ALKALINE PHOSPHATASE BR CE

REF 1103005 2 x 50 mL **CONTENTS** R1. Reagent 2 x 40 mL R2. Reagent 1 x 20 mL	REF 1103010 3 x 100 mL **CONTENTS** R1. Reagent 3 x 80 mL R2. Reagent 1 x 60 mL
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.

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

This test has been formulated according the standarized method described by 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.
All the kit compounds are stable until the expiry date stated on the label. Do not use reagents over the expiration date.
Store the vials tightly closed, protected from light and prevented contaminations during the use.

Discard If appear signs of deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 405 nm > 1.000 in 1cm cuvette.

REAGENT PREPARATION

Working reagent. Mix 4 mL of **R1** + 1 mL of **R2**. Stable for 5 days at 20-25ºC or 15-30 days at 2-8ºC, depending on the remaining caducity of both reagents.
Protect from light.

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

- Lipemia (intralipid 20 g/L) do not interfere.
- Bilirubin (20 mg/dL) do not interfere.
- Hemoglobin (>2 g/L) may affect the results.
- Other drugs and substances may interfere[2,3].

MATERIALS REQUIRED

- Photometer or spectrophotometer with a thermostatted cell compartment set at 25/30/37ºC, capable of reading 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

QUALITY SYSTEM CERTIFIED
ISO 9001 ISO 13485

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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.

If the values are found outside of the defined range, check the instrument, reagents and procedure.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

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 synthesize 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

- ***Detection Limit :*** 14.35 U/L
- ***Linearity :*** Up to 800 U/L
- ***Precision:***

U/L	Within-run		Between-run	
Mean	313	490	313	490
SD	1.45	11.17	10.23	12.47
CV%	0.46	2.32	3.27	2.54
N	10	10	10	10

- ***Sensitivity:*** 0,3 mA/min/ U/L phosphatase.
- ***Correlation:*** This assay (y) was compared with a similar commercial method (x). The results were:
  N = 50 r = 0.98 y = 1.11x - 9.19

The analytical performances have been generated using on automatic instrument. Results may vary depending on the instrument.

NOTES

1. This method may be used with different instruments. Any application to an instrument should be validated to demonstrate that results meets the performance characteristics of the method. It is recommended to validate periodically the instrument. Contact to the distributor for any question on the application method.
2. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

REFERENCES

1. German Society for Clinical Chemistry: Recommendations of the Enzyme Commision. Z. Klin. Chem. Klin. Biochem. 10 : 281 (1972).
2. Young, D.S. Effects of Drugs on Clinical Laboratory Tests. 4th Edition. AACC Press (1995).
3. Tietz. N.W. Clinical Guide to Laboratory Tests, 3rd Edition. W.B. Saunders Co. Philadelphia, PA. (1995).
4. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.

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