

**Enzymatic Assay of PHYTASE**  
**(EC 3.1.3.26)**

**PRINCIPLE:**

Phytic Acid + H<sub>2</sub>O  $\xrightarrow{\text{Phytase}}$  1L-myo-inositol 1,2,3,4,5-PKP + P<sub>i</sub>

Abbreviations:

L-Myo-inositol 1,2,3,4,5-PKP = L-Myo-inositol 1,2,3,4,5-Pentakisphosphate

Phytic Acid = Myo-Inositol Hexakisphosphate

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

**CONDITIONS:** T = 55°C, pH 5.15, A<sub>660nm</sub>, Light path = 1 cm

**METHOD:** Colorimetric

**REAGENTS:**

- A. 200 mM Sodium Acetate Buffer, pH 5.15 at 55°C  
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 5.15 at 55°C with 1 M Acetic Acid.)
- B. 100 mM Magnesium Sulfate Solution (MgSO<sub>4</sub>)  
(Prepare 100 ml in deionized water, using Magnesium Sulfate, Heptahydrate, Sigma Prod. No. M-9397.)
- C. 6.82 mM Phytic Acid Solution, pH 5.15 at 55°C  
(Prepare 25 ml in Reagent A using Phytic Acid, Dodecasodium Salt, Sigma Prod. No. P-8810. Adjust the pH to 5.15 at 55°C with 0.1 N Acetic Acid.)<sup>1</sup>
- D. 10% (v/v) Trichloroacetic Acid Solution (TCA)  
(Prepare 50 ml in deionized water using Trichloroacetic Acid, 6.1 N Solution, Sigma Stock No. 490-10.)
- E. Phosphorus Standard Solution (P Std)  
(Use Phosphorus Standard Solution, Sigma Stock No. 661-9. The phosphorus concentration is 20 µg/ml, 0.645 µmoles/ml.)

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**REAGENTS:** (continued)

- F. 10% (w/v) Ammonium Molybdate Solution (Amm Moly)  
(Prepare 25 ml in 10 N Sulfuric Acid using Molybdic Acid, Ammonium Tetrahydrate Salt, Sigma Prod. No. M-0878 and Sulfuric Acid, Sigma Prod. No. S-1526.)
- G. Tausky-Shorr Color Reagent Solution (TSCR)  
(Prepare by adding 10 ml Reagent F to 70 ml of deionized water. Then add 5 g Ferrous Sulfate, Heptahydrate, Sigma Prod. No. F-0131, and mix until dissolved. Add enough deionized water to a final volume of 100 ml. Store in the dark.)
- H. Phytase Enzyme Solution  
(Immediately before use, prepare a solution containing 20 mg solid/ml in cold deionized water.)

**PROCEDURE:**

Prepare the following Standards (in milliliters):

	Std	Blank	Std1	Std2	Std3	Std4	Std5
Reagent E (P Std)	0.00	0.20	0.40	0.60	0.80	1.00	
Deionized Water	2.00	1.80	1.60	1.40	1.20	1.00	
Reagent D (TCA)	1.00	1.00	1.00	1.00	1.00	1.00	

Pipette (in milliliters) the following reagents into a 50 ml Erlenmeyer flask:

	<u>Test</u>
Deionized Water	5.00
Reagent A (Buffer)	10.00
Reagent B (MgSO <sub>4</sub> )	0.40
Reagent C (Phytic Acid)	4.40

Mix by swirling and equilibrate at 55°C for several minutes. At time zero, add:

Reagent H (Enzyme)	0.20
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**PROCEDURE:** (continued)

Immediately mix gently by swirling, stopper the flasks, and incubate at 55°C. At each of the times 0, 10, 20, 30, 40, 50 and 60 minutes, remove a 2.0 ml aliquot and transfer to an appropriately labeled test tube that contains 1.0 ml of Reagent D (TCA). After collecting all the aliquots, add to each of the aliquots and the phosphorus standards (labeled Std Blank to Std6) the following reagents (in milliliters):

Deionized Water	2.0
Reagent G (TSCR)	5.0

Mix by swirling. After approximately 5 minutes, read the  $A_{660\text{nm}}$  of each of the test tubes.<sup>2</sup>

**CALCULATIONS:**

Standard Curve:

$$\Delta A_{660\text{nm}} \text{ Standard} = A_{660\text{nm}} \text{ Standard} - A_{660\text{nm}} \text{ Standard Blank}$$

Prepare a standard curve by plotting  $\Delta A_{660\text{nm}}$  Standard versus  $\mu\text{moles}$  of phosphate.

Sample Determination:

$$\Delta A_{660\text{nm}} \text{ Test} = A_{660\text{nm}} \text{ Test} - A_{660\text{nm}} \text{ Test Blank}$$

Determine the  $\mu\text{moles}$  of phosphate liberated using the Standard curve.

$$\text{Units/ml enzyme} = \frac{(\mu\text{moles of Phosphate released})(df)(20)}{(T)(0.20)(2.0)}$$

df = Dilution factor

T = 10, 20, 30, 40, 50, or 60 minutes

0.20 = Volume (in milliliters) of enzyme used

20 = Volume of (milliliters) of initial Reaction

Mixture volume

2.0 = Volume of Reaction Mixture taken for Phosphorus quantitation

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

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**UNIT DEFINITION:**

One unit will liberate 1.0  $\mu$ mole of inorganic phosphorus from  $1.5 \times 10^{-3}$  M phytate per minute at pH 5.15 at 55°C.

**FINAL ASSAY CONCENTRATION:**

In a 20.00 ml reaction mix, the final concentrations are 144 mM acetate, 2.0 mM magnesium sulfate, 1.5 mM phytate and 4 mg phytase.

**REFERENCE:**

Peers, F.G. (1953) *Biochemical Journal* **53**, 102-110

Taussky, H.H. and Skorr, E. (1953) *Journal of Biological Chemistry* **202**, 675-685

**NOTE:**

1. It is important that the phytic acid concentrations be corrected for purity and water content since the substrate concentrations are critical.
2. The color is not stable, so each test tube should be read at approximately the same time after the addition of Reagent G (TSCR).
3. This assay is based on the cited references.
4. All products 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.**