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# ACID PHOSPHATASE Kinetic method

Reagent for quantitative determination of total (ACP) and prostatic acid phosphatase (P ACP) activity [EC 3.1.2] in human serum.

| | | |
|---|---|---|
| REF 82560 | **R1** 10 x 15mL | **R2** 5 x 15mL |
| | **R3** 10 x 15mL | **R4** 1 x 5 mL |

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CE

IVD

**Made In France**

I: corresponds to significant modifications

## INTENDED USE

This reagent is designated for professional use in laboratory (manual or automated method).
It allows the quantification of of total (ACP) and prostatic acid phosphatase (P ACP) activity [EC 3.1.2] in human serum.

## PRINCIPLE (4) (5)

Hillmann modified method. Reaction scheme is as follows:

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

$\alpha$-naphtol + Fast Red TR Salt $\longrightarrow$ Azo dye

The rate at which the diazo compound is formed, measured at 405 nm, is proportional to the ACP activity in the specimen.
The NP ACP activity (Non prostatic ACP activity, tartrate resistant) is measured in the presence of Tartrate. The difference between the assay of the ACP and the one of the NP ACP gives the P ACP activity.

## REAGENTS COMPOSITION

| | | |
|---|---|---|
| **R1** | **ACID PHOSPHATASE** | Citrate Buffer |
| | | Concentration in the test |
| Citrate buffer pH 5.4 | | 150 mmol/L |
| 1,5-Pentanediol | | 114 mmol/L |
| Surfactant, preservative | | |
| **R2** | **ACID PHOSPHATASE** | Citrate/Tartrate Buffer |
| Na Tartrate | | 75 mmol/L |
| Citrate buffer pH 5.4 | | 150 mmol/L |
| 1,5-Pentanediol | | 114 mmol/L |
| Surfactant, preservative | | |

Danger: Eye Dam. 1: H318 - Causes serious eye damage. Met. Corr. 1: H290 - May be corrosive to metals. Skin Irrit. 2: H315 - Causes skin irritation

P280: Wear protective gloves/protective clothing/eye protection/face protection. P302+P352: IF ON SKIN: Wash with plenty of water. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Classification due to Dodecan-1-ol, ethoxylated 2,5 - < 10%. For more details, refer to SDS (Safety data sheet).

| | | |
|---|---|---|
| **R3** | **ACID PHOSPHATASE** | Substrate |
| $\alpha$-naphtyl phosphate | | 12.5 mmol/L |
| Fast Red TR Salt | | 1.6 mmol/L |
| (diazo 2, chloro 5 toluene) | | |

Warning: Eye Irrit. 2: H319 - Causes serious eye irritation. Skin Irrit. 2: H315 - Causes skin irritation.

P280: Wear protective gloves/protective clothing/eye protection/face protection. P302+P352: IF ON SKIN: Wash with plenty of water. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Classification due to Monosodium 1-Naphthyl Phosphate Monohydrate 10 - < 25%. For more details, refer to SDS (Safety data sheet).

| | | |
|---|---|---|
| **R4** | **ACID PHOSPHATASE** | Stabilizer |
| Acetic acid | 1.5 | mol/L (in vial r4) |

Warning: Flam. Liq. 3: H226 - Flammable liquid and vapour

P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P233: Keep container tightly closed. Classification due to acetic acid 2.5 - < 10%. For more details, refer to SDS (Safety data sheet).

## GENERALITIES (1)

Determination of phosphatase acid activity in serum is almost always directed toward the prostatic enzyme. Indeed, it is recommended that tumour markers measurements such as PSA determination (Prostatic Specific Antigen) be combined with both patient clinical examinations. P ACP activity in serum then allows confirming and evaluating a positive diagnosis of prostatic carcinoma.

## SAFETY CAUTIONS

- Refer to current Material Safety Data Sheet available on request or on www.biolabo.fr
- Verify the integrity of the contents before use.
- Waste disposal: Respect legislation in force in the country.
- All specimens or reagents of biological origin should be handled as potentially infectious. Respect legislation in force in the country.

I Any serious incident that has occurred in connection with the device is notified to the manufacturer and the competent authority of the Member State in which the user and/or patient is based.

## REAGENTS PREPARATION

- Vial R3: Use a non-sharp instrument to remove the cap.
- ACP Reagent: Add promptly the contents of vial R3 into R1
- NP ACP Reagent: Add promptly the contents of vial R3 into R2
- Mix gently and wait for complete dissolution.
- Vial R4 is ready for use.

## STABILITY AND STORAGE

**Stored away from light, well caped in the original vial at 2-8°C, when stored and used as described, reagents are stable:**

Unopened:

- Until expiry date stated on the label of the kit.

Once opened:

- reconstituted reagents are stable for 10 days at 2-8°C.
- Discard any reagents if cloudy or if reagent blank at 405 nm > 0.600.

## SPECIMEN COLLECTION AND HANDLING (2)

Unhemolysed serum. Separate from the clot as soon as possible after collection and promptly assayed.

Acidify at pH 5.4-6.2 ,adding a drop (20 µL) of vial R4 (Stabiliser) for 1 mL of serum.
The activity decreases of 50% in 8 hours in non-acidified serum.

Acid Phosphatase activity is stable in the acidified serum for:

- 7 days at 2-8°C.

## LIMITES (2) (3) (6) (7)

Oxalate and fluorides inhibit Acid Phosphatase activity.
Discard any icteric specimens

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

## MATERIAL REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. Spectrophotometer or Biochemistry Clinical Analyzer

## QUALITY CONTROL

- REF 95010 EXATROL-N Level I
- REF 95011 EXATROL-P Level II
- External quality control program.

It is recommended to control in the following cases:

- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If control is out of range, apply following actions:

1. Prepare a fresh control serum and repeat the test.
2. If control is still out of range, use a new vial of calibrator or a fresh calibrator and repeat the test.
3. If control is still out of range, repeat the tests with a new vial of reagent.

If control is still out of range, please contact BIOLABO technical support or your local Agent.

## EXPECTED VALUES (2) (8)

| (2) Prostatic Acid Phosphatase (30°C or 37°C) | | |
|---|---|---|
| 0-0.8 IU/L | | 0-0.01 µkat/L |
| **(8) Total Acid Phosphatase (37°C)** | | |
| Men | < 6.6 IU/L | < 0.110 µkat/L |
| Women | < 6.6 IU/L | < 0.110 µkat/L |
| **(8) Prostatic Acid Phosphatase (37°C)** | | |
| Men | < 3,5 U/L | < 0,058 µkat/L |

Each laboratory should establish its own normal ranges for the population it serves.

## PERFORMANCE CHARACTERISTICS

On Cobas Mira at 37°C, 405 nm.

Linearity Range:
PAC : between 10 IU/L and 150 UI/L (2,5 µKat/L)
PANP : between 10 and 75 UI/L (1,25 µKat/L)

Detection limit: approx. 1 IU/L

**Precision: TOTAL ACID PHOSPHATASE**

| ***Within-run N = 20*** | *Low level* | *Normal level* | *High level* |
|---|---|---|---|
| Mean (IU/L) | 7.4 | 22.9 | 48.3 |
| S.D. (IU/L) | 0.1 | 0.17 | 0.68 |
| C.V. % | 1.4 | 0.8 | 1.4 |

| ***Between run N = 20*** | *Low level* | *Normal level* | *High level* |
|---|---|---|---|
| Mean (IU/L) | 11.8 | 27.1 | 51.1 |
| S.D. IU/L | 0.47 | 1.1 | 1.65 |
| C.V. % | 4.0 | 4.0 | 3.2 |

**Precision: PROSTATIC ACID PHOSPHATASE**

| ***Within-run N = 20*** | *Low level* | *Normal level* | *High level* |
|---|---|---|---|
| Mean (IU/L) | 3.0 | 10.3 | 18.4 |
| S.D. (IU/L) | 0.22 | 0.21 | 0.24 |
| C.V. % | 2.2 | 2.0 | 1.3 |

| ***Between run N = 20*** | *Low level* | *Normal level* | *High level* |
|---|---|---|---|
| Mean (IU/L) | 9.2 | 15.1 | 17.1 |
| S.D. IU/L | 0.67 | 0.45 | 1.02 |
| C.V. % | 7.3 | 3.0 | 6.0 |

Analytical Sensitivity: approx. 0.009 abs/min for 10 IU/L at 405 nm

Comparison with commercially available reagent:

ACP: y = 0,9042 x + 0,7177 r = 0,9995
NP ACP: y = 1,0728 x – 3,5025 r = 0,9907

Interferences:

| | |
|---|---|
| Turbidity | No interference up to 0.282 abs |
| Ascorbic acid | Positive interference from 10000 mg/dL |
| Total bilirubin | Negative interference from 240 µmol/L |
| Glucose | No interference up to 1060 mg/dL |

Other substances may interfere (see § Limits)

Calibration Stability:

Make a new calibration when changing reagent batch, if quality control results are found out of the range and after maintenance operations

## CALIBRATION

- REF 95015 Multicalibrator traceable to *an Internal Masterlot*

The calibration frequency depends on proper instrument functions and on the preservation of reagent

## PROCEDURE

Manual Method

Let stand reagents and specimens at room temperature.

| Pipette into 1 cm path length thermostated cuvette (37°C): | **Assay 1 (ACP)** | **Assay 2 (NP ACP)** |
|---|---|---|
| **ACP Reagent (R1+R3)** | 1 mL | |
| **NP ACP Reagent (R2+R3)** | | 1 mL |
| **Standard / Control or Specimen** | 100 µL | 100 µL |
| Mix. Record initial absorbance at 405 nm after 5 minutes, and ΔAbs /min every minutes during 3 minutes. | | |

1- Performances with manual procedure should be validated by user.

2- Kenza applications and other applications proposal are available on request.

## CALCULATION

P ACP Activity = Assay 1 Activity– Assay 2 Activity

With serum Multicalibrator

$$\text{ALP Activity} = \frac{(\Delta\text{Abs/min) Assay}}{(\Delta\text{Abs/min) Calibrator}} \times \text{Calibrator Concentration}$$

With theoretical factor:

Activity (IU/L) = ΔAbs/min x Factor

$$\text{Factor} = \frac{\text{VR} \times 1000}{15.07 \times \text{VE} \times \text{P}}$$

With:
VR = Total reactional volume (mL)
VE = Specimen volume (mL)
15.07 = Molar extinction coefficient for PNPP at 405 nm
P = Path length (cm).

Example, with manual Procedure,
(Path length 1 cm, 37°C, 405 nm):

IU/L = (ΔAbs/min) x 730

$$\text{µkat/L} = \frac{\text{IU/L}}{60}$$

## REFERENCES


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|---|---|---|---|---|---|
| Manufacturer | Expiry date | In vitro diagnostic | Storage temperature | Dematerialized water | Biological risk |
| Product Reference | See Insert | Batch number | Store away from light | Sufficient for | Dilute with |