Thermostable Inorganic Pyrophosphatase

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

M0296S 009140916091

M0296S

250 units **Lot: 0091409** **Exp: 9/16**

2,000 U/ml **Store at –20°C**

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

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

Source: An *E. coli* strain carrying a plasmid encoding pyrophosphatase from the extreme thermophile *Thermococcus litoralis*.

Supplied in: 100 mM KCl, 20 mM Tris-HCl (pH 8.0), 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 75°C in 50 mM Tricine [pH 8.5], 1 mM $MgCl_2$, 0.32 mM PPi, reaction volume of 0.5 ml).

Quality Control Assays

Exonuclease Activity: The PPase is incubated with 1 µg of a mixture of single and double-stranded, ^{3}H-labeled *E. coli* DNA (200,000 cpm/µg) in a reaction volume of 0.05 ml. NEBuffer 1, a low salt buffer, is used for the test. Incubations are at 75°C for 4 hours (under oil). Exonuclease contamination is indicated by the percent of the labeled DNA in the reaction that has been rendered TCA-soluble. When 100 units of enzyme were incubated under these conditions, < 0.1% radioactivity was released.

Nicking Activity: To assay for non-specific endonuclease contamination, PPase is incubated with a supercoiled plasmid substrate (ϕX174 DNA). A single non-specific nick in the RF I DNA converts it to the RF II form (nicked circle). Aliquots are incubated with 1 µg of RF I (supercoiled form) DNA in a reaction volume of 0.05 ml. NEBuffer 2 (10 mM Tris-HCl, 10 mM $MgCl_2$, 50 mM NaCl, 1 mM dithiothreitol, (pH 7.9 @ 25°C) is used because high salt inhibits contaminating activity. Incubations are peformed for 4 hours at 75°C (under oil). The two forms are easily distinguished on a 1% agarose gel. Under these conditions, 100 units of the PPase results in < 20% conversion to RF II.

Endonuclease Activity: The PPase is incubated overnight at 75°C in NEBuffer 2 with 1 µg of Hind III fragments of λ DNA in a volume of 0.05 ml (under oil). A sharp, unaltered pattern of DNA bands under these conditions is an indication that the enzyme preparation is free of detectable levels of non-specific DNases. Under these reaction conditions, 100 units of enzyme incubated for 16 hours resulted in the same pattern of DNA bands as a reaction without enzyme.

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 thermostable 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 75°C. Conversion of p-nitrophenylphosphate to p-nitrophenol is measured spectrophotometrically at A_{405} after 30 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 100 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 @ 75°C for 1 hour in CircumVent™ Sequencing Buffer with a mixture of dNTPs, each at 200 µM. The reaction products

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are analyzed by the AAM assay and compared to a standard curve of known phosphate concentrations generated in the same assay buffer. Incubation under these conditions with 500 units of PPase liberated < 0.01 µmole of inorganic phosphate from dNTPs.

Heat Stability: PPase is extremely thermostable, retaining > 100% activity after incubation at 100°C for 4 hours.

Heat Inactivation: No

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1. Heinonen, J.K. and Lahti, R.J. (1981), *Analytical Biochemistry* 113, 313–317

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