

ProductInformation

SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of PHOSPHATASE, ALKALINE¹ (EC 3.1.3.1) Diethanolamine Assay

PRINCIPLE:

p-Nitrophenyl Phosphate + H₂O Alkaline Phosphatase > p-Nitrophenol + P_i

Abbreviation used: P_i = Inorganic Phosphate

CONDITIONS: $T = 37^{\circ}C$, pH = 9.8, A_{405nm} , Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 1.0 M Diethanolamine Buffer with 0.50 mM Magnesium Chloride, pH 9.8 at 37°C (Prepare 50 ml using Diethanolamine, Sigma Prod. No. D-8885, and Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250. Dissolve the Magnesium Chloride complete in deionized water before adding the Diethanolamine. Adjust to pH 9.8 at 37°C with 5 M HCl. PREPARE FRESH.)
- B. 150 mM p-Nitrophenyl Phosphate Solution (PNPP) (Prepare 2 ml in deionized water using Sigma 104 Phosphatase Substrate, Sigma Stock No. 104-0. PREPARE FRESH.)
- C. Phosphatase, Alkaline Enzyme Solution (Immediately before use, prepare a solution containing 0.1 0.2 unit/ml of Alkaline Phosphatase in cold Reagent A.)

PROCEDURE:

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

	<u> 165t</u>	DIAITK
Reagent A (Buffer)	2.70	2.80
Reagent B (PNPP)	0.30	0.30

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PROCEDURE: (continued)

Mix by inversion and equilibrate to 37° C. Monitor the A_{405nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

	<u>Test</u>	<u>Blank</u>
Reagent C (Enzyme Solution)	0.10	

Immediately mix by inversion and record the increase in A_{405nm} for approximately 5 minutes. Obtain the ΔA_{405nm} /minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

Units/ml enzyme =
$$\frac{(\Delta A_{405nm}/min \text{ Test - } \Delta A_{405nm}/min \text{ Blank})(3.1)(df)}{(18.5)(0.1)}$$

3.1 = Volume (in milliliters) of assay

df = Dilution factor

18.5 = Millimolar extinction coefficient of p-Nitrophenol at 405 nm

0.1 = Volume (in milliliters) of enzyme used

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

UNIT DEFINITION:

One unit will hydrolyze 1.0 µmole of p-nitrophenyl phosphate per minute at pH 9.8 at 37°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.10 ml reaction mix, the final concentrations are 903 mM diethanolamine, 0.45 mM magnesium chloride, 14 mM p-nitrophenyl phosphate and 0.01 - 0.02 unit alkaline phosphatase.

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REFERENCES:

Walter, K. and Schütt, C. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed) 2nd ed., Volume II, pp 860-864, Academic Press, Inc., NY

NOTES:

- 1. This enzyme assay is not to be used to assay Phosphatase, Alkaline, in which the specific activity is cited only in glycine units.
- 2. This assay is based on the cited reference.
- 3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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