Pyrophosphatase, Inorganic (yeast)

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

M2403S 003140916091

M2403S

10 units **Lot: 0031409** **Exp: 9/16**

100 U/ml **Store at –20°C**

Description: Inorganic pyrophosphatase (PPase) catalyzes the hydrolysis of inorganic pyrophosphate to form orthophosphate.

$$P_2O_7^{-4} + H_2O \rightarrow 2HPO_4^{-2}$$

A variety of metabolic reactions generate inorganic pyrophosphate as a reaction byproduct. Such reactions are rendered irreversible when the pyrophosphate is degraded by pyrophosphatase (1). RNA and DNA synthesis are examples of reactions that can be pulled far in the synthesis direction by the action of inorganic pyrophosphatase.

Source: An *E. coli* strain containing a genetic fusion of the *Saccharomyces cerevisiae ppa* gene and the gene coding for *Mycobacterium xenopi* GyrA intein. Developed by Biohelix Corporation, a New England Biolabs-affiliated company.

Applications:

- Enhancing yields of RNA in transcription reactions (2)

Molecular Weight: 71 kDa (homodimeric)

Supplied in: 20 mM Tris-HCl (pH 8.0), 100 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol and 50% glycerol.

Unit Definition: One unit is the amount of enzyme that will generate 1 µmol of phosphate per minute from inorganic pyrophosphate under standard reaction conditions (a 10 minute reaction at 25°C in 100 mM Tris-HCl, [pH 7.2], 2 mM $MgCl_2$ and 2 mM PPi in a reaction volume of 0.5 ml).

Quality Assurance: Free of endonuclease, exonuclease and RNase activities.

Quality Control Assays

Exonuclease Activity: Incubation of a 50 µl reaction containing 6 units of Pyrophosphatase, with 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA (105 cpm/µg) for 4 hours at 37°C released < 0.1% of the total radioactivity.

Endonuclease Activity: Incubation of a 50 µl reaction containing 6 units of Pyrophosphatase, with 1 µg of ϕX174 DNA for 4 hours at 25°C resulted in < 1% conversion to RF II as determined by agarose gel electrophoresis.

DNase Activity: Incubation of a 50 µl reaction containing 6 units of Pyrophosphatase, with 1 µg of λ DNA (HindIII digest) for 16 hours at 37°C resulted the same pattern of DNA bands as a reaction without enzyme as determined by agarose gel electrophoresis.

Alkaline Phosphatase Activity: This colorimetric assay tests for the presence of alkaline phosphatase which removes 5´ phosphates from DNA, RNA, rNTPs and dNTPs. Phosphatase contamination is revealed if p-nitrophenylphosphate is hydrolyzed to p-nitrophenol (yellow color). The PPase is incubated in a reaction buffer (1 ml) of 1 M diethanolamine-HCl (pH 9.8), 0.5 mM $MgCl_2$ and 10 mM p-nitrophenylphosphate at 37°C. Conversion of p-nitrophenylphosphate to p-nitrophenol is measured spectrophotometrically at A_{405} after 60 minutes. One unit of alkaline phosphatase is defined as the amount of enzyme that hydrolyzes 1 µmole of p-nitrophenylphosphate to p-nitrophenol in 1 minute. When 9 units of PPase are incubated under the above conditions < 0.0001 unit of alkaline phosphatase activity is revealed.

dNTPase Activity: dNTPase contamination is measured as the removal of β or γ phosphates from dATP, dCTP, dGTP, or dTTP using the AAM assay (1) for inorganic phosphate. The PPase is incubated in a volume of 0.5 ml @ 25°C for 1 hour in CircumVent™ Sequencing Buffer with a mixture of dNTPs, each at 200 µM. Incubation under these conditions with 9 units of PPase liberated < 0.05 µmole of inoganic phosphate from dNTPs.

(see other side)

CERTIFICATE OF ANALYSIS

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(see other side)

CERTIFICATE OF ANALYSIS

RNase Assay: Incubation of a 50 µl reaction containing 6 units of Pyrophosphatase, Inorganic (yeast) with 1 µg of MS2RNA for 1 hour at 25°C resulted in no detectable degradation of the RNA as determined by agarose gel electrophoresis.

Heat Inactivation: No

References:

1. Kornberg, A. (1980) *DNA Replication* (pp. 54–55, 94) W.H. Freeman and Company.
2. Cunningham, P.R. and Ofengand, J. (1990) *Biotechniques*, 9, 713–714.
3. Cooperman, B.S. (1982) *Methods Enzymol*. 87 (pt. C), 526–548.

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