ALKALINE PHOSPHATASE
Insert

Ref.: **40**

Intended use . System for alkaline phosphatase determination in blood samples by fixed-time-kinetic method and end point measurement.

Test principle . The serum alkaline phosphatase hydrolyses thymolphthalein monophosphate releasing thymolphthalein that has a blue color in alkaline solution. The color is proportional to the enzyme activity, and is measured at 590 nm. The final product of the reaction has a mixture of blue and the substrate color.

Summary . Based on King[2] and Roy[3] studies, Labtest focused all efforts researching thymolphthalein monophosphate, an ester of phosphoric acid substrate. Using thymolphthalein monophosphate, it is possible to measure directly the hydrolysis product, altering only the pH. pH modification induces at the same time the interruption of the enzyme activity and the blue color formation that is a characteristic of the reaction product what is measured photometrically.

Thymolphthalein monophosphate is stable at room temperature without presenting spontaneous hydrolysis and the system of measurement, with end point colorimetry, allows manual assay in a large scale.

The measurement of the enzyme activity is performed for 10 minutes with zero-order kinetic with concentrations up to 500 U/L, presenting linear results to enzymes activities 12 times higher than the reference values.

Methodology . Roy modified.

Reagents

1. R1 - Substrate- Store at 15 - 25 ºC.
Reagent label bears expiration date. Thymolphthalein monophosphate (22 mmol/L). Do not refrigerate. The reagent may present precipitate or may be turbid what does not interfere on its quality. Mix before using. Keep the bottle tightly closed to avoid evaporation.

2. R2 - Buffer - Store at 15 - 25 ºC.
Reagent label bears expiration date. Buffer pH 10.1 (300mmol/L).

3. R3 - Color Reagent - Store at 15 - 25 ºC.
Reagent label bears expiration date. Sodium carbonate (94 mmol/L) and sodium hydroxide (250 mmol/L).

4. CAL - Standard 45 U/L - Store at 15 - 25 ºC.
Reagent label bears expiration date.

Precautions and warnings

For in vitro diagnostic use.

Disposal of all waste material should be in accordance with local guidelines.

The usual security cares should be applied on the reagent handling.

Color Reagent contains sodium hydroxide that is caustic and may result in burns. Avoid ingestion. In case of eyes, mucosa and skin contact immediately flush with plenty of water and get medical assistance.
Use the appropriate protection equipments for handling caustic reagents.

As in all enzymatic reaction, cares regarding reaction time, temperature of incubation and pipetting are extremely important for the quality of results. One minute difference during the incubation time of this measurement induces 10% error in the results.

Storage and stability . Unopened reagents, when stored at indicated temperature, are stable up to expiration date shown on the label. In order to avoid evaporation of the Standard, keep the bottle tightly closed.

Deterioration . Microbial or chemical contamination may decrease reagents stability.

Specimen collection and preparation

Use serum or plasma (heparin). Alkaline phosphate is reportedly stable in serum for about 7 days at 2 - 8 ºC and several months at -20 ºC.

No known test method can offer complete assurance that human blood samples will not transmit infectious diseases. Therefore, all blood derivatives should be considered potentially infectious.

Interference

Citrate, oxalate, fluoride and EDTA are inhibitors of alkaline phosphate activity because they form a complex with magnesium that is an important activator of enzymatic action.

Bilirubin up to 38 mg/dL, and triglycerides up to 250 mg/dL do not interfere significantly.

Hemolytic samples with hemoglobin values up to 30 mg/dL may be tolerate, but samples with high level hemolysis should not be accepted because provide false decreased results.

Triglycerides values over than 250 mg/dL yield false increased results due photometric interference. The action of this interference may be minimized using the blank of the sample, using 0.85% NaCl as diluent, applicable for triglycerides values up to 1800 mg/dL.

Labtest

Materials required not provided

1. A constant temperature water bath (37 ºC).
2. Photometer capable of measuring absorbance at 580 - 590 nm.
3. Pipettes to measure reagents and samples.
4. Timer.

Manual procedure

See notes 1 and 2.

Set up three tubes and proceed as follows:

	Blank	Unknown	Standard
Substrate (n° 1)	0.05 mL	0.05 mL	0.05 mL
Buffer (n° 2)	0.5 mL	0.5 mL	0.5 mL
Standard (nº 4)	----	----	0.05 mL

Incubate in a water bath at 37 ºC during 2 minutes. Do not remove the tubes of the water bath for adding the sample.

Sample	----	0.05 mL	----

Mix and incubate in a water bath at 37 ºC during 10 minutes.

Color Reagent (n° 3)	2.0 mL	2.0 mL	2.0 mL

Mix and measure the absorbance of the Unknown and Standard against Blank at 590 nm (580 to 590). The color is stable during 120 minutes.

Quality control . For quality control use Qualitrol H Level 1 and Qualitrol H Level 2 or other suitable control material. The limits and control interval must be adapted to the laboratory requirements. Each laboratory should establish corrective actions to be taken if values fall outside the control limits.

Calculations

$$\text{Alkaline Phosphatase (U/L)} = \frac{A_{Unknown}}{A_{Standard}} \times 45$$

Due the great reproductive results of the assay system, it is possible to use the factor method:

$$\text{Calibration factor} = \frac{45}{A_{standard}}$$

$$\text{Alkaline Phosphatase (U/L)} = A_{Unknown} \times \text{Factor}$$

Measurement/reportable range

Up to 500 U/L with zero-order kinetic.

If the Unknown absorbance is more than 2.0, dilute the Unknown and Blank with the Color Reagent. Multiply the result by the appropriate dilution factor. If even after the dilution, the value is equal to or more than 500 U/L, repeat the measurement reducing the incubation time after adding the sample for 2 minutes. Multiply the result by 5.

Expected range . Each laboratory should evaluate the transferability of the expected values to its own patient population and, if necessary, estimate its own reference interval.

Adults . 13 to 43 U/L.

Children up to 12 years old . 56 to 156 U/L.

Unit definition: one unit is equal to the enzyme amount that releases by hydrolysis, 1 mol of thymolphthalein per minute, per liter of serum in the test conditions.

Performance characteristics[6]

Recovery studies . In two samples with alkaline phosphatase concentrations of 56 and 159 U/L were added different quantities of the analyte. Subsequent analyses provided recoveries ranging from 100 to 112%. The mean proportional systematic error at 50 U/L decision level was 2.5 U/L or 5.0%.

Method comparison . A group of 80 sera were assayed by the proposed method and the PNP Labtest method. Serum alkaline phosphatase values ranged from 17 -182 U/L. The comparisons yielded a correlation coefficient of 0.96 and regression equation was $y = 13.4 + 0.307x$. It is evident a positive correlation among both methods observing a 42% systematic difference when using a decision level of 50 U/L, that is explained by the difference among the substrates and the methodologies used.

Imprecision - Within Run

	N	Mean (U/L)	SD (U/L)	(%) CV
Sample 1	20	50	1.20	2.4
Sample 2	20	91	1.47	1.6

Imprecision - Run-to-Run

	N	Mean (U/L)	SD (U/L)	(%) CV
Sample 1	20	52	1.16	2.4
Sample 2	20	88	2.30	2.6

Analytical sensitivity . Detection limit: 0.125 U/L. Using the Standard absorbance as parameter, the photometric detection limit is 0.125 U/L, corresponding to an absorbance equal to 0.001.

Labtest

Notes

1. The material cleaning and drying are fundamental factors to the reagent stability and to obtain correct results.

2. The deionized or distilled water in the laboratory to prepare reagents, use in the measurements and for final glass washing must have resistivity ≥1 megaohm.cm, or conductivity ≤1 microsiems/cm and silicates concentration must be <0.1mg/L.

3. It is suggested to consult "www.fxol.org" in order to review physiopathological source and drugs interference in results and methodology.

References

1. Coleman CM, Stroje RC. Clin Chim Acta 1966; 13:401.
2. King EJ. J Path Bact. 1943; 55:31.
3. Roy AV. Clin Chem 1970; 16:431.
4. Tonks DB. Quality Control in Clinical Laboratories, Warner-Chilcott Laboratories, Diagnostic Reagents Division, Scarborough, Canada, 1972.
5. Westgard JO, Barry PL, Hunt MR, Groth T. 1981, 27:493-501.
6. Labtest: data on file.

Presentation

Product	Reference	Contents	
Alkaline Phosphatase	40	R 1	1 X 5 mL
		R 2	1 X 50 mL
		R 3	1 X 200 mL
		CAL	1 X 3 mL

Consumer information

[Warranty conditions]

Labtest Diagnóstica warrants the performance of this product under the specifications until the expiration date shown in the label since the application procedures and storage conditions, indicated on the label and in this insert, have been followed correctly.

Labtest Diagnóstica S.A.
CNPJ: 16.516.296 / 0001 - 38
Av. Paulo Ferreira da Costa, 600 - Vista Alegre - CEP 33400-000
Lagoa Santa . Minas Gerais Brasil - **www.labtest.com.br**
Consumer Service | e-mail: sac@labtest.com.br

Revision: June, 2009
Ref.: 170309

Símbolos utilizados com produtos diagnósticos in vitro

Símbolos usados con productos diagnósticos in vitro
Symbols used with ivd devices

 **Conteúdo suficiente para < n > testes** Contenido suficiente para < n > tests Contains sufficient for < n > tests	 **Risco biológico** Riesgo biológico Biological risk
 **Data limite de utilização (aaaa-mm-dd ou mm/aaaa)** Estable hasta (aaaa-mm-dd o mm/aaaa) Use by (yyyy-mm-dd or mm/yyyy)	 **Marca CE** Marcado CE CE Mark
 **Material Calibrador** Material Calibrador Calibrator Material	 **Tóxico** Tóxico Poison
 **Material Calibrador** Material Calibrador Calibrator Material	 **Reagente** Reactivo Reagent
 **Limite de temperatura (conservar a)** Temperatura limite (conservar a) Temperature limitation (store at)	 **Fabricado por** Elaborado por Manufactured by
 **Representante Autorizado na Comunidade Europeia** Representante autorizado en la Comunidad Europea Authorized Representative in the European Community	 **Número do lote** Denominación de lote Batch code
 **Consultar instruções de uso** Consultar instrucciones de uso Consult instructions for use	 **Controle** Control Control
 **Número do catálogo** Número de catálogo Catalog Number	 **Controle negativo** Control negativo Negative control
 **Adições ou alterações significativas** Cambios o suplementos significativos Significant additions or changes	 **Controle positivo** Control positivo Positive control
 **Produto diagnóstico in vitro** Dispositivo de diagnóstico in vitro In vitro diagnostic device	 **Controle** Control Control
 **Liofilizado** Liofilizado Lyophilized	 **Corrosivo** Corrosivo Corrosive

Ref.: 170309

Labtest