SIGMA

ProductInformation

SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of PHOSPHATASE, ACID (EC 3.1.3.2)

PRINCIPLE:

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

Abbreviation:
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, pH 4.8 at 37°C
(Prepare 100 ml in deionized water using Citric Acid, Trisodium, Dihydrate, Sigma Prod. No. C-7254, or Citrate Buffer Solution, Sigma Stock No. 104-4. Adjust to pH 4.8 at 37°C with 1 M NaOH or 1 M HCl.)

B. 15.2 mM p-Nitrophenyl Phosphate (PNPP)
(Prepare 5 ml in deionized water using Sigma 104 Phosphatase Substrate, Sigma Stock No. 104-0.)

C. 100 mM Sodium Hydroxide Solution (NaOH)
(Prepare 50 ml in deionized water using Sodium Hydroxide, Anhydrous, Sigma Prod. No. S-5881.)

D. Acid Phosphatase Enzyme Solution
(Immediately before use, prepare a solution containing 0.15 - 0.25 unit/ml of Phosphatase, Acid in cold deionized water.)

PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	0.50	0.50
Reagent B (PNPP)	0.50	0.50

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PROCEDURE: (continued)

Mix by inversion and equilibrate to 37°C. Then add:

	Test	Blank
Reagent D (Enzyme Solution)	0.10	------

Immediately mix by inversion and incubate at 37°C for exactly 10 minutes. Then add:

Reagent C (NaOH)	4.00	4.00
Reagent D (Enzyme Solution)	------	0.10

Mix by inversion and record the A_{410nm} for both the Test and Blank in a suitable spectrophotometer.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(A_{410nm}\ \text{Test} - A_{410nm}\ \text{Blank})(5.1)(df)}{(10)(18.3)(0.1)}$$

5.1 = Total volume (in milliliters) of solution
df = Dilution factor
10 = Time of assay (in minutes) as per the Unit Definition
18.3 = Millimolar extinction coefficient of p-Nitrophenol at 410 nm
0.1 = Volume (in milliliter) of enzyme used

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will hydrolyze 1.0 µmole of p-nitrophenyl phosphate per minute at pH 4.8 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 1.10 ml reaction mix, the final concentrations are 41 mM citric acid, 6.9 mM p-nitrophenyl phosphate and 0.015 - 0.025 unit phosphatase, acid.

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REFERENCE:

Bergmeyer, H.U., Gawehn, K., and Grassl, M. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer H.U.) Volume I, 2nd ed., 495-496, Academic Press, Inc., New York, NY

NOTES:

1. This assay is based on the cited reference.

2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.