Acid Phosphatase Reagent
Alpha-Naphthylphosphate Method

PRODUCT SUMMARY

Stability	:	5 days at 2-8°C
Linear Range	:	Up to 80 U/L
Specimen Type	:	Serum
Method	:	Kinetic
Reagent Preparation	:	Add specified volume of distilled or deionized water.

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorised Representative		Temperature Limitation
IVD	For in vitro diagnostic use		Use by/Expiration Date
LOT	Batch code/Lot number		CAUTION. CONSULT INSTRUCTIONS FOR USE.
REF	Catalogue number		
	Consult instructions for use		Manufactured by
REAG A	Reagent A		Xi - Irritant
REAG B	Reagent B	REAG C	Reagent C

INTENDED USE
This reagent is intended for the in vitro quantitative determination of total and prostatic acid phosphatase in human serum on both manual or automated systems.

CLINICAL SIGNIFICANCE
Significant levels of acid phosphatase are found in the spleen, erythrocytes, platelets and prostate gland. Elevation of the prostatic fraction of acid phosphatase results from carcinoma of the prostate and operative trauma. Elevations of total acid phosphatase occur in various liver and bone diseases, Gaucher's disease and excessive destruction of platelets.[1]

METHODOLOGY
Acid phosphatase (ACP) catalyses the hydrolysis of alpha-naphthylphosphate liberating the alpha-naphthol and phosphate. The alpha-naphthol is then coupled with diazotised 4-chloro-2-methylbenzene (Fast Red TR) to form a diazo dye which has a strong absorbance at 405nm and the increase in absorbance is directly proportional to the level of acid phosphatase in the sample. The addition of L-Tartrate inhibits prostatic acid phosphatase but does not inhibit other isoenzymes. The difference between the two assays (Total acid Phosphatase and Non-Prostatic acid phosphatase) would be the level of prostatic acid phosphatase in serum.[2]

$$\alpha\text{-naphthylphosphate} + H_2O \xrightarrow{ACP} \alpha\text{-naphthol} + PO_4$$

$$\alpha\text{-naphthol} + \text{Fast Red TR} \longrightarrow \text{Diazonium Dye}$$

REAGENT COMPOSITION

Active Ingredient	Concentration
Reagent A:	
α-naphthylphosphate	3 mmol/L
Fast Red TR	1 mmol/L
Citric Acid	20 mmol/L
Sodium Citrate	60 mmol/L
pH 5.3 ± 0.1 at 20°C	
Reagent B:	
Sodium L-Tartrate	2 mol/L
Reagent C:	
Acetate Buffer	3 mol/L

WARNING: Do not ingest. Avoid contact with skin and eyes. If spilt, thoroughly wash affected areas with water. Flush with plenty of water when disposing. For further information consult the Acid Phosphatase Reagent Material Safety Data Sheet. **The Packaging of This Product Contains Dry Natural Rubber.** Exercise precaution when handling crimps and broken glass vials, as sharp edges can injure the user.

Reagent A:
R41 Risk of serious damage to eyes.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

REAGENT PREPARATION
Reagent A:
Reconstitute the reagent with the volume of distilled or deionized water stated on the vial label. Mix gently until dissolved.
Reagent B:
Reconstitute the reagent with the volume of distilled or deionized water stated on the vial label. Mix gently until dissolved.
Reagent C:
The stabilizer solution is provided ready to use.

STABILITY AND STORAGE
1. All reagents should be stored refrigerated (2-8°C) and can be used until the expiration date indicated on the label.
2. Reconstituted Reagent A is stable for 5 days refrigerated (2-8°C), when stored in an amber vial protected from direct light.
3. Reconstituted Reagent B is stable for 90 days when stored refrigerated (2-8°C). The solution may be warmed to 45-55°C if crystallization occurs on storage.

Indications of Reagent Deterioration:
- Turbidity;
- Failure to recover control values within the assigned range; and/or
- Reagent A absorbance >0.3 AU at 405nm (1 cm light path).

SPECIMEN COLLECTION AND HANDLING
Serum: Use non-haemolysed serum.
Plasma: Not recommended. Oxalate and fluoride anticoagulants will interfere with the assay.
Storage: Acid phosphatase is very unstable at the pH of serum.[3] Stabilize the sample by the addition of 0.020 mL of stabilizer (Reagent C) to every 1 mL of serum. The enzyme activity will be stable for three days at 2-8°C.

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED
- A clinical chemistry analyzer capable of maintaining constant temperature (37°C) and measuring absorbance at 405nm.
- Analyzer specific consumables, e.g.: sample cups.
- Distilled or deionized water for reagent preparation and related equipment, e.g.: pipettes.
- Normal and Abnormal assayed control material.

ASSAY PROCEDURE
The following system parameters are recommended. Individual instrument applications are available upon request from the Technical Support Group.

SYSTEM PARAMETERS

Total Acid Phosphatase

Temperature	30°/37°C
Primary Wavelength	405 nm (405-420 nm)
Secondary Wavelength	500-650 nm
Assay Type	Rate/Kinetic
Direction	Increase
Sample : Reagent Ratio	1 : 10
e.g.: Sample Vol	0.2 mL
Reagent Vol	2.0 mL
Delay/Lag Time	5 minutes
Read Time	10 minutes
Reagent Blank Limits	Low 0.0 AU
(405nm, 1cm lightpath)	High 0.3 AU
Linearity	0-80 U/L
(refer to linearity section)	
Sensitivity	1.2 ΔmA/min per U/L
(405nm, 1cm lightpath)	

Non-Prostatic Acid Phosphatase

Temperature	30°/37°C
Primary Wavelength	405 nm (405-420 nm)
Secondary Wavelength	500-650 nm
Assay Type	Rate/Kinetic
Direction	Increase
Sample : Reagent Ratio	1 : 10.1
e.g.: Sample Vol	0.2 mL
Reagent A Vol	2.0 mL
Reagent B Vol	0.02 mL
Delay/Lag Time	5 minutes
Read Time	10 minutes
Reagent Blank Limits	Low 0.0 AU
(405nm, 1cm lightpath)	High 0.3 AU
Linearity	0-80 U/L
(refer to linearity section)	
Sensitivity	1.2 ΔmA/min per U/L
(405nm, 1cm lightpath)	

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CALCULATIONS

Results are calculated, usually automatically by the instrument, as follows:

Activity in U/L = ΔAbs/min x Factor

$$\text{Factor} = \frac{\text{TV} \times 1000}{\text{SV} \times \text{E} \times \text{P}}$$

Where:

TV = Total reaction volume in mL
SV = Sample volume in mL
E = millimolar extinction coefficent of Diazo dye at 405 nm = 12.9
P = Cuvette pathlength in cm.

Example:

1. Total Acid Phosphatase (T-ACP):
   ΔAbs/min = 0.017
   Factor = 853
   Acid Phos = 0.017 x 853 = 14.5 U/L
2. Non-Prostatic Acid Phosphatase (N-ACP):
   ΔAbs/min = 0.010
   Factor = 860
   Acid Phos = 0.010 x 860 = 8.6 U/L
3. Prostatic Acid Phosphatase is obtained by subtracting the results of the Non-Prostatic Acid Phosphatase assay from the results of the Total Acid Phosphatase assay on the same sample.
   Prostatic ACP (U/L) = T-ACP (U/L) - N-ACP (U/L)
   Prostatic ACP (U/L) = 14.5 - 8.6 = 5.9 U/L

NOTES

1. The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
2. If the change in absorbance is greater than 0.1 A/min repeat the assay with less sample or dilute with saline. Remember to adjust the factor for smaller sample volume or multiply the final result by the dilution factor.
3. Valid results depend on accurately calibrated instrument, timing and temperature control.
4. Unit conversion: U/L x 16.67 x 10^{-3} = µkat/L.

CALIBRATION

Not required. The rate of reaction is converted to U/L of activity by a calculation factor. Refer to the calculation section of this package insert.

QUALITY CONTROL

To ensure adequate quality control, normal and abnormal control with assayed values should be run as unknown samples:-

- At least every eight hours.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.

Control results falling outside the upper or lower limits of the established ranges indicate the assay may be out of control.

The following corrective actions are recommended in such situations:-

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
- If results are still out of control, contact Technical Services or your local distributor.

LIMITATIONS

1. Avoid the use of haemolysed samples.
2. Studies to determine the level of interference from bilirubin were carried out. The following results were obtained:
   Bilirubin: No interference from bilirubin up to 68 µmol/L (4 mg/dL).
3. Avoid the use of lipaemic samples.
4. Young DS[4] has published a comprehensive list of drugs and substances which may interfere with this assay.

EXPECTED VALUES[1,5]

Total Acid Phosphatase

At 37°C 0 - 6.0 U/L (0.0 - 0.10 µkat/L)
At 30°C 0 - 4.5 U/L (0.0 - 0.075 µkat/L)

Prostatic Acid Phosphatase

At 37°C 0 - 2.0 U/L (0.0 - 0.03 µkat/L)
At 30°C 0 - 1.5 U/L (0.0 - 0.025 µkat/L)

The quoted values are representative of the expected range for this method and should serve as a guide only. It is recommended that each Laboratory verify this range or derives a reference interval for the population that it serves.[6]

PERFORMANCE DATA

The following data was obtained using the Acid Phosphatase Reagent on a well maintained automated clinical chemistry analyzer. Users should establish product performance on their specific analyzer used.

IMPRECISION

Within Run:	LEVEL I	LEVEL II
Number of data points	20	20
Mean (U/L)	2.9	24.5
SD (U/L)	0.24	0.95
CV (%)	8.3	3.9

Between Day:	LEVEL I	LEVEL II
Number of data points	20	20
Mean (U/L)	3.0	27.7
SD (U/L)	0.48	0.73
CV (%)	16.0	2.6

ACCURACY

Comparison studies were carried out using another similar commercially available acid phosphatase reagent. Serum samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained.

Total Acid Phosphatase (T-ACP)

Number of sample pairs	43
Range of sample results	1.3 - 64.0 U/L
Mean of reference method results	6.4 U/L
Mean of T-ACP results	6.4 U/L
Slope	1.20
Intercept	-1.3 U/L
Correlation coefficient	0.999

Prostatic Acid Phosphatase (N-ACP)

Number of sample pairs	41
Range of sample results	0.1 - 56.0 U/L
Mean of reference method results	3.5 U/L
Mean of N-ACP results	3.5 U/L
Slope	1.14
Intercept	-0.5 U/L
Correlation coefficient	0.999

LINEARITY

When run as recommended, the assay is linear between 0 - 80 U/L (0.0-1.33µkat/L).

Linearity on automated instruments will be dependent upon the ratio of sample volume to reagent volume used and the timing of measurements. The specific instrument application should be consulted.

SENSITIVITY

When run as recommended the sensitivity of this assay is 1.2 ΔmA/min per U/L.

REFERENCES

1. Kaplan, L.A., Pesce, A.J., Clinical Chemistry Theory Analysis and Correlation, C.V. Mosby Company, St. Louis, 1984 pp. 1087.
2. Hillman, G.Z., Klin Chem., Klin. Biochem, 3, 273, 1971.
3. Doe, R.P., Mellinger, G.T., and Seal U.S. Clin. Chem. 11, 943, 1965.
4. Young DS. Effects of Drugs on Clinical Laboratory Tests Third Edition 1990; 3: 4-5.
5. Tietz, N.W., Fundamentals of Clinical Chemistry, Saunders, Philadelphia, 1986.
6. Wachtel M et al, Creation and Verification of Reference Intervals. Laboratory Medicine 1995; 26:593-7.

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REF

Reorder Information

	REAG A	REAG B	REAG C
TR27010	18 x 10 mL	1 x 10 mL	1 x 10 mL
TR27015	18 x 20 mL	1 x 20 mL	1 x 20 mL