



## Product Information

### SIGMA QUALITY CONTROL TEST PROCEDURE

#### Enzymatic Assay of PHOSPHATASE, ALKALINE<sup>1</sup> (EC 3.1.3.1) Diethanolamine Assay

##### PRINCIPLE:

p-Nitrophenyl Phosphate + H<sub>2</sub>O  $\xrightarrow{\text{Alkaline Phosphatase}}$  p-Nitrophenol + P<sub>i</sub>

Abbreviation used:  
P<sub>i</sub> = Inorganic Phosphate

**CONDITIONS:** T = 37°C, pH = 9.8, A<sub>405nm</sub>, 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:

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.70	2.80
Reagent B (PNPP)	0.30	0.30

**Enzymatic Assay of PHOSPHATASE, ALKALINE<sup>1</sup>**  
**(EC 3.1.3.1 )**  
**Diethanolamine Assay**

**PROCEDURE:** (continued)

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

	<u>Test</u>	<u>Blank</u>
Reagent C (Enzyme Solution)	0.10	-----

Immediately mix by inversion and record the increase in  $A_{405\text{nm}}$  for approximately 5 minutes. Obtain the  $\Delta A_{405\text{nm}}/\text{minute}$  using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

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

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  $\mu\text{mole}$  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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**Diethanolamine Assay**

**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.

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