ALKALINE PHOSPHATASE BR CE

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For *in vitro* diagnostic use only	

ALKALINE PHOSPHATASE BR

DGKC
Colorimetric method

KINETIC

PRINCIPLE

Alkaline phosphatase (ALP) catalyze the hydrolysis of 4-nitrophenylphosphate (4-NPP) with the formation of free 4-nitrophenol and inorganic phosphate, acting the alkaline buffer as a phosphate-group acceptor.
The reaction is monitored kinetically at 405 nm by the rate of formation of 4-nitrophenol, proportional to the activity of ALP present in the sample.

$$\text{4-Nitrophenilphosphate} + H_2O \xrightarrow[pH > 9]{ALP,\ Mg^{++}} \text{4-Nitrophenol} + P_i$$

The method follows the proposed optimised formulation of the DGKC.[1]

REAGENT COMPOSITION

R1 **ALP buffer.** DEA buffer 1.25 mol/L pH 10.2, magnesium chloride 0.6 mmol/L. Biocides.

R2 **ALP substrate**. 4-NPP 50 mmol/L. Biocides.

STORAGE AND STABILITY

Store at 2-8ºC.
The Reagents are stable until the expiry date stated on the label.

REAGENT PREPARATION

Working reagent. Mix 4 mL of **R1** + 1 mL of **R2**. Stable for 5 days at 20-25ºC or 30 days at 2-8ºC. Protect from light.
Discard the reagent if the blank presents an absorbance over 0.800 at 405 nm. against distilled water or if it fails to recover the declared values of control sera.

SAMPLES

Serum or heparinized plasma, free of hemolysis. Other anticoagulants such as EDTA, oxalate and citrate inhibit the enzyme by complexing Mg^{++} and should not be used.
Alkaline phosphatase in serum or plasma is stable for 7 days at 2-8ºC.

INTERFERENCES

- Bilirubin (>20 mg/dL) and triglycerides (>10 g/L) do not interfere.
- A list of drugs and substances wich cause changes in ALP levels or interfere with its measurement can be found published.[2]

MATERIALS REQUIRED

- Photometer or spectrophotometer with a thermostatted cell compartment set at 25/30/37ºC, capable to read at 405 nm.
- Stopwatch, strip-chart recorder or printer.
- Cuvettes with 1-cm pathlength.
- Pipettes to measure reagent and samples.

PROCEDURE

1. Preincubate working reagent, samples and controls to reaction temperature.
2. Set the photometer to 0 absorbance with distilled water.
3. Pipette into a cuvette:

Working reagent	1.0 mL
Sample or control	20 µL

4. Mix gently by inversion. Insert cuvette into the cell holder and start stopwatch.
5. Incubate for 1 minute and record initial absorbance reading.
6. Repeat the absorbance readings exactly after 1, 2 and 3 minutes.
7. Calculate the difference between absorbances.
8. Calculate the mean of the results to obtain the average change in absorbance per minute (ΔA/min).

CALCULATIONS

U/L = ΔA/min x 2764

Samples with ΔA/min exceeding 0.250 at 405 nm should be diluted 1:2 with saline and assayed again. Multiply the results by 2.

If results are to be expressed as SI units apply:
U/L x 0.01667 = µkat/L

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REFERENCE VALUES[3]

Serum, plasma

	25ºC
Children, up to	480 U/L (8.0 µktal/L)
Adults, up to	180 U/L (3.0 µktal/L)

	30ºC
Children, up to	590 U/L (9.8 µktal/L)
Adults, up to	220 U/L (3.7 µktal/L)

	37ºC
Children, up to	800 U/L (13.3 µktal/L)
Adults, up to	270 U/L (4.5 µktal/L)

It is recommended that each laboratory establishes its own reference range.

QUALITY CONTROL

To ensure adequate quality control (QC), each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns.

REF **1980005** HUMAN MULTISERA NORMAL
Borderline level of ALP. Assayed.

REF **1985005** HUMAN MULTISERA ABNORMAL
Elevated level of ALP. Assayed.

CLINICAL SIGNIFICANCE

Serum ALP measurements are of particular interest in the investigation of two groups of conditions: *bone disease* and *hepatobiliary disease*.
Among the bone diseases, the highest levels are found in Paget´s disease and in patients with osteogenic bone cancer, and moderate raises in osteomelacia and rickets, the latter falling to normal on treatment with vitamin D.
Physiological bone growth elevates ALP in serum of growing children and a transient elevation may be found during healing of bone fractures.

Causes of decreased plasma ALP level are: cretinisme, vitamin D deficiency and hypophosphatasia, an hereditary bone disease.

The response to the liver to any form of biliary tree obstruction is to sinthesize more ALP. Intrahepatic obstruction of the bile flow by invading cancer or drugs raises serum ALP. Any drug that is hepatotoxic or induces cholestasis will greatly increase serum ALP. Well over 200 drugs have been shown to increase serum ALP in susceptible patients.[4]

ANALYTICAL PERFORMANCE

- ***Linearity.*** Up to 800 U/L
- ***Precision***

U/L	Within-run		
Mean	49	186	301
SD	0.7	2.8	5.2
CV%	1.43	1.5	1.72
N	21	21	21

Replicates: 21 for each level.
Instrument: PHILIPS

- ***Sensitivity.*** Using this reagent and method an ΔA/min of 0.010 reac at 405 nm is equivalent to 1,60 U/L of phosphatase activity.
- ***Correlation.*** This assay (y) was compared with a similar commercial method (x). The results were:
  N = 25 r = 0.999 y = 0.961x + 5.431

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B1103-1/0312
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