

Acid Phos Kinetic

1.0 INTENDED USE

This reagent is intended for the quantitative determination of Acid Phosphatase (ACP) in serum.

2.0 BACKGROUND

2.1 METHOD AND HISTORY

Several substrates have been used for determination of acid phosphatase activity (10.3-10.6.) However, a-naphthyl phosphatase has been recommended as the most suitable substrate for automated assays (10.7, 10.8.) This method measures the acid phosphatase activity using a-naphthyl phosphate as substrate. The reagents may be employed to determine both the tartrate resistant and tartrate sensitive fractions of the enzyme.

2.2 TEST PRINCIPLE

Acid phosphatase (ACP) catalyzes the hydrolysis of a-naphthyl phosphate to a-naphthol and inorganic phosphate. a-Naphthol immediately reacts with fast red TR salt to produce a yellow chromophore with an absorbance maximum at 405nm. The rate of increase in absorbance at 405nm is directly proportional to ACP activity in the sample. ACP activity is determined in the presence and absence of L-tartrate, the difference in activity being attributed to prostatic acid phosphatase activity.

ACP

a-Naphthyl Phosphate + H₂O ----> a-Naphthol + inorganic phosphate

a-Naphthol + Fast Red TR ----> Chromophore

2.3 CLINICAL SIGNIFICANCE

Acid phosphatase (orthophosphorus phosphohydrolase) is found in a variety of tissues, notable in the prostate, stomach, liver, muscle, skin, spleen and erythrocytes (10.1.) However, acid phosphatase activity in human serum from healthy donors is relatively low compared to the activity of alkaline phosphatase. Serum acid phosphatase activity is increased in some prostate cancer patients, especially the enzyme activity which is tartrate sensitive. Therefore, determination of serum acid phosphatase activity is of established value in the detection of metastasizing carcinoma of the prostate (10.2.)

3.0 SPECIMEN COLLECTION AND HANDLING

3.1 PATIENT PREPARATION

No special patient preparation is required.

3.2 SPECIMEN COLLECTION.

Fresh, clear, unhemolyzed serum is the preferred specimen. Plasma should not be used. Separate serum from clot promptly after blood collection.

Use a standard venipuncture tube to draw patient sample.

The amount of sample required will depend on the analyzer used. The amount of serum required is in the range of 5-100 µl. Call Biotron's technical service department at 1-800-595 TRON for the recommended sample volume for your analyzer.

Record the patient's name, date and time of sample collection and preparation.

3.3 SPECIMEN STORAGE

Acid phosphatase at the normal pH of serum is very labile. To stabilize the enzyme, add 20µl ACP Stabilizer to 1ml serum. Mix and store serum in refrigerator (2-8°C) until ready to assay. Acid phosphatase activity in serum treated in this manner will remain stable for 7 days.

4.0 MATERIALS

(10 X 10 ml)

(6 X 50 ml)

Reagents necessary for the determination of acid phosphatase are included in the kit.

4.1 REAGENT

4.1.1 ACP Reagent contains:

a-naphthyl phosphate 4 mmol/L
Fast Red TR 1 mmol/L
nonreactive stabilizers and fillers

4.1.2 ACP Tartrate Reagent contains:

L-Tartrate 2 mol/L
nonreactive stabilizers and fillers

4.1.3 ACP Stabilizer contains:

Acetate Buffer 5 mol/L

4.2 WARNINGS AND PRECAUTIONS

For In Vitro Diagnostic Use. Not for Internal use in Humans or Animals. In Vitro Diagnostics reagents may be hazardous. Avoid ingestion and skin or eye contact.

4.3 REAGENT PREPARATION

Reconstitute ACP reagent with volume of deionized water indicated on the vial label. Replace rubber stopper and allow to sit for 5 minutes. Swirl gently until dissolution is complete. Reconstitute ACP tartrate reagent with 5 ml of deionized water. Replace rubber stopper and allow to sit for 5 minutes. Swirl gently until dissolution is complete.

Record the date and time of reconstitution.

The ACP Stabilizer is supplied in liquid form and requires no further preparation.

4.4 REAGENT STORAGE AND STABILITY

When stored at 2°-8°C, away from light, unopened reagents are stable until the expiration date printed on the label.

Reconstituted ACP reagent is stable for 2 weeks at 2°-8°C or 24 hours at 18°-26°C.

Reconstituted ACP Tartrate reagent is stable indefinitely at 2°-8°C if free from bacterial contamination. If crystallization occurs, warm reagent (40-50° C) to dissolve.

ACP reagent is not suitable for use if the absorbance of the reconstituted solution is greater than 0.5 at 405nm. Discard vial if reagent exhibits caking due to possible moisture penetration, does not dissolve completely upon reconstitution or if solution appears turbid.

ACP tartrate reagent and ACP stabilizer are not suitable for use if the solution appears turbid.

4.5 ADDITIONAL MATERIALS REQUIRED

4.5.1 A spectrophotometer or colorimeter capable of reading absorbance accurately at 405 nm.

4.5.2 1 cm cuvettes or a flow cell capable of transmitting light at 405 nm.

4.5.3 Test tubes capable of holding 2 ml.

4.5.4 Pipettes capable of delivering 1 ml and 100 µl.

4.5.5 Timer with one minute increments.

4.5.6 Constant temperature heat source which can be adjusted to 30° C or 37° C.

4.5.7 Normal and abnormal control for quality control.

5.0 TEST PROCEDURE

The following is a general procedure for use on a manual instrument.

5.1 PROCEDURE CONDITIONS

Wavelength	405 nm
Temperature	30° C or 37° C
Pathlength	1 cm
Mode	Kinetic
Reaction Time	10 min.
Sample Volume	100 µl
Reagent volume	1 ml
Total Volume	1.1 ml
Sample to reagent ratio	1/10

5.2 INSTRUMENT

Any instrument capable of reading absorbance accurately with a sensitivity of 0.001 absorbance at 405 nm may be used. The band width should be 10 nm or less, stray light 0.5% or less, and the wavelength accuracy within 2 nm.

5.3 CALIBRATION

No routine reagent calibration is necessary as the acid phosphatase activity is calculated by use of the molar absorptivity of a-naphthol fast red TR complex which is taken as 12.9 at 405nm.

5.4 PROCEDURE FOR TOTAL ACID PHOSPHATASE

- 5.4.1 Prepare the required volume of acid phosphatase working reagent. (See 4.3 Reagent Preparation section.)
- 5.4.2 Add 1 ml of ACP reagent.
- 5.4.3 Pipette 100 µl of serum to be assayed and incubate for 5 minutes at 30° C or 37° C.
- 5.4.4 Record the absorbance (A1).
- 5.4.5 Record the absorbance (A2) at the end of an additional 5 minutes.

5.5 CALCULATION AND RESULTS FOR TOTAL ACID PHOSPHATASE

Note: One unit of activity is defined as that amount of enzyme which will hydrolyze one micromole of a-naphthyl phosphate per minute under conditions of the assay

$$\text{Total ACP U/L} = \frac{\Delta A/5 \text{ min} \times \text{assay volume} \times 1000}{12.9 \times \text{sample volume} \times 5 \times \text{light path}} = \Delta A/5 \text{ min} \times 171$$

$\Delta A/5 \text{ min}$ = change in absorbance per 5 minutes at 405nm
assay volume (ml) = 1.1 ml
sample volume (ml) = 0.1 ml
12.9 = Millimolar absorptivity of a-naphthol-fast red TR complex at 405nm
light path = length of light path (usually 1 cm)
1000 = Conversion of units per ml to units per liter
5 = Conversion of ΔA per 5 min to ΔA per 1 min
Example:
A1 = 0.159, A2 = 0.180

$$\text{Total ACP Activity (U/L)} = (0.180 - 0.159) \times 171 = 3.6 \text{ U/L}$$

5.6 PROCEDURE FOR NON PROSTATIC ACID PHOSPHATASE

- 5.6.1 Prepare the required volume of acid phosphatase working reagent. (See 4.3 Reagent Preparation section.)
- 5.6.2 Add 1 ml of ACP reagent and 0.01 ml of ACP tartrate reagent. Mix by inversion.
- 5.6.3 Pipette 100 µl of serum to be assayed and incubate for 5 minutes at 30° C or 37° C.
- 5.6.4 Record the absorbance (A1).
- 5.6.5 Record the absorbance (A2) at the end of an additional 5 minutes.

5.7 CALCULATION AND RESULTS FOR PROSTATIC ACID PHOSPHATASE

Prostatic ACP activity = Total ACP activity - Non Prostatic ACP activity

$$\text{Non Prostatic ACP U/L} = \frac{\Delta A/5 \text{ min} \times \text{assay volume} \times 1000}{12.9 \times \text{sample volume} \times 5 \times \text{light path}} = \Delta A/5 \text{ min} \times 172$$

$\Delta A/5 \text{ min}$ = change in absorbance per 5 minutes at 405nm
assay volume (ml) = 1.11 ml
sample volume (ml) = 0.1 ml
12.9 = Millimolar absorptivity of a-naphthol-fast red TR complex at 405nm
light path = length of light path (usually 1 cm)
1000 = Conversion of units per ml to units per liter
5 = Conversion of ΔA per 5 min to ΔA per 1 min
Example:
A1 = 0.151, A2 = 0.166

$$\text{Non Prostatic ACP Activity (U/L)} = (0.166 - 0.151) \times 172 = 2.6 \text{ U/L}$$

$$\text{Prostatic ACP activity} = 3.6 - 2.6 = 1.0 \text{ U/L}$$

5.8 TEMPERATURE CONVERSION FACTORS

To convert ACP activity from 30° C to 37° C, multiply the result by 1.33.
To convert ACP activity from 37° C to 30° C, multiply the result by 0.76.

6.0 INTERPRETATION OF RESULTS

6.1 EXPECTED VALUES (10.4)

The range of expected values for adults (25-45 years) is:

	30° C	37° C
Total ACP	2.2 - 4.3 U/L	2.9 - 5.7 U/L
Prostatic ACP	0.05 - 0.9 U/L	0.07 - 1.2 U/L

Note: ACP activity is age dependent.

These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located.

6.2 MEDICAL ALERT VALUES (10.11)

Each laboratory should establish low and high values beyond which the patient would require immediate attention by a physician. If a "medical alert value" is reached, always repeat the test to confirm the result and notify a physician if the result is confirmed.

6.3 LIMITATIONS OF PROCEDURE

High levels of serum bilirubin reportedly inhibit acid phosphatase activity (10.9.) Oxalates and fluoride also inhibit the enzyme activity, while heparin and EDTA cause turbidity in the sample. Certain drugs and other substances are known to influence circulating levels of acid phosphatase (10.10.)

7.0 QUALITY CONTROL

Standard practice for quality control should be applied to this system. Commercially available lyophilized controls can be used to monitor the daily acceptable variations. Normal and abnormal controls should be assayed at the beginning of each run of patient samples, whenever a new reagent or a different lot number is being used, and following any system maintenance.

A satisfactory level of performance is achieved when the analyte values obtained are within the "acceptable range" established by the laboratory.

8.0 CALIBRATION PROCEDURES

No routine reagent calibration is necessary as the acid phosphatase activity is calculated by use of the molar absorptivity of a-naphthol fast red TR complex which is taken as 12.9 at 405nm.

9.0 PERFORMANCE CHARACTERISTICS

9.1 PRECISION

The estimates of precision shown below were obtained from assays of human control serum.

Samples	Mean (U/L)	Within-Run	
		SD (U/L)	CV (%)
15	2.86	± 0.17	5.9
15	18.4	± 0.29	1.6
Between-Run			
Samples	Mean (U/L)	SD (U/L)	CV (%)
25	2.88	± 0.22	7.6
25	19.1	± 0.45	2.4

9.2 CORRELATION

A correlation study was done comparing this method and a similar acid phosphatase method. The samples range between 1.9 and 18.3 U/L.

Number of Samples	Regression Equation $y = \text{Biotron}, x = \text{Comparative}$	Correlation Coefficient
28	$y = 1.085x - 0.422$	0.995

9.3 LINEARITY

This procedure is linear through 40 U/L beyond which the specimen should be diluted 1 to 1 with isotonic saline. Reassay the specimen and multiply the results by 2.

9.4 SENSITIVITY

The average sensitivity for this method is 0.006 $\Delta A/5 \text{ min}$ per unit of concentration (U/L).

10.0 REFERENCES

- 10.1 Frankel S, Reitman S, Sonnenwirth AC: Gradwohl's Clinical Laboratory Methods and Diagnosis, vol 1, 7th ed., CV Mosby, St Louis (MO), 1970, p 115
- 10.2 Warren RJ, Moss DW, Clin Chem Acta 77:179, 1977
- 10.3 Kind PRN, King EJ, J Clin Pathol 7:322, 1954.
- 10.4 Babson AL, Read PA, Phillips GE, Am J Clin Pathol 32:83, 1959.
- 10.5 Seligman AM, Chauncey HH, Nachlas MM, Manheimer LH, Ravin HA, J Biol Chem 190:7, 1951.
- 10.6 Hudson PB, Brendler H, Scott WW, J Urol 58:89, 1947.
- 10.7 Bais R, Edwards JB, Clin Chem 22:2025, 1976.
- 10.8 Cooper JDH, Turnell DC, Price CP, Clin Chim Acta 126:297, 1982.
- 10.9 Shaw LM, Brummund W, Dorio RJ, Am J Clin Pathol 68:57, 1977
- 10.10 Effects of Drugs on Clinical Laboratory Tests, 3rd ed. DS Young, Editor, AACC Press, Washington (DC), 1990
- 10.11 G.J. Kost, "Critical Limits for Urgent Clinician Notification at U.S. Medical Centers"; JAMA, Feb. 2, 1990; Vol 263, No.5, p.704

