

# **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.)

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# 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>l est</u>	Blank
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/ml enzyme = 
$$\frac{(\Delta A_{405nm}/min \text{ Test - } \Delta A_{405nm}/min \text{ Blank})(3)(df)}{(18.5) (0.1)}$$

$$3 = \text{Volume (in milliliters) of assay}$$

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.

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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:

- 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.

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