p-Nitrophenyl Phosphate (PNPP)

1-800-632-7799
info@neb.com
www.neb.com

P0757S 008120814081

P0757S

1 ml **Lot: 0081208** **Exp: 8/14**

500 mM **Store at –20°C**

Description: *p*-Nitrophenyl Phosphate (PNPP) is a non-proteinaceous, non-specific substrate used to assay protein, alkaline and acid phosphatases. The PNPP phosphatase activity is measured using a continuous or single-point spectrophotometric assay based on the ability of phosphatases to catalyze the hydrolysis of PNPP to *p*-nitrophenol, a chromogenic product with absorbance at 405 nm (1). The assay can be used for the quick analysis of the protein phosphatase activity under any non-standard conditions.

The advantage of the PNPP phosphatase activity assay is that unlike radioactive assays the substrate concentration can be much higher than the K_m. The initial velocity can be recorded in the continuous assay, but the assay volume is larger than in a radioactive assay (about 1 ml to fill a 1 ml spetrophotometer cuvette) (1,2). The reaction volume in a single-point assay can be very small because the reaction is stopped with the amount of NaOH enough to fill the cuvette (1,3,4).

Supplied in: Sterile purified water.

Molecular Weight: 461.4 daltons [di(tris)salt].

Purity: >99% pure.

Suggested Working Concentration:
50–100 mM

Store protected from light

Notes on Use in Protein Phosphatase Assay: The PNPP phosphatase activity assay is very simple, non-expensive, and routinely used for the unit determination of all NEB protein phosphatases. PNPP has apparent K_m values for protein phosphatases in the range of 0.5 –10 mM (2-5).

The PNPP phosphatase activity is assayed in a reaction mixture (50 µl) containing 50 mM PNPP and a protein phosphatase buffer supplemented with additional components when required. The reaction is initiated by addition of enzyme and quenched after 5-10 minutes by addition of 1 ml of 1 N NaOH (or 1 ml of 0.5 M EDTA for Mn^{2+}-dependent protein phosphatases, λ-PPase and PP1). The amount of product, *p*-nitrophenol, is determined by reading the absorbance at 405 nm and using a molar extinction coefficient of 18,000 M^{-1} cm^{-1} (16,000 $M^{-1}cm^{-1}$ for 0.5 M EDTA) (1, 3).

One unit of the protein phosphatase activity is defined as the amount of enzyme that hydrolyzes 1 nanomole of PNPP in one minute at 30°C in a total reaction volume of 50 µl under standard reaction condition.

To estimate the protein phosphatase activity accurately it is essential to ensure linear kinetics of dephosphorylation.

References:

1. MacKintosh, C. (1993). In D.G. Hardie (Ed.), *Protein Phosphorylation: A Practical Approach* (p. 221). New York: IRL Press.
2. Zhuo, S. et al. (1993) *J. Biol. Chem.* 268, 17754–17761.
3. Zhang, Z.-Y. et al. (1992) *J. Biol. Chem.* 267, 23759–223766.
4. Pot, D.A. et al. (1991) *J. Biol. Chem.* 266, 19688–19696.
5. Takai, A. and Mieskes, G. (1991) *Biochem. J.* 275, 233–239.

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