



BIOLABO
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ALKALINE PHOSPHATASE

Colorimetric Method

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

REF 80014 R1 2 x 56 mL R2 1 x 2,5 mL
R3 1 x 27 mL R4 1 x 27 mL

TECHNICAL SUPPORT AND ORDERS

Tel : (33) 03 23 25 15 50

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Latest revision : www.biolabo.fr

IVD

Made In France

I: corresponds to significant modifications

I INTENDED USE

This reagent is designated for professional use in laboratory (manual or automated method).

It allows the quantitative determination of alkaline phosphatase activity [EC 3.1.3.1] in human serum and plasma to screen its level.

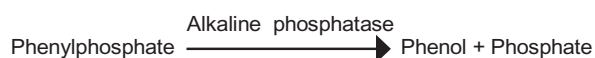
I GENERALITIES (1)

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 and bone diseases associated with increased osteoblastic activity.

ALP determined reflects total serum levels and does not distinguish the source of the isoenzyme. Clinicians must therefore rely on other parameters of liver or other organ function or a more specific determination of ALP to assess its source.

PRINCIPLE (4) (5)

Colorimetric determination of the ALP activity as follows :



Free phenol liberated by hydrolysis of the substrate reacts then with 4-amino-antipyrine in the presence of alkaline potassium ferricyanide to form a red-coloured complex which absorbance measured at 510 nm is directly proportional to the ALP activity in the specimen.

REAGENTS

R1 ALKALINE PHOSPHATASE Substrate-Buffer
Disodium Phenyl phosphate 5 mmol/L
Carbonate-bicarbonate buffer pH 10 50 mmol/L
Stabilizer

R2 ALKALINE PHOSPHATASE Standard
Phenol corresponding to 20 U King and Kind

R3 ALKALINE PHOSPHATASE Blocking Reagent
4-Amino-antipyrine 60 mmol/L
Sodium arsenate 240 mmol/L

DANGER: Acute Tox. 4: H302 - Harmful if swallowed.
Aquatic Chronic 2: H411 - Toxic to aquatic life with long lasting effects.
Carc. 1A: H350 - May cause cancer.

P264: Wash thoroughly after handling, P273: Avoid release to the environment, P280: Wear protective gloves/face protection/protective clothing/respiratory protection/protective footwear, P308+P313: IF exposed or concerned: Get medical advice/attention, P501: Dispose of contents/container in accordance with regulations on hazardous waste or packaging and packaging waste respectively. Classification is due to sodium arsenate 2,5 - < 10%. For more details, see Safety Data Sheet (SDS)

R4 ALKALINE PHOSPHATASE Dye Reagent
Potassium ferricyanide 150 mmol/L

R1, R2, R4: These reagents are not classified as hazardous according to CLP Regulation (EC) No 1272/2008.

I 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.

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

Reagents are ready to use.

STABILITY AND STORAGE

Store at 2-8°C and away from light.

- Unopened :
Reagents are stable upon expiry date stated on the label.
- Once reconstituted :
Reagents are stable at least for 3 months when free from contamination.

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.

LIMITS (3)

Avoid haemolysed serum.

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

MATERIALS REQUIRED BUT NOT PROVIDED

- Basic medical analysis laboratory equipment.
- Spectrophotometer and water bath thermostated at 37°C

QUALITY CONTROL

- 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. Check the temperature and 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, use a new vial of Standard and repeat the test.
4. If control is still out of range, calibrate with a new vial of reagent.
5. If control is still out of range, please contact BIOLABO technical support or your local Agent.

REFERENCE INTERVALS (2)

Kind and King Units /dL		IU/L (37°C)
Birth	5-15	[36-107]
Premature	(1.5 to 2 x adult value)	
1 month	10-30	[71-213]
3 years	10-20	[71-142]
10 years	15-30	[107-213]
Adults	4.5-13	[32-92]

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

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

CALIBRATION

- Standard enclosed in the Kit (vial R2)

PROCEDURE

Manual method

Let stand reagent and specimens at room temperature.

Prepare tubes as follows :	Reagent blank	Specimen blank	Standard	Assay
Reagent R1	2 mL	2 mL	2 mL	2 mL
Incubate 5 minutes at 37°C.				
Specimen				50 µL
Reagent R2 (Standard)			50 µL	
Let stand exactly 15 minutes at 37°C.				
Reagent R3	0,5 mL	0,5 mL	0,5 mL	0,5 mL
Mix well.				
Reagent R4	0,5 mL	0,5 mL	0,5 mL	0,5 mL
Specimen		50 µL		
Demineralised water	50 µL			
Mix. Incubate 10 minutes at room temperature and away from light. Read absorbances of the blank specimen, standard and assay at 510 nm against reagent blank. Coloration is stable for 45 minutes away from light.				

Notes:

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

CALCULATION

- 1) Results (Kind and King unit):

Quantity of enzyme which, on reaction's conditions, liberates 1 mg of phenol in 15 minutes at 37°C.

$$\text{ALP activity (Kind and King units/ 100 mL)} = \frac{\text{Abs Assay} - \text{Abs Specimen blank}}{\text{Abs Standard}} \times 20$$

- 2) Result (IU/L) = 7,09 x Result (Kind and King Unit/100 mL)

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 et p.1429-1431.
- (2) Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006) p. 78-83
- (3) YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) P.3-26 à 3-35
- (4) Kind P. R. N., King E. J, Estimation of plasma Phosphatase by determination of hydrolysed phenol with amino-anti-pyridine, J. Clin. Path. (1954), 7, p.322-326
- (5) Belfield A, Goldberg D. M., Revised assay for serum phenylphosphatase activity using 4-amino-antipyrine, Enzyme (1971), 12, p.561-573

 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