

## *p*-Nitrophenyl Phosphate (PNPP)



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P0757S 009130615061

# P0757S

**1 ml**      **Lot: 0091306**      **Exp: 6/15**

**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.

Store protected from light

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 spectrophotometer 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

**Notes on Use in Protein Phosphatase Assay:** The PNPP phosphatase activity assay is very simple,

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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  $\mu$ l) 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,  $\lambda$ -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  $\mu$ l 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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