Acid phosphatase (ACP)

Colorimetric kinetic test.
α-Naphtyl phosphate. Hillmann

Code HBE01 **18 x 2 ml**

Store at 2-8°C.

CYPRESS DIAGNOSTICS

CE

Clinical significance

Non-specific acid phosphatase activity is widely distributed throughout the living world. This enzyme is secreted by the human prostate gland. Elevate levels of non-prostatic acid phosphate have been observed in patients with Paget's disease, hyperparathyroidism with skeletal involvement, and in cancers wich have invaded the bones.

Principle

Acid phosphatase (ACP) activity present in the sample is determined according to the modified method of Hillmann.

$$\alpha\text{-naphtyl-phosphate} + H_2O \xrightarrow{ACP} \alpha\text{-naphtol} + \text{phosphate}$$

$$\alpha\text{-naphtol} + \text{Fast Red TR} \longrightarrow \text{Diazo Dye}$$

The rate of colour formation is proportional to the Acid phosphatase activity in the sample.

Reagent composition

Reagent 1 Buffer	Sodium citrate pH 5,2……50 mmol/l
Reagent 2 Substrate	α-naphtylphosphate……10 mmol/l Fast Red TR……6 mmol/l
Reagent 3 Tartrate	Sodium tartrate……2 mmol/l
Reagent 4	Acetic acid……0,5 mol/l

For *in vitro* diagnostic use only.

Preparation

Dissolve one tablet R2 substrate in 2 ml of R1 buffer. Cap and mix gently to dissolve the contents.
The stability of working reagent is 2 days at 2-8°C or 6 hours at room temperature.
R3 and R4: ready to use.

Storage and stability

All the components of the kit are stable at 2-8°C up to the date of expiration as specified, when stored tightly closed, protected from light and contaminations prevented during their use. Do not use the tablets if they appear to be broken.
The reagent should be a clear solution. If turbidity or precipitation has occurred or if blank absorbance at 405 nm ≥ 0,44, the reagent should be discarded.

Additional equipment

- Spectrophotometer or colorimeter measuring at 405 nm
- Thermostatic bath at 30°C or 37°C (± 0,1 °C)
- Matched cuvettes 1,0 cm light path
- General laboratory equipment

Samples

Clear serum, separated from the clot as soon as possible. Do not use plasma or haemolytic serum.
Acid phosphatase is extremely labile. stabilize by adding 50 µl of acetic acid (R4) per ml of the sample. Stability: 7 days at 2-8°C.

Procedure

1. Wavelength 405 nm; Constant Temperature 30°C/37°C; Cuvette 1 cm light path.
2. Adjust the instrument to zero with distilled water or air.
3. Pipette into a cuvette:

	Total ACP	ACP Non Prostatic
Working reagent	1,0 ml	1,0 ml
Tartrate R.3	---	10 µl
Sample	100 µl	100 µl

Mix and incubate 5 min. Read the initial absorbance (abs), start the stopwatch and read absorbance every minute for 3 minutes. Calculate the difference between the absorbances and the average absorbance differences per minute (Δ abs./min).

Calculation

Total ACP (U/l) = 750 x Δ Abs/min

Prostatic acid phosphatase = Total ACP –non prostatic ACP.

ACP prostatic (U/l)
= 750 x (Δ Abs/min total ACP - Δ Abs/min ACP non prostatic)

One international unit (IU) is the amount of enzyme that transforms 1 µmol of substrate per minute, in standard conditions. The concentration is expressed in units per litre of sample (U/l).

Quality control

Control sera are recommended to monitor the performance of assay procedures. If control values are found outside the defined range, check the instrument, reagents and calibrator for problems. Each laboratory should establish its own QC scheme and corrective actions if controls do not meet the acceptable tolerances.

Normal and pathological human (HBC01, HBC02) or bovine (HBC04, HBC05) sera are available.

Reference values

	30°C.	37°C.
Total acid phosphatase: Men Women	 < 4,3 U/l < < 3,1 U/l	 < 5,4 U/l < 4,2 U/l
Prostatic acid phosphatase	< 1,5 U/l	< 1,7 U/l

These values are for orientation purpose. Each laboratory should establish its own reference range.

Performance characteristics (Total ACP)

Measuring range: from 0,13 U/l (detection limit) to 150 U/l (linearity limit). If the obtained results are greater than 150 U/l, dilute the sample 1:2 with saline solution, repeat the determination, and multiply the result by factor 2.

Presicion:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (U/l)	23,67	2,56	23,6	2,6
SD	0,22	0,07	0,22	0,07
CV (%)	0,95	2,90	0,92	2,76

Sensitivity: 1 U/l = 0,0034 ΔAbs/min
Accuracy: Results obtained using CYPRESS DIAGNOSTICS reagents did not show systematic differences when compared with other commercial reagents.

Interferences

Hemolysis interferes in the assay due to the high ACP concentration in red cells. A list of drugs and other interfering substances with ACP determination has been reported by Young et al.

Bibliography

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Langdorp, 02.2005

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