Reagent kit for determination of acid phosphatase activity in serum (Hillmann’s colorimetric method).

Acid phosphatase consists of several isoenzymes originating from different tissues. Most of the enzymes are found in lysosomes. Isoenzymes differ from one another in their catalytic activity, substrate specificity and sensitivity to inhibitors. All isoenzymes are labile at alkaline pH. From diagnostic point of view the isoenzyme of prostatic origin is of primary importance which in contrast to that of erythrocytic origin present in high concentration in serum can be inhibited by tartrate. Immunochemical methods (RIA, FIA, ELISA, immunoelectrophoresis) have recently been developed that allow the estimation of the concentration of isoenzymes instead of their activities. Monovalent immunoserum do not cross react with different isoenzymes of acid phosphatase. Increased activities of total and specific prostatic acid phosphatase are associated with prostatic disorders, prostatic cancer and its metatases. Elevations in total enzyme activity are observed also in cases of myeloma, thymocytopenia and some liver diseases.

Principle
Acid phosphatase present in serum splits α-naphthyl phosphate into α-naphthol and inorganic phosphate. The α-naphthol liberated during the reaction gives a diazo-dyestuff with Fast Red TR. The enzyme fraction of prostatic origin (prostatic acid phosphatase) can be inhibited by tartrate.

Reference values
Total: Male: max. 10 U/l (0.17 pkat/l)
Female: max. 7.8 U/l (0.13 pkat/l)
Prostatic: max. 4.3 U/l (0.072 pkat/l)
It is recommended that each laboratory should assign its own normal range.

Reagents
1. Reagent (R1) Citrate buffer, pH=5.20 150 mmol/l
2. Reagent (R2) α-Naphthyl phosphate 10 mmol/l
Fast Red TR 6 mmol/l
3. Reagent (R3) Sodium tartrate 400 mmol/l

Samples
Serum free of hemolysis. NO plasma can be used.

PROCEDURE
Preparation of working reagent
Dissolve one vial of reagent (R2) in an appropriate volume of buffer solution (R1).

Stability of working reagent
20–25°C: 6 hours
2–8 °C: 2 days

Light sensitive, store protected from light!
If the absorbance of working reagent is higher than 0.7 at 405 nm the reagent can not be used.

Assay conditions
Wavelength: 405 nm
Temperature: 37 °C
Cuvette: 1 cm pathway
Method: kinetic (increasing)

Pipette into cuvette

Mix and incubate for 5 minutes, then read the absorbance in every minute for 3 minutes. Calculate the change of absorbance per minute (ΔA/min).

Calibration:
- (37 °C, Hillman’s colorimetric method)
- S1: Distilled water
- S2: Random Calibration Serum Level I
- S3: Random Calibration Serum Level II

Calibration frequency
Calibration is recommended:
- after reagent lot change,
- as required following quality control procedures.

Calculation using calibration

\[ \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \times C_{\text{standard}} = C_{\text{sample}} \]

A = Absorbance, C = Concentration

ACID PHOSPHATASE
FREEZE DRIED
Cat. No.: 42401 42411
20x3 ml 12x10 ml

Calculation using factor
Total acid phosphatase activity (U/l) = (ΔA/min x 750)
Prostatic acid phosphatase activity (U/l) = (ΔA/min total-ΔA/min(tartrate resistant)) x 750
Prostatic acid phosphatase activity (pKat/l) = (ΔA/min total-ΔA/min(tartrate resistant)) x 12,5

Quality control
A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

PERFORMANCES DATA
The following data were obtained using the Olympus 600 analyzer.

Linearity
The test is linear up to 150 U/l (2.5 pKat/l) acid phosphatase activity.

Sensitivity
It is recommended that each laboratory establishes its own range of sensitivity as this is limited by the sensitivity of the spectrophotometer used. Under manual conditions however, a change of 0.001 Abs units/min is equivalent to 0.75 U/l (0.0125 pKat/l).

Total acid phosphatase activity at 405 nm.

Precision
Total acid phosphatase

<table>
<thead>
<tr>
<th>Reproducibility</th>
<th>Average activity (U/l)</th>
<th>SD</th>
<th>CV%</th>
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<tbody>
<tr>
<td>Sample I.</td>
<td>23.4</td>
<td>0.320</td>
<td>1.36</td>
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<tr>
<td>Sample II.</td>
<td>37.5</td>
<td>0.283</td>
<td>0.76</td>
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Correlation
Comparative studies were done to compare our reagent with another commercial acid phosphatase assay.
The results from these studies are detailed below.

Correlation coefficient: r=0.9938
Linear regression: y=0.913x – 0.364
(x= other commercial reagent y= own reagent).

Specificity
Bilirubin 128.3 μmol/l (7.5mg/dl), lipid 10.0 mg/dl, glucose 55.5 mmol/l (1000mg/dl), and ascorbic acid 2.84 mmol/l (0.05mg/dl) don’t interfere with the assay up to the given levels.

Note
Do not pipeette reagents by mouth. Hemolytic as well as icteric sera interfere with the test. Test should be performed as soon as possible after blood sampling. 20 µl acetate buffer solution (5 mol/l) should be added to every ml of serum if samples have to be stored. Do not use reagents after the expiry date stated on each reagent container label. Do not use products, test solutions and reagents described above for any purpose other than described herein.

For in vitro diagnostic use only.

The following symbols are used on labels

<table>
<thead>
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<th>IVD</th>
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<tr>
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Bibliography

Acid phosphatase, freeze dried
Date of revision: 2009-07