

p-Nitrophenyl Phosphate (PNPP)



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



P0757S 011160918091

P0757S

1 ml **Lot: 0111609** **Exp: 9/18**
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,

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 μ 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, 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 μ 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.



NEW ENGLAND BIOLABS® is a registered trademark of New England Biolabs, Inc.

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

CERTIFICATE OF ANALYSIS

p-Nitrophenyl Phosphate (PNPP)



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



P0757S 011160918091

P0757S

1 ml **Lot: 0111609** **Exp: 9/18**
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,

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 μ 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, 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 μ 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.



NEW ENGLAND BIOLABS® is a registered trademark of New England Biolabs, Inc.

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

CERTIFICATE OF ANALYSIS