

### **ProductInformation**

### SIGMA QUALITY CONTROL TEST PROCEDURE

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

#### PRINCIPLE:

p-Nitrophenyl Phosphate + H<sub>2</sub>O Alkaline Phosphatase > p-Nitrophenol + P<sub>i</sub>

Abbreviation:

P<sub>i</sub> = Inorganic Phosphate

**CONDITIONS:**  $T = 37^{\circ}C$ , 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<sub>2</sub>) (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.)

P8639 SPPNPP01 Revised: 07/12/94 Page 1 of 3

# Enzymatic Assay of PHOSPHATASE ALKALINE<sup>1</sup> (EC 3.1.3.1) Glycine with Zinc Assay

#### 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  $37^{\circ}C$ . Monitor the  $A_{405nm}$  until constant, using a suitably thermostatted spectrophotometer. Then add:

	<u>l est</u>	Blank
Reagent C (MgCl <sub>2</sub> )		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  $\Delta A_{405nm}$ /minute using the maximum linear rate for both the Test and Blank.

#### **CALCULATIONS:**

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

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

#### **UNIT DEFINITION:**

One unit will hydrolyze 1.0 µmole of p-nitrophenyl phosphate per minute at pH 10.4 at 37°C.

P8639 SPPNPP01 Revised: 07/12/94

# Enzymatic Assay of PHOSPHATASE ALKALINE<sup>1</sup> (EC 3.1.3.1 ) Glycine with Zinc Assay

#### 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:

- 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.
- Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

Sigma warrants that the above procedure information is currently utilized at Sigma and that Sigma products conform to the information in Sigma publications. Purchaser must determine the suitability of the information and products for its particular use. Upon purchase of Sigma products, see reverse side of invoice or packing slip for additional terms and conditions of sale.

P8639 SPPNPP01 Revised: 07/12/94