

ProductInformation

SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of PHOSPHATASE, ALKALINE¹ (EC 3.1.3.1) Glycine with Zinc Assay

PRINCIPLE:

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

Abbreviation:

P_i = Inorganic Phosphate

CONDITIONS: $T = 37^{\circ}C$, pH = 10.4, A_{405m} , Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Glycine Buffer with 1.0 mM Magnesium Chloride and 1.0 mM Zinc Chloride, pH 10.4 at 37 °C

(Prepare 50 ml in deionized water using Glycine, Free Base, Sigma Prod. No. G-7126; Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250; and Zinc Chloride, Sigma Prod. No. Z-4875. Adjust to pH 10.4 at 37°C with 1 M NaOH. **PREPARE FRESH**.)

- B. 60 mM p-Nitrophenyl Phosphate Solution (PNPP)
 (Prepare 5 ml in deionized water using Sigma 104 Phosphatase Substrate, Sigma Stock No. 104-0. PREPARE FRESH.)
- C. 1.0 mM Magnesium Chloride Solution (MgCl₂) (Prepare 50 ml in deionized water using Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250.)
- D. Phosphatase, Alkaline Enzyme Solution (Immediately before use, prepare a solution containing 0.1 - 0.2 unit/ml of Alkaline Phosphatase in cold Reagent C.)

P3765 SPPNPP01 Revised: 07/12/94

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PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.60	2.60
Reagent B (PNPP)	0.30	0.30

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

	<u>l est</u>	Blank
Reagent C (MgCl ₂)		0.10
Reagent D (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 =
$$\frac{(\Delta A_{405nm}/min \text{ Test - } \Delta A_{405nm}/min \text{ Blank})(3)(df)}{(18.5) (0.1)}$$

$$3 = \text{Volume (in milliliters) of assay}$$

$$df = \text{Dilution factor}$$

18.5 = Millimolar extinction coefficient of p-nitrophenol at 405 nm 0.1 = Volume (in milliliter) of enzyme used

UNIT DEFINITION:

One unit will hydrolyze 1.0 µmole of p-nitrophenyl phosphate per minute at pH 10.4 at 37°C.

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FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 87 mM glycine, 0.90 mM magnesium chloride, 0.87 mM zinc chloride, 6.0 mM p-nitrophenyl phosphate and 0.01 - 0.02 unit alkaline phosphatase.

REFERENCES:

Bergmeyer, H.U., Grassl, M., and Walter, H.E. (1983) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U., ed) 3rd ed., Volume II, 269-270, Verlag Chemie, Deerfield Beach, FL

NOTES:

- This assay is not to be used for Phosphatase, Alkaline, Type XXIII from Trout Intestine, Sigma Prod. No. P-6271, Phosphatase, Alkaline-Acrylic Beads, Sigma Prod. No. P-0927, Phosphatase, Alkaline, Affinity Filtration Cartridge, Sigma Prod. No. P-9548, Phosphatase, Alkaline-Agarose, Sigma Prod. No. P-0762, Phosphatase, Alkaline-Biotinamidocaproyl, Sigma Prod. No. P-1318, Phosphatase, Alkaline, from Shrimp, Sigma Prod. No. P-8302, or for Phosphatase, Alkaline, Bacterial (Escherichia coli), Sigma Prod. No. P-4069, or any phosphatase, alkaline in which the specific activity is described in DEA 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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P3765 SPPNPP01 Revised: 07/12/94