Acid Phosphatase Reagent
Alpha-Naphthylphosphate Method

**PRODUCT SUMMARY**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stability</td>
<td>5 days at 2-8°C</td>
</tr>
<tr>
<td>Linear Range</td>
<td>Up to 80 U/L</td>
</tr>
<tr>
<td>Specimen Type</td>
<td>Serum</td>
</tr>
<tr>
<td>Method</td>
<td>Kinetic</td>
</tr>
<tr>
<td>Reagent Preparation</td>
<td>Add specified volume of distilled or deionized water.</td>
</tr>
</tbody>
</table>

**SYMBOLS IN PRODUCT LABELLING**

**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.

**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.

- α-naphthylphosphate + H₂O → α-naphthol + PO₄
- α-naphthol + Fast Red TR → Diazonium Dye

**REAGENT COMPOSITION**

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

**SYSTEM PARAMETERS**

**Total Acid Phosphatase**
- Temperature: 30°C/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)
- Linearity: High 0.3 AU (405nm, 1cm lightpath)

**Non-Prostatic Acid Phosphatase**
- Temperature: 30°C/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)
- Linearity: High 0.3 AU (405nm, 1cm lightpath)
- Sensitivity: 1.2 μm/min per U/L

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

**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 B is stable for 90 days when stored refrigerated (2-8°C).
3. 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. 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.

**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.

**CAUTION. CONSULT INSTRUCTIONS FOR USE.**
CALCULATIONS
Results are calculated, usually automatically by the instrument, as follows:

Activity in U/L = ΔAbs/min x Factor

Factor = TV x 1000
SV x E X P

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

Example:
1. Total Acid Phosphatase (T-ACP):
   \[ \Delta \text{Abs/min} = 0.017 \]
   Factor = 853
   Acid Phos = 0.017 x 853 = 14.5 U/L
2. Non-Prostatic Acid Phosphatase (N-ACP):
   \[ \Delta \text{Abs/min} = 0.010 \]
   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.
   ProstaticACP (U/L) = T-ACP (U/L) - N-ACP (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.

QUALITY CONTROL
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.

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 mAU/min per U/L.

REFERENCES