

**Enzymatic Assay of PHOSPHODIESTERASE I
(EC 3.1.4.1)**

PRINCIPLE:

bis(p-Nitrophenyl) Phosphate + H₂O $\xrightarrow{\text{Phosphodiesterase I}}$ PNP + PNPP

Abbreviations used:

PNP = p-Nitrophenol

PNPP = p-Nitrophenyl Phosphate

CONDITIONS: T = 25°C, pH = 8.9, A_{405nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 200 mM Tris HCl Buffer, pH 8.9 at 25°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 8.9 at 25°C with 1 M HCl.)
- B. 6.0 mM bis(p-Nitrophenyl) Phosphate Solution (bPNPP)
(Prepare 10 ml in Reagent A using bis (p-Nitrophenyl) Phosphate, Hemicalcium Salt, Sigma Prod. No. N-2877.)
- C. Phosphodiesterase I Enzyme Solution
(Immediately before use, prepare a solution containing 0.15 - 0.30 unit/ml of Phosphodiesterase I in cold deionized water.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent B (bPNPP)	3.00	3.00

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PROCEDURE: (continued)

Equilibrate to 25°C. Monitor the A_{405nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

	<u>Test</u>	<u>Blank</u>
Reagent C (Enzyme Solution)	0.06	-----
Deionized Water	-----	0.06

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

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{405\text{nm}}/\text{min Test} - \Delta A_{405\text{nm}}/\text{min Blank})(3.06)(\text{df})}{(18.5)(0.06)}$$

3.06 = Volume (in milliliters) of assay

df = Dilution factor

18.5 = Millimolar extinction coefficient of p-Nitrophenol at 405 nm

0.06 = 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 μmole of bis(p-nitrophenyl) phosphate per minute at pH 8.9 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 3.06 ml reaction mix the final concentrations are 196 mM Tris, 5.9 mM bis (p-nitrophenyl) phosphate, and 0.009 - 0.02 unit phosphodiesterase I.

REFERENCE:

Landt, M. and Butler, L.G. (1978) *Biochemistry* **17**, 4130-4135.

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NOTE:

1. This assay is based on the cited reference.
2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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