

Enzymatic Assay of PHOSPHODIESTERASE I¹
(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 = 37°C, pH = 8.8, A_{410nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Tris HCl Buffer, pH 8.8 at 37°C.
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 8.8 at 37°C with 1 M HCl.)
- B. 10 mM Ammonium Acetate Solution (NH₄OAc)
(Prepare 20 ml in deionized water using Ammonium Acetate, Sigma Prod. No. A-7262.)
- C. 1.0 mM bis(p-Nitrophenyl) Phosphate Solution (bPNPP)
(Prepare 10 ml in Reagent B using bis(p-Nitrophenyl) Phosphate, Hemicalcium Salt, Sigma Prod. No. N-2877. Stir gently at a temperature of no greater than 50°C with sonication every 5 minutes.)
- D. 300 mM Magnesium Acetate Solution (Mg(OAc)₂)
(Prepare 10 ml in deionized water using Magnesium Acetate, Tetrahydrate, Sigma Prod. No. M-0631.)
- E. Phosphodiesterase I Enzyme Solution
(Immediately before use, prepare a solution containing 0.1 - 0.2 unit/ml of Phosphodiesterase I in cold deionized water.)

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PROCEDURE:

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

| | <u>Test</u> | <u>Blank</u> |
|-----------------------------------|-------------|--------------|
| Deionized Water | 0.60 | 0.70 |
| Reagent A (Buffer) | 1.00 | 1.00 |
| Reagent C (bPNPP) | 1.00 | 1.00 |
| Reagent D (Mg(OAc) ₂) | 0.30 | 0.30 |

Mix by inversion and equilibrate to 37°C. Monitor the A_{410nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

| | <u>Test</u> | <u>Blank</u> |
|-----------------------------|-------------|--------------|
| Reagent E (Enzyme Solution) | 0.10 | ----- |

Immediately mix by inversion and record the increase at A_{410nm} for approximately 10 minutes. Obtain the ΔA_{410nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{410\text{nm}}/\text{min Test} - \Delta A_{410\text{nm}}/\text{min Blank})(3)(\text{df})}{(18.3)(0.1)}$$

3 = Volume (in milliliters) of assay

df = Dilution factor

18.3 = Millimolar extinction coefficient of p-Nitrophenol at 410 nm

0.1 = 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 8.8 at 37°C.

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FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix the final concentrations are 33 mM Tris, 3.3 mM ammonium acetate, 0.33 mM bis (p-nitrophenyl) phosphate, 30 mM magnesium acetate and 0.01 - 0.02 unit phosphodiesterase I.

REFERENCE:

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

NOTE:

1. This assay is not to be used to assay Phosphodiesterase I from *Crotalum durissus terrificus*, Sigma Prod. Nos. P-5785 and P-7027.
2. This assay is based on the cited reference.
3. 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.