

## Thermostable Inorganic Pyrophosphatase



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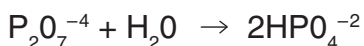
M0296S 009140916091

# M0296S



**250 units** Lot: 0091409 Exp: 9/16  
**2,000 U/ml** Store at  $-20^{\circ}\text{C}$

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



**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  $\mu\text{mol}$  of phosphate per minute from inorganic pyrophosphate under standard reaction conditions (a 10 minute reaction at  $75^{\circ}\text{C}$  in 50 mM Tricine [pH 8.5], 1 mM  $\text{MgCl}_2$ , 0.32 mM PPI, reaction volume of 0.5 ml).

### Quality Control Assays

**Exonuclease Activity:** The PPase is incubated with 1  $\mu\text{g}$  of a mixture of single and double-stranded,  $^3\text{H}$ -labeled *E. coli* DNA (200,000 cpm/ $\mu\text{g}$ ) in a reaction volume of 0.05 ml. NEBuffer 1, a low salt buffer, is used for the test. Incubations are at  $75^{\circ}\text{C}$  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 ( $\phi\text{X174}$  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  $\mu\text{g}$  of RF I (supercoiled form) DNA in a reaction volume of 0.05 ml. NEBuffer 2 (10 mM Tris-HCl, 10 mM  $\text{MgCl}_2$ , 50 mM NaCl, 1 mM dithiothreitol, (pH 7.9 @  $25^{\circ}\text{C}$ ) is used because high salt inhibits contaminating activity. Incubations are performed for 4 hours at  $75^{\circ}\text{C}$  (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  $75^{\circ}\text{C}$  in NEBuffer 2 with 1  $\mu\text{g}$  of Hind III fragments of  $\lambda$  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  $\text{MgCl}_2$  and 10 mM p-nitrophenylphosphate at  $75^{\circ}\text{C}$ . 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  $\mu\text{mole}$  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  $\beta$  or  $\gamma$  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 @  $75^{\circ}\text{C}$  for 1 hour in CircumVent™ Sequencing Buffer with a mixture of dNTPs, each at 200  $\mu\text{M}$ . The reaction products

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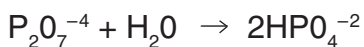
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are analyzed by the AAM assay and compared to a standard curve of known phosphate concentrations generated in the same assay buffer. Incubation under these conditions with 500 units of PPase liberated < 0.01  $\mu$ mole of inorganic phosphate from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

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

**Heat Inactivation:** No

**Reference:**

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

U.S. Patent No. 5,861,296

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