

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

PRINCIPLE:

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

Abbreviation:

P_i = Inorganic Phosphate

CONDITIONS: T = 37°C, pH = 8.8, A_{410m}, Light path = 1 cm

METHOD: Stopped Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Glycine Buffer with 1.0 mM Magnesium Chloride, pH 8.8 at 37°C
(Prepare 50 ml in deionized water using Glycine, Free Base, Sigma Prod. No. G-7126 and Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250. Adjust to pH 8.8 at 37°C with 1 M NaOH. **PREPARE FRESH.**)
- B. 15.2 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. 20 mM Sodium Hydroxide Solution (NaOH)
(Prepare 100 ml in deionized water using Sodium Hydroxide, Anhydrous, Sigma Stock No. 505-8.)
- D. Phosphatase, Alkaline Enzyme Solution
(Immediately before use, prepare a solution containing 0.1 - 0.2 unit/ml of Alkaline Phosphatase in cold deionized water.)

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PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	0.50	0.50
Reagent B (PNPP)	0.50	0.50

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

Reagent D (Enzyme Solution)	0.10	-----
Deionized Water	-----	0.10

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

Reagent C (NaOH)	10.00	10.00
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Mix by swirling and transfer the solutions to suitable cuvettes and record the $A_{410\text{nm}}$ for both Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(A_{410\text{nm}} \text{ Test} - A_{410\text{nm}} \text{ Blank})(11.1)(\text{df})}{(18.3) (0.1) (10)}$$

11.1 = Volume (in milliliters) of assay

df = Dilution factor

18.3 = Millimolar extinction coefficient of p-nitrophenol at 410 nm

0.1 = Volume (in milliliters) of enzyme used

10 = Time of assay (in minutes) as per the Unit Definition

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

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

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UNIT DEFINITION:

One unit will hydrolyze 1.0 μ mole of p-nitrophenyl phosphate per minute at pH 8.8 at 37°C.

FINAL ASSAY CONCENTRATIONS:

In a 1.10 ml reaction mix, the final concentrations are 45 mM glycine, 0.45 mM magnesium chloride, 6.9 mM p-nitrophenyl phosphate and 0.01 - 0.02 unit alkaline phosphatase.

REFERENCES:

Bernt, E. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U., ed) 2nd ed., Volume II, pp 868-870, 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.

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