SIGMA

ProductInformation

SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of PHOSPHATASE, ALKALINE[1] (EC 3.1.3.1) Diethanolamine Assay

PRINCIPLE:

p-Nitrophenyl Phosphate + H_2O $\xrightarrow{\text{Alkaline Phosphatase}}$ p-Nitrophenol + P_i

Abbreviation used:
P_i = Inorganic Phosphate

CONDITIONS: T = 37°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:

	Test	Blank
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:

	Test	Blank
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:

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

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

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

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

UNIT DEFINITION:

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