SIGMA

ProductInformation

SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of PHOSPHATASE, ALKALINE[1] (EC 3.1.3.1) Glycine with Zinc Assay

PRINCIPLE:

p-Nitrophenyl Phosphate + H_2O $\xrightarrow{\text{Alkaline Phosphatase}}$ 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 ($MgCl_2$)
(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:

	Test	Blank
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:

	Test	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:

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

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, 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.