ACID PHOSPHATASE
ENZYMATIC COLORIMETRIC TEST

**Principle:** (1,2)

The presence of Acid Phosphatase activity is demonstrated by the following reaction:

\[
p\text{-nitrophenylphosphate} + H_2O \rightarrow \text{p-nitrophenol} + \text{phosphate}
\]

**Reagents Concentration:**

- **Buffer:** Citrate Buffer pH 4.8 55mmol/l
- **Substrate:** p-nitrophenylphosphate 5.5mmol/l
- **Sodium Tartrate:** 200mmol/l
- **Sodium Hydroxide:** 200mmol/l

**Preparation and stability of solutions:**

1. **Buffer**
   Contents ready for use. Stable until expiry date when stored at 2 - 8 ºC.
2. **Substrate**
   Reconstitute the contents of one bottle with 10ml of Buffer 1. Stable for 5 days at 2 - 8 ºC.
3. **Tartrate**
   Contents ready for use stable up to the expiry date when stored at 2 - 8 ºC.
4. **Sodium Hydroxide**
   For 10 assays dilute 10ml of solution 4 with 90ml redistilled water. Stable up to the expiry date when stored at 2 - 8 ºC.

**Sample:**

Serum. Specimens must be absolutely free from haemolysis. Stabilize sample with 5 mg NaHSO₄H₂O per ml of serum.

**Procedure:**

- **Wavelength:** Hg 405 nm
- **Temperature:** +37ºC
- **Cuvette:** 1cm light path
- **Zero adjustment:** Against reagent Blank

<table>
<thead>
<tr>
<th>Pipette into test tube:</th>
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<tbody>
<tr>
<td>Reagent Blank</td>
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<tr>
<td>Solution 2</td>
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<tr>
<td>Solution 3</td>
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</table>

Incubate for exactly 5 min at +37ºC. Add at 30sec intervals:

| Sample   | --- | 0.2ml | 0.2ml |

Incubate for exactly 30 min at +30ºC. Add at 30 sec intervals:

| Diluted NaOH | 10.0ml | 10.0ml | 10.0ml |
| Sample       | 0.2ml  | ---    | ---    |

Mix, read absorbance of sample against Reagent Blank.

**Calculation:**

To calculate the acid phosphatase activity use the following formulae:

| Total Acid Phosphatase: | 101 x A sample 1 |
| Prostatic Phosphatase: | 101 x (A sample 1 - A sample 2) |

**Normal values (3):**

| Total Acid Phosphatase     | up to 11 U/I (37 ºC) |
| Prostatic Phosphatase      | up to 4 U/I (37 ºC)  |

**Quality control:**

For accuracy and reproducibility control:-
- Assayed Multi-Sera Normal and Elevated.
- For reproducibility control:-
  Multi-Sera Low, Normal and Elevated.

**Linearity:**

If the absorbance change exceeds 0.800 dilute 0.2 ml of sample with 0.6 ml of 0.9% NaCl solution and repeat the assay multiplying the result by 4. dilute 0.1 ml of sample with 0.9 ml of 5% albumin multiplying the result by 10.

**Presentation:**

#ACP 5650 10x10ml, (10 x 10 tests)
1. Buffer 1 x 105 ml
2. Substrate 10 x 10 ml
3. Tartrate 1 x 5 ml
4. Sodium Hydroxide 1 x 102 ml

**Safety precautions:**

For in vitro diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents. The reaction of the substrate liberates p-nitrophenol. Avoid contact with skin and do not swallow or inhale vapour. Health and Safety data sheets available on request.

**References:**