
Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support.

CALCULATION

Calculate the result as follows:

With theoretical factor:

IU/L = (ΔAbs/min) x 5450

$$\text{µkat/L} = \frac{\text{IU/L}}{60}$$

With serum multicalibrator

$$\text{ALP Activity} = \frac{(\Delta\text{Abs/min) Assay}}{(\Delta\text{Abs/min) Calibrator}} \times \text{Calibrator Concentration}$$

REFERENCES

(1) *TIETZ N.W. Text book of clinical chemistry, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 676-684 and p.1429-1431.*
(2) *Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006) p. 80-83*
(3) *YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p.3-26 to 3-35*
(4) *Scandinavian Journal of clinical and laboratory investigation (1974), vol.33, p.291-306*
(5) *Recommendations of the German Society for Clin. Chemistry Z .Klin. Chem. Klin. Biochem. (1972), 10, p.290-291*

Manufacturer | Use by | IVD In vitro diagnostic | Temperature limitation | REF Catalogue number | See insert | LOT Batch number | Store away from light | sufficient for | dilute with