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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

CE

IVD

Made In France

I: corresponds to significant modifications

INTENDED USE

This reagent is designated for professional use in laboratory (automated method).
It allows the quantification of global activity of the alkaline phosphatase enzyme in human serum or plasma.

GENERALITIES (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 malignancy) and bone diseases associated with increased osteoblastic activity (child's rickets with D vitamin deficiency, Paget's disease, hyperparathyroidism in the skeleton, metastatic carcinoma).

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 catalyzes 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

R1 ALKALINE PHOSPHATASE Buffer

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

Danger Eye Dam..1: H318 – Causes serious eye damage

P280: Wear protective gloves/protective clothing/eye protection/face protection, P305+P351+P338 IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing,
P310: Immediately call a POISON CENTER or doctor/physicians. For more details refer to current Material Safety Data Sheet (MSDS).
Classification due to: Diethanolamine 2,5 - < 10%

For more details, refer to SDS (Safety data sheet)

R2 ALKALINE PHOSPHATASE Substrate

p-nitrophenyl phosphate	10	mmol/L

According to 1272/2008 regulation, this reagent is not classified as dangerous

Once reconstituted: Working Reagent is classified as vial R1.

SAFETY CAUTIONS

- Refer to current Material Safety Data Sheet available on request or on www.biolabo.fr
- Verify the integrity of the contents before use.
- Waste disposal: Respect legislation in force in the country.
- All specimens or reagents of biological origin should be handled as potentially infectious. Respect legislation in force in the country.

I Any serious incident that has occurred in connection with the device is notified to the manufacturer and the competent authority of the Member State in which the user and/or patient is based.

REAGENTS PREPARATION

Use a non-sharp instrument to remove the cap.

Add promptly the contents of vial R2 into vial R1.

Mix gently and wait for complete dissolution.

STABILITY AND STORAGE

Stored away from light, well caped in the original vial at 2-8°C, when stored and used as described, reagents are stable:

Unopened:

- Until expiry date stated on the label of the kit.

Once opened:

- Reconstitute immediately substrate (vial R2)
- Contents of vial R1 (Buffer) is stable at least 6 months.

Once reconstituted:

- Transfer requested quantity and store in the original vial at 2-8°C.
- Working reagent is stable at least for 30 days.
- Discard reagent if cloudy or if absorbance at 405 nm is > 0.800.
- Don't use working reagent after expiry date of the Kit.

SPECIMEN COLLECTION AND HANDLING (2)

Unhemolysed serum or heparinized plasma immediately refrigerated.

ALP activity is stable in the specimen for:

- 2-3 days at 2-8°C.
- 1 month at –25°C.

LIMITATIONS (3)

Avoid hemolyzed serum.
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. Spectrophotometer or Biochemistry Clinical Analyzer

Manufacturer	Expiry date	In vitro diagnostic	Storage temperature	Dematerialized water	Biological risk
Product Reference	See Insert	Batch number	Store away from light	Sufficient for	Dilute with

QUALITY CONTROL

- REF 95010 EXATROL-N Level I
- REF 95011 EXATROL-P Level II
- 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. Prepare a fresh control serum and repeat the test.
2. If control is still out of range, use a new vial of calibrator or a fresh calibrator and repeat the test.
3. If control is still out of range, repeat the tests with a new vial of reagent.

If control is still out of range, please contact BIOLABO technical support or your local Agent.

REFERENCE INTERVAL (2)

at 37°C	Men (IU/L)	Women (IU/L)
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 at 37°C on KENZA 240TX

Refer to the application of analyzer used.

Linearity Range: between 50 and 1500 IU/L

Detection limit: approx. 35 IU/L

Precision:

Within-run N = 20	*Low level*	*Normal level*	*High level*
Mean (IU/L)	41	172	404
S.D. (IU/L)	1.0	1.9	4.5
C.V. %	2.3	1.1	1.1

Between run N = 20	*Low level*	*Normal level*	*High level*
Mean (IU/L)	49	171	399
S.D. IU/L	2.3	5.2	15.5
C.V. %	4.6	3.1	3.9

Comparison studies with commercially available reagent:

Realized on serum specimens (n=67) between 53 and 459 IU/L

y = 0.9811 x + 2.5 r = 0.9964

Analytical Sensitivity: approx. 0.005 abs/min for 10 IU/L

Interferences:

Turbidity	No interference up to 0.291 abs
Ascorbic acid	No interference up to 2500 mg/dL
Total bilirubin	Negative interference from 390 µmol/L
Direct bilirubin	No interference up to 373 µmol/L
Hemoglobin	Negative interference from 152 µmol/L
Glucose	No interference up to 1088 mg/dL

Other substances may interfere (see § Limits)

On the board stability: 13 days

Calibration Stability: 5 days

Make a new calibration when changing reagent batch, if quality control results are found out of the range and after maintenance operations

CALIBRATION

- REF 95015 Multicalibrator traceable to *an Internal Masterlot*

The calibration frequency depends on proper instrument functions and on the preservation of reagent

PROCEDURE

Manual Method

Let stand reagent and specimens at room temperature.

Pipette into 1 cm path length thermostated cuvette (37°C):	
Reagent	1000 µL
Standard / Control or Specimen	10 µL
Mix. After 1 minute at 405 nm, record the absorbance each minute during 3 minutes.	
Calculate absorbance change per minute (ΔAbs/min).	

Notes:

1- Performances with manual procedure should be validated by user.

2- Kenza applications and other applications proposal are available on request.

CALCULATION

With serum Multicalibrator

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

With theoretical factor:

Activity (IU/L) = ΔAbs/min x Factor

$$\text{Factor} = \frac{VR \times 1000}{18.35 \times VE \times P}$$

With:
VR = Total reactional volume (mL)
VE = Specimen volume (mL)
18.35 = Molar extinction coefficient for PNPP at 405 nm
P = Path length (cm).

Example, with manual Procedure,

(Path length 1 cm, 37°C, 405 nm):

IU/L = (ΔAbs/min) x 5450

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

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*