

**Enzymatic Assay of PHOSPHATASE, ALKALINE  
(EC 3.1.3.1)  
Glycine Assay**

**PRINCIPLE:**

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

**CONDITIONS:** T = 37°C, pH = 10.4, A<sub>410nm</sub>, Light path = 1 cm

**METHOD:** Spectrophotometric Stop Rate Determination

**REAGENTS:**

- A. 100 mM Glycine Buffer with 1 mM Magnesium Chloride, pH 10.4 at 37°C  
(Prepare 50 ml in deionized water using Glycine, Prod. No. G-7126, and Magnesium Chloride Hexahydrate, Prod. No. M-0250. Adjust to pH 10.4 at 37°C with 1 M NaOH. **PREPARE FRESH.**)
- B. 15.2 mM p-Nitrophenyl Phosphate Solution (PNPP)  
(Prepare 2 ml in deionized water using Sigma Phosphatase Substrate, Stock No. 104-0. **PREPARE FRESH.**)
- C. Phosphatase, Alkaline Enzyme Solution  
(Immediately before use prepare a solution containing 0.1 - 0.2 units/ml of Alkaline Phosphatase in cold deionized water.)
- D. 20 mM Sodium Hydroxide Solution (NaOH)  
(Prepare 100 ml in deionized water using Sodium Hydroxide, Stock No. 505-8.)

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**PROCEDURE:**

Pipette (in milliliters) the following reagents into suitable cuvettes:

|                    | <u>Test</u> | <u>Blank</u> |
|--------------------|-------------|--------------|
| Deionized Water    | ----        | 0.10         |
| Reagent A (Buffer) | 0.50        | 0.50         |
| Reagent B (PNPP)   | 0.50        | 0.50         |

Mix by inversion and equilibrate to 37°C. Monitor the A<sub>410nm</sub> until constant, using a suitably thermostatted spectrophotometer. Then add:

|                             |      |      |
|-----------------------------|------|------|
| Reagent C (Enzyme Solution) | 0.10 | ---- |
|-----------------------------|------|------|

Immediately mix by inversion and incubate for exactly 10 minutes. Then add:

|           |       |       |
|-----------|-------|-------|
| Reagent D | 10.00 | 10.00 |
|-----------|-------|-------|

Record the A<sub>410nm</sub> for both the test and blank.

**CALCULATIONS:**

$$\text{Units/mg protein} = \frac{(\Delta A_{410\text{nm}} \text{ Test} - \Delta A_{410\text{nm}} \text{ Blank}) (11.1)}{(10) (18.3) (\text{mg protein/RM})}$$

11.1 = total volume

10 = Time of Assay (Unit Definition)

18.3 = Millimolar extinction coefficient for p-nitrophenol

RM = Reaction Mix

**UNIT DEFINITION:**

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

**FINAL ASSAY CONCENTRATIONS:**

In a 1.1 ml reaction mix, the final concentrations are 45 mM glycine, 0.45 mM magnesium chloride, 6.9 mM p-nitrophenyl phosphate and 0.01 to 0.02 units alkaline phosphatase.

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**NOTES:**

1. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

**This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.**