

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

PRINCIPLE:

p-Nitrophenyl Phosphate + H₂O $\xrightarrow{\text{Alkaline Phosphatase}}$ p-Nitrophenol + P_i

Abbreviation:

P_i = Inorganic Phosphate

CONDITIONS: T = 37°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.)

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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°C. Monitor the A_{405nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

	<u>Test</u>	<u>Blank</u>
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:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{405\text{nm}}/\text{min Test} - \Delta A_{405\text{nm}}/\text{min Blank})(3)(\text{df})}{(18.5)(0.1)}$$

3 = Volume (in milliliters) of assay

df = Dilution factor

18.5 = Millimolar extinction coefficient of p-nitrophenol at 405 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 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:

1. 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.
2. This assay is based on the cited reference.
3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.