SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

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

PRINCIPLE:

$$\text{p-Nitrophenyl Phosphate} + H_2O \xrightarrow{\text{Prostatic Acid Phosphatase}} \text{p-Nitrophenol} + P_i$$

Abbreviation used:
P_i = Inorganic phosphate

CONDITIONS: T = 37°C, pH = 4.8, A_{410nm}, 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.)[1]

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.)

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PROCEDURE:

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

	Test1	Test2	Blank
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:

	Test1	Test2	Blank
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 =

$$\frac{(\Delta A_{410nm}/\text{min Test}_2 - \Delta A_{410nm}/\text{min Blank})(5.0)(df)}{(30)(18.3)(0.20)}$$

Prostatic Acid Phosphatase Activity:

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

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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 μmole 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.