TECO DIAGNOSTICS
1268 N. Lakeview Ave.
Anaheim, CA 92807
1-800-222-9880

ALKALINE PHOSPHATASE REAGENT SET (COLORIMETRIC ENDPOINT METHOD)

INTENDED USE
For the direct colorimetric determination of alkaline phosphatase in human serum.

INTRODUCTION
Distributed in almost every tissue of the body, serum alkaline phosphatase (ALP) levels are of interest in the diagnosis of hepatobiliary disorder and bone disease.[1] Most of the ALP in normal adults serum is from the liver or biliary tract.[2] Normal alkaline phosphatase levels are age dependent, and levels are elevated during periods of active bone growth. Moderate elevations of ALP (not involving the liver or bone) may be attributed to Hodgins' disease, congestive heart failure, and abdominal bacterial infections.[3] Elevations also occur in the third trimester of pregnancy.

Earlier methods were based on the measurement of phosphate liberated by the action of the enzyme on a beta-glycerolphosphate substrate or on the measurement of phenol liberated from disodium phenyl phosphate substrate. Many of these substrates are unstable in solution and need to be prepared fresh daily. The substrate developed by Roy, which uses sodium thymolphthalein monophosphate is stable for one year when properly stored.[4]

PRINCIPLE
The alkaline phosphatase acts upon the AMP-buffered sodium thymolphthaIein monophosphate. The addition of an alkaline reagent stops enzyme activity and simultaneously develops a blue chromogen, which is measured photometrically.

REAGENT COMPOSITION
1. Alkaline Phosphatase Substrate: 3.6 mM, Sodium Thymolphthalein Monophosphate in 0.2 M 2-Amino-2-Methyl-l-Propanol buffer. Magnesium Chloride l.0 mM, wetting agent, inactive ingredients, preservatives; pH 10.2 ± 0.1. ***WARNING: MAY CAUSE SKIN IRRITATION.***

2. Alkaline Phosphatase Color Developer: 0.1 M Sodium Hydroxide, 0.1 M Sodium Carbonate.
   DANGER: CAUSES BURNS

3. Alkaline Phosphatase Standard: Thymolphthalein in n-Propanol 0.5 mM/L. Equivalent to 50 U/L enzyme activity when used according to the Alkaline Phosphatase Procedure.

WARNINGS AND PRECAUTIONS:
1. For In vitro diagnostic use.
   CAUTION: In vitro diagnostic reagents may be hazardous. Handle in accordance with good laboratory procedures which dictate avoiding ingestion, and eye or skin contact.
2. Specimens should be considered infectious and handled appropriately.
3. In case of contact with Alkaline Phosphatase Color Developer, wash with copious amounts of water. Do not ingest.

STORAGE AND STABILITY
Store reagent set at 2 - 8°C (refrigerated)

REAGENT DETERIORATION
1. The Alkaline Phosphatase Substrate should be a clear amber solution. A precipitation or blue-green color would indicate deterioration.
2. The Alkaline Color Developer should be a clear colorless solution.
3. Failure of the Alkaline Phosphatase Standard to achieve assayed values of freshly prepared control sera would indicate deterioration.

SPECIMEN COLLECTION
Unhemolyzed serum is the preferred sample. Heparinized plasma may also be used. Oxalate, fluoride and EDTA inhibit ALP, so are unsuitable as anticoagulants.[5] Samples should be kept cold and assayed as soon as possible after collection. A timed routine for sample collection and analysis should be established in each laboratory because ALP levels in serum or plasma, or in reconstituted control serum, rise significantly when stored at 2 - 8°C or at room temperature.

INTERFERING SUBSTANCES
EDTA, citrate, fluoride and oxalate inhibit ALP. Young, et al. give a list of drugs and other substances, which may interfere with the determination of ALP activity.[6]

MATERIALS REQUIRED BUT NOT PROVIDED
1. Pipetting devices.
2. Test tubes/rack.
3. Timer.
4. Spectrophotometer with a temperature controlled cuvette.
5. Heating bath/ block.

PROCEDURE (MANUAL)
1. For each sample, dispense 0.5 mL of Alkaline Phosphatase Substrate into labeled test tubes and equilibrate to 37°C for three (3) minutes.
2. At timed intervals, add 0.05 mL (50 µl) of each standard, control, and sample to its respective test tube. Mix gently. Use deionized water as sample for Reagent Blank.
3. Incubate for exactly ten (10) minutes at 37°C.
4. Following the same sequence as in Step 2 add 2.5 mL Alkaline Phosphatase Color Developer at timed intervals. Mix well.
5. Set the wavelength of the spectrophotometer at 590 nm. Zero with Reagent Blank. (Wavelength range: 580-630).
6. Read and record absorbance of samples.

TC – MULTI-PURPOSE CALIBRATOR MAY BE USED TO REPLACE STANDARD.

NOTE:
1. If the activity is greater than 100 IU/L repeat the assay with test specimen diluted with normal saline and multiply the test results by the dilution factor.
2. The final colored product is stable for 60 minutes at controlled room temperature (15 - 30°C).

CALCULATION

Abs of Unknown × Value of Std. (IU/L) = Unk. (IU/L)
Abs of Standard

Example: Unknown Absorbance = 0.224
Standard Absorbance = 0.313
Standard Value = 50 IU/L

0.224 × 50 IU/L = 35.7 IU/L
0.313

PROCEDURAL LIMITATIONS

This methodology measures total ALP irrespective of tissue or organ of origin. Further tests may be necessary to assist in differential diagnosis.

QUALITY CONTROL

It is recommended that controls be included in each set of assays. Commercially available control material with established ALP activity may be used for quality control. The assigned value of the control material must be confirmed by the chosen application. Failure to obtain the proper range of values in the assay of control material may indicate either reagent deterioration, instrument malfunction, or procedural errors.

EXPECTED VALUES

Adults: 9-35 IU/L at 37°C. Children have a higher normal value. It is strongly suggested that each laboratory establish its own normal range.[7]

PERFORMANCE CHARACTERISTICS

1. *Linearity:* 100 IU/L
2. *Sensitivity:* Based on instrument resolution of A = 0.001, the present procedure has a sensitivity of 0.16 IU/L.
3. *Comparison:* A study performed between the present procedure and one commercial product resulted in a coefficient of correlation of 0.99 with a regression of y = 1.02x + 0.75.
4. *Precision studies:*

	Within Run	
Mean (IU/L)	S.D.	C.V.
34.8	1.1	3.1%
85.6	2.6	3.1%

	Run to Run	
Mean (IU/L)	S.D.	C.V.
34.9	1.5	4.2%
84.3	2.6	3.1%

REFERENCES

1. Kochmar, J.F., and Moss, D.W.: *Fundamentals of Clinical Chemistry*, N.W. Tietz (ed), p. 604, W.B. Saunders and Company, Philadelphia, PA (1976).
2. Kaplan, M.M., and Righetti, A.: *J. Clin. Inv.* 34:126 (1955).
3. Kaplan, M.M.: *New England J. Med.* 286:200 (1972).
4. Roy, A.V., *Clin. Chem.* 16:431 (1970).
5. Demetrious, J.A., et al.: *Enzymes in Clinical Chemistry Principles and Technics*, 2nd Ed. Hagerstown (MD), Harper and Row 927 (1974).
6. Young, D.S., et al*: Clin. Chem* 21:5 (1975).
7. Tietz, N.: *Fundamentals of Clinical Chemistry* 602-609 (1976).

Manufactured by:

TECO DIAGNOSTICS
1268 N. LAKEVIEW AVE.
ANAHEIM, CA 92807
U.S.A.

CE EC REP
EMERGO EUROPE
Prinsessegracht 20
2514 AP The Hague
The Netherlands