Alkaline Phosphatase Reagent

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Order No.	Description
R85120	20 × 6.5 mL
R83064	10 × 20 mL
R83065	10 × 50 mL

INTENDED USE

This reagent is intended for the in vitro kinetic determination of the activity of alkaline phosphatase in serum or plasma.

TEST SUMMARY

The substrate employed in this procedure is p-nitrophenyl-phosphate (4-NPP). The first diagnostically useful application of this substrate was in the procedure by Bessey, Lowry and Brock (1). This compound is today the most widely accepted substrate for the determination of the activity of alkaline phosphatase. Optimized procedures have been published, which employ this substrate in kinetic assays, where the buffer used also acts as a phosphate acceptor and thus has an accelerating effect on the hydrolysis. Among these studies are those in references 2, 3 and 4. A recom-mended method employing this same substrate is the one published by the Scandinavian Society for Clinical Chemistry and Clinical Physiology (5). The buffer employed in the last reference is diethanolamine, which has a much higher accelerating effect on alkaline phosphatase activity than other buffers. A reference method for measurement of alkaline phosphatase in human serum has been recently published by the Study Group on Alkaline Phosphatase of the American Association for Clinical Chemistry (6). The method presented here is a modification of this procedure. We employ as buffer 2-amino-2-methyl-1, 3-propanediol (2A2M1,3PD) in the place of 2-amino-2-methyl-1-propanol. Zinc sulfate and N-(2-hydroxyethyl) ethylenediamine triacetic acid (HEDTA) have been included in the reagent.

The colorless substrate is hydrolyzed by alkaline phosphatase to yield inorganic phosphate and p-nitrophenol, which has an intense color in alkaline solution at 405 nm. The formation of the color can be followed kinetically in a spectrophotometer and its intensity is proportional to the activity of alkaline phosphatase in the sample. The reaction is accelerated by the buffer.

REAGENT PREPARATION

The reagent is provided in the form of dry powder, single vial, readily soluble in water.

Dissolve the dry reagent in the vial with the volume of deionized or distilled water specified on the vial label. Cap vials and invert several times. The reagent should go into solution in approximately 3 minutes. Occasional swirling of vials will aid solution.

Store the dissolved reagent in the refrigerator.

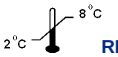
REAGENT COMPOSITION

Reactive ingredients:

p-Nitrophenylphosphate	16 mmol/L

Non-reactive ingredients:

Buffers, stabilizers and fillers



REAGENT STORAGE AND STABILITY

The unreconstituted reagent is stable in the unopened vials until the expiration date on the label when stored in the refrigerator (2–8 °C)

When reconstituted as directed, the reagent is stable for 48 hours at room temperature (18–25 °C) and for 30 days in the refrigerator (2–8 °C).

Do not use the reagent if the absorbance of the freshly dissolved powder is greater than 0.800 at 405 nm when measured against water in a cuvette with 1 cm light path.

Avoid exposure to strong sunlight, since the reagent is light sensitive.

PRECAUTIONS

Good laboratory safety practices should be followed when handling any laboratory reagent. Refer to a recognized laboratory safety program for additional information. (See GP17-T, Clinical Laboratory Safety; Tentative Guideline (1994), National Committee on Clinical Laboratory Standards, Wayne, PA.)

Intended for in vitro diagnostic use only.

SPECIMEN COLLECTION, PREPARATION AND STORAGE

Serum or heparin plasma can be used for this assay (2). EDTA, citrate and oxalate are unsuitable as anticoagulants because they inhibit alkaline phosphatase.

Alkaline phosphatase has been found to be stable, with only slight increase in the sample, for at least 7 days in the refrigerator (2). It has been found that the enzyme activity increases in samples stored at room temperature (6). An increase in activity of greater magnitude has been reported in reconstituted control sera stored both at room temperature and in the refrigerator (7).

INTERFERING SUBSTANCES

EDTA, citrate and oxalate inhibit the enzyme (2). Young (8) has published a comprehensive list of drugs and substances which cause changes in levels of alkaline phosphatase or interfere with its determination.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Spectrophotometer or colorimeter capable of accurately measuring absorbance changes at 405 nm.
2. Matched cuvettes, preferably with 1 cm light path.
3. Constant temperature bath. If the assay is followed in the cuvette compartment of a spectrophotometer, this should be thermostated.
4. Distilled or deionized water.
5. Pipettes to measure water, reagent and samples.

MATERIALS PROVIDED

Alkaline phosphatase reagent in the form of dry powder in single vials of 6.5 mL, 20 mL, or 50 mL.

TEST PROCEDURE

Bring needed volume of reagent and samples to assay temperature.

Wavelength:	405 nm
Temperature:	30 °C
Blank:	Water
Test:	Reagent 1 mL
Bring to 30 °C Temperature	
Add:	Sample 0.02 mL

Mix. Incubate for approximately 15 seconds at 30 °C. With the instrument adjusted to 0 absorbance with the blank, take absor-bance readings at 30 second intervals. Determine the ΔA/minute from the linear part of the assay.

CALCULATIONS

$$\Delta A/\text{min.} \times \frac{TV \times 1000}{18.45 \times LP \times SV} = \text{mU/mL}$$

Where:

TV	=	Total volume in mL.
18.45	=	Millimolar extinction coefficient of 4-nitrophenol.
LP	=	Light path in cm (1 if 1 cm cell was used).
1000	=	To convert units into milliunits.
SV	=	Sample volume in mL.

The calculation factor is (using cells with 1 cm light path): 2764. You must multiply this factor by the ΔA/min. to obtain the mU/mL. If different conditions of assay were used (such as different sample to reagent ratio or light path) substitute the appropriate values in the formula above.

QUALITY CONTROL

Serum controls are recommended to monitor the performance of manual and automated assay procedures, providing a continued screening of the instrument, reagents and technique. Commercially available control material with established values for alkaline phosphatase activity may be used. Cliniqa Assayed Control Serum, Level 1 (R83082) and Level 2 (R83083) are recommended for this purpose.

LIMITATIONS OF THE PROCEDURE

1. Other temperatures, such as 25 °C or 37 °C, can be employed in performing this assay. The change in absorbance per minute will

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increase with the increase in temperature, as will the expected range. Also the pH of the reagent will be different at various temperatures. As a result, the extinction coefficient of p-nitrophenol may vary slightly. The calculations will, however, remain the same.

2. If the change in absorbance is greater than 0.300/minute, or if the initial reading is higher than 1.5, dilute the sample with 4 parts of physiological saline and repeat the assay. Multiply the results by 5 to obtain the original activity.
3. Even though the reagent is capable of measuring absorbance changes greater than 0.300/minute (equivalent to 830 mU/mL) we do not advise to do so since any deviation from accurate timing in readings will result in significant errors if a faster activity is measured.

PERFORMANCE

1. Linearity: If the assay is higher than 830 mU/mL, repeat the assay with a sample diluted with physiological saline.
2. Results obtained using this reagent were compared with those obtained on the same samples using other reagents as reference. A total of 98 samples ranging in activity between 26 and 520 mU/mL were assayed. The results against other reagents as designated were:

   Reagent as in reference 6:

   Correlation coefficient: 0.999

   Regression equation: y = 0.975x - 0.621

   Reagent as in reference 5:

   Correlation coefficient: 0.994

   Regression equation: y = 0.466x - 0.183
3. Precision:

   Within Run

Mean	48 mU/mL	188 mU/mL	302 mU/mL
SD	0.7 mU/mL	2.6 mU/mL	5.0 mU/mL
CV	1.5%	1.4%	1.7%
N	21	21	18

As reported above, alkaline phosphatase levels vary during storage. A run to run precision study would therefore be invalidated by this unpredictable stability of the enzyme.

REFERENCE RANGE

The reference range was obtained on 120 samples from apparently normal donors as established by multiphasic screening.

at 25 °C: 20 to 77 mU/mL

at 30 °C: 26 to 99 mU/mL

at 37 °C: 36 to 141 mU/mL

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

REFERENCES

1. Bessey, O.A., Lowry, O.H. and Brock, M.J., *J. Biol. Chem*, 164, 321, 1946.
2. Bowers, G.N., Jr. and McComb, R.B., *Clin Chem* 12, 70, 1966.
3. McComb, R.B. and Bowers, G.N., Jr., *Clin Chem* 18, 97, 1972.
4. Moss, D.W., *Proc 2nd International Symposium on Clinical Enzymology*, Mount Sinai Medical Center, Chicago, Illinois, 1975.
5. Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology, Recommended Methods For The Determination Of Four Enzymes In Blood, *Scand. J. Clin. Lab. Invest.* 33, 291, 1974.
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8. Young, D.S., *Effects of Drugs on Clinical Laboratory Tests, Third Edition,* AACC Press, Washington, D.C., 1990.

IVD **For in vitro diagnostic use**

See package insert for proper use

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RE-ORDER INFORMATION
Alkaline Phosphatase Reagent

Catalog No.	Catalog No.	Catalog No.
REF R83064	REF R83065	REF R85120

Made in the USA

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