Thermophilic Inorganic Pyrophosphatase

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 phosphoric acid 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 MgCl₂, 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, 2°-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.

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

Nicking Activity: To assay for non-specific endonuclease contamination, PPase is incubated with a supercoiled plasmid substrate (pX174 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 MgCl₂, 50 mM NaCl, 1 mM dithiothreitol, pH 7.9 @ 25°C) is used because high salt inhibits contaminating activity. Incubations are performed for 4 hours at 75°C (under oil). The two forms are easily distinguished on an 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 MgCl₂, and 10 mM p-nitrophenylphosphate at 75°C. Conversion of p-nitrophenylphosphate to p-nitrophenol is measured spectrophotometrically at A₄₀₅ 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 5' or 3' 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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CERTIFICATE OF ANALYSIS
are analyzed by the AAM assay and compared
to a standard curve of known phosphate
conzentations 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

**Reference:**
*Analytical Biochemistry* 113, 313–317