SPECTRUM

The Creative Approach to Bioscience

Alkaline Phosphatase - Colorimetric

REF: 216 001 50 Test

SYMBOLS IN PRODUCT LABELLING

EC REP Authorised Representative	Use by/Expiration Date
IVD For in-vitro diagnostic use	CAUTION. Consult instructions for use
LOT Batch Code/Lot number	
REF Catalogue Number	Manufactured by
Consult instructions for use	(Xi) - Irritant
Temperature Limitation	

Intended Use

Spectrum Alkaline Phosphatase Colorimetric reagent is intended for the in-vitro quantitative, diagnostic determination of ALP in human serum on both automated and manual systems.

Background

Alkaline phosphatase (ALP) catalyzes the hydrolysis of a wide variety of physiologic and non-physiologic phosphoric acid esters in alkaline medium (pH optimum 10). The liver and biliary tract are the source of alkaline phosphatase in normal sera. Normal alkaline phosphatase levels are age dependent being higher in children and adolescents in comparison to adults. ALP is one of the tests of choice for evaluating cholestasis and obstructive juandice. Elevated levels are found in many diseases including hepatitis, cirrhosis, malignancy, and in bone diseases.

Method

ALP – (Colorimetric method).

Assay Principle

Colorimetric determination of alkaline Phosphatase activity according to the following reaction:

$$\text{Phenylphosphate} \xrightarrow[\text{pH 10}]{ALP} \text{Phenol + Phosphate}$$

Phenol liberated is measured in the presence of 4-aminoantipyrine and Potassium ferricyanide. The presence of sodium arsenate in the reagent stops the enzymatic reaction.

Reagents

Reagent 1 (R1 Buffer) pH 10

Disodium phenylphosphate	5.0	mmol/L
Carbonate-bicarbonate buffer	50	mmol/L

Reagent 2 (R2 Standard)

Phenol — Equal to 20 kind and king U

Reagent 3 (R3 Blocking reagent)

4-aminoantipyrine	60	mmol/L
Sodium arsenate	240	mmol/L
Buffer pH 10		

Toxic reagent

R 45 : may cause cancer.
R 23/25 : toxic by inhalation and if swallowed.
S 28 : after contact with skin, wash immediately with plenty of water.
S 45 : in case of accident or if you feel unwell, seek medical advice immediately (show the label when possible).

Reagent 4 (R4 Color reagent)

Potassium ferricyanide	150	mmol/L

Precautions and Warnings

Do not ingest or inhalate. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.

Reagent preparation, Storage, and Stability

The reagents are supplied ready-to-use and stable up to the expiry date labeled on the bottles when stored at 2 – 8 ^{o}C.

Specimen Collection and Preservation

Serum and Plasma

Nonhemolyzed fresh serum is the preferred specimen. Heparin is the only acceptable anticoagulant. Complexing anticoagulants such as citrate, oxalate, and EDTA must be avoided.
Alkaline Phosphatase activity may slowly increase in serum samples stored at room temperature.
Stability: 2 months at – 20 ^{o}C; 4 weeks at 4 – 8 ^{o}C;
7 days at 20 – 25 ^{o}C

System Parameters

Wavelength	510 nm (Hg 492)
Optical path	1 cm
Assay type	Endpoint
Direction	Increase
Temperature	37 ^{o}C and 20 – 25 ^{o}C
Zero adjustment	Reagent Blank

Procedure

Set up the following tubes

	Serum Sample	Serum blank	Standard	Reagent blank
R1	2 ml	2ml	2ml	2ml
Incubate for 5 minutes at 37 ^{o}C				
R2	.....	.....	50 µl	.....
Serum	50 µl	.....	.....	.....
Incubate for exactly 15 minutes at 37 ^{o}C				
R3	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Mix well or preferably vortex.				
R4	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Serum	.....	50 µl	.....	.....
Dist.Water	.....	.....	.....	50 µl

Mix,let stand for 10 minutes in the dark then measure.
The color intensity is stable for 45 minutes.

Calculation

$$\frac{\text{OD serum sample} - \text{OD serum blank}}{\text{OD Standard}} \times n$$

n = 20 (Kind and king U/100 ml)
n = 142 (IU/L)

Performance Characteristics

Precision

Within run (Repeatability)

	Level 1	Level 2
n	20	20
Mean (U/L)	88.9	180
SD	3.21	5.5
CV%	3.61	3.06

Run to run (Reproducibility)

	Level 1	Level 2
n	20	20
Mean (U/L)	90.3	183
SD	3.24	6.24
CV%	3.59	3.41

Quality Control

Normal & abnormal commercial control serum of known concentrations should be analyzed with each run.

Methods Comparison

A comparison between Alkaline phosphatase reagent and a commercial reagent of the same methodology was performed on 200 human sera. A correlation of 0.978 was obtained.

Sensitivity

When run as recommended, the minimum detection limit of the assay is 1 U/100mL.

Linearity

For activities < 40 kind and king U/100 ml (285 IU/L) reassay using a smaller volume such as 20 or 10 µl. multiply the result by 2.5 or 5 respectively.

Expected Values

Children: 10 - 20 Kind and King U/100ml
71 - 142 IU/L

Adutlts: 3 - 13 Kind and King U/100ml
21 - 92 IU/L

Note

One Kind and King unit is the amount of enzyme which in the given conditions liberates 1 mg of phenol in 15 minutes at 37 ^{o}C

Waste Disposal

This product is made to be used in professional laboratories.
Please consult local regulations for a correct waste disposal.
S56: dispose of this material and its container at hazardous or special waste collection point.
S57: use appropriate container to avoid environmental contamination.
S61: avoid release in environment. refer to special instruction/safety data sheets.

References

1. Kind Pra and King EJ: J clin patho 7:322, 1954.
2. Marsh WH, Finger hut B, Kirsch E: clin chem. 5:119, 1959.
3. Belfield A, GOLDBERG D.M - Enzyme 1971, 12,561.

ORDERING INFORMATION	
CATALOG NO.	**QUANTITY**
216 001	50 Test

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