

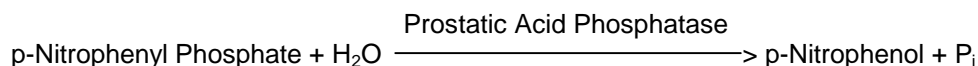


## SIGMA QUALITY CONTROL TEST PROCEDURE

## Product Information

### Enzymatic Assay of PHOSPHATASE, ACID, PROSTATIC (EC 3.1.3.2)

#### PRINCIPLE:



Abbreviation used:

P<sub>i</sub> = Inorganic phosphate

**CONDITIONS:** T = 37°C, pH = 4.8, A<sub>410nm</sub>, Light path = 1 cm

**METHOD:** Spectrophotometric Stop Rate Determination

#### REAGENTS:

- A. 90 mM Citrate Buffer Solution, pH 4.8 at 37°C  
(Use Citrate Buffer Solution, Sigma Stock No. 104-4.)
- B. 40 mM Tartrate Buffer, with 90 mM Citrate, pH 4.8 at 37°C.  
(Use Tartrate Acid Buffer Solution, Sigma Stock No. 104-12.)<sup>1</sup>
- C. 11.3 mM p-Nitrophenyl Phosphate (PNPP)  
(Prepare 5 ml in deionized water using p-Nitrophenyl Phosphate Sigma 104 Phosphatase Substrate, Sigma Stock No. 104-0.)
- D. 100 mM Sodium Hydroxide Solution (NaOH)  
(Prepare 50 ml in deionized water using Sodium Hydroxide, Anhydrous Sigma Prod. No. S-5881.)
- E. Prostatic Acid Phosphatase Enzyme Solution  
(Immediately before use, prepare a solution containing 0.015 - 0.02 unit/ml of Acid Phosphatase in cold deionized water.)

**Enzymatic Assay of PHOSPHATASE, ACID, PROSTATIC  
(EC 3.1.3.2)**

**PROCEDURE:**

Pipette (in milliliters) the following reagents into suitable containers:

|                             | <u>Test1</u> | <u>Test2</u> | <u>Blank</u> |
|-----------------------------|--------------|--------------|--------------|
| Reagent A (Citrate Buffer)  | 0.50         | -----        | 0.50         |
| Reagent B (Tartrate Buffer) | -----        | 0.50         | -----        |
| Reagent C (PNPP) 0.50       | 0.50         | 0.50         |              |

Mix and equilibrate to 37°C. Then add:

|                             | <u>Test1</u> | <u>Test2</u> | <u>Blank</u> |
|-----------------------------|--------------|--------------|--------------|
| Reagent E (Enzyme Solution) | 0.20         | 0.20         | -----        |

Immediately mix and incubate at 37°C for exactly 30 minutes. Then add:

|                             |       |       |      |
|-----------------------------|-------|-------|------|
| Reagent D (NaOH) 3.80       | 3.80  | 3.80  |      |
| Reagent E (Enzyme Solution) | ----- | ----- | 0.20 |

Mix and record the  $A_{410nm}$  for both the Tests and Blank in a suitable spectrophotometer.

**CALCULATIONS:**

Total Acid Phosphatase Activity:

$$\text{Units/ml Total enzyme} = \frac{(\Delta A_{410nm}/\text{min Test}_1 - \Delta A_{410nm}/\text{min Blank})(5.0)(df)}{(30)(18.3)(0.2)}$$

Nonprostatic Acid Phosphatase Activity:

Units/ml Nonprostatic enzyme =

$$\frac{(\Delta A_{410nm}/\text{min Test}_2 - \Delta A_{410nm}/\text{min Blank})(5.0)(df)}{(30)(18.3)(0.2)}$$

Prostatic Acid Phosphatase Activity:

Units/ml Prostatic Enzyme = Units/ml Total Enzyme - Units/ml Nonprostatic Enzyme

## Enzymatic Assay of PHOSPHATASE, ACID, PROSTATIC (E C 3.1.3.2)

### CALCULATIONS: (continued)

5.0 = Total volume (in milliliters) of solution

30 = Time of assay (in minutes)

18.3 = Millimolar extinction coefficient of p-Nitrophenol at 410 nm at an Alkaline pH

0.2 = Volume (in milliliter) of enzyme used

df = Dilution factor

$$\text{Units/mg solid} = \frac{\text{units/ml Prostatic Enzyme}}{\text{mg solid/ml Total Enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml Prostatic Enzyme}}{\text{mg protein/ml Total Enzyme}}$$

### UNIT DEFINITION:

One unit will hydrolyze 1.0  $\mu$ mole of p-nitrophenyl phosphate per minute at pH 4.8 at 37°C.

Prostatic acid phosphatase activity is the difference between the total acid phosphatase activity and the acid phosphatase activity in the presence of 20 mM tartrate.

### FINAL ASSAY CONCENTRATION:

In a 1.20 ml reaction mix, the final concentrations are 38 mM citric acid, 4.7 mM p-nitrophenyl phosphate and 0.004 unit prostatic acid phosphatase.

### NOTES:

1. Tartrate Buffer negates roughly 95% of the prostatic acid phosphatase.
2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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