

Product Information	
Thermostable Pyrophosphatase	
Part Number	Y9370L
Concentration	2,000 U/mL
Unit Size	1,250 U
Storage Temperature	-25°C to -15°C
Lot Number	
Reference Number	

Product Description: Thermostable pyrophosphatase is a recombinant enzyme from *Sulfolobus acidocaldarius* which catalyzes the Mg^{2+} -dependent reaction of $P_2O_7^{4-} + H_2O \rightarrow 2HPO_4^{2-}$. It has a low K_m (5.4 μM) for pyrophosphate, is active between pH 7 and 9, has an optimal temperature for activity at 75°C and is functional under PCR conditions (1-3).

Product Specifications						
P7620						
Assay	SDS Purity	Specific Activity	SS Exonuclease	DS Exonuclease	DS Endonuclease	<i>E. coli</i> DNA Contamination
Units Tested	n/a	n/a	50	50	50	50
Specification	>95%	3,500 U/mg	<1.0% Released	<1.0% Released	No Conversion	<10 copies

Source of Protein: Recombinant *E. coli* strain carrying the Thermostable Pyrophosphatase gene from *S. acidocaldarius*.

Unit Definition: 1 unit is the amount of enzyme that will liberate 1 μmol of phosphate per minute from inorganic pyrophosphate at 75°C and pH 8.5

Molecular weight: 19.4 kDa

Quality Control Analysis:

Unit Activity The assay is based on that described by Taussky and Shorr (4). Briefly, enzyme dilutions are added to 30 mM Tris HCl pH 8.5, 1.5 mM $MgCl_2$ and 1.5 mM sodium pyrophosphate. After a 10 minutes incubation at 75°C, the product formed, 2-orthophosphate, is reacted with ammonium molybdate to form phosphomolybdic acid. The phosphomolybdic acid is then reduced by ferrous sulfate under weak acidic conditions to form a blue color, the absorbance of which is measured at 660 nm. The amount of product formed is extrapolated from a phosphate standard curve generated from the ammonium molybdate/ferrous sulfate reaction.

Protein Concentration (OD₂₈₀) is determined by OD₂₈₀ absorbance.

Physical Purity is evaluated by SDS-PAGE of concentrated and diluted enzyme solutions followed by silver stain detection. Purity is assessed by comparing the aggregate mass of contaminant bands in the concentrated sample to the mass of the protein of interest band in the diluted sample.

Single-Stranded Exonuclease is determined in a 50 μL reaction containing a radiolabeled single-stranded DNA substrate and 10 μL of enzyme solution incubated for 4 hours at 37°C.

Double-Stranded Exonuclease is determined in a 50 μL reaction containing a radiolabeled double-stranded DNA substrate and 10 μL of enzyme solution incubated for 4 hours at 37°C.

Double-Stranded Endonuclease is determined in a 50 μL reaction containing 0.5 μg of plasmid DNA and 10 μL of enzyme solution incubated for 4 hours at 37°C.

***E. coli* 16S rDNA Contamination** is evaluated using 5 μL replicate samples of enzyme solution denatured and screened in a TaqMan qPCR assay for the presence of contaminating *E. coli* genomic DNA using oligonucleotide primers corresponding to the 16S rRNA locus.

Supplied in:

10 mM Tris-HCl, 50 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol (pH 7.5 at 25°C)

Usage Instructions:

The Thermostable Pyrophosphatase can be used to reduce inhibitory effects of accumulated PPI in polymerase chain reactions (PCRs) (5-7). As a starting point for PCR, add 0.01-0.1 U/μL of Thermostable Pyrophosphatase to identify the optimal concentration.

Notes:

Thermostable Pyrophosphatase catalysis is Mg²⁺-dependent, therefore, it is important to have Mg²⁺ in the reaction buffer. It is compatible with most PCR buffers that contains Mg²⁺.

References:

1. Leppänen, V.M. et al. (1999) Protein Sci., 8(6):1218-31.
2. Hansen, T. et al. (1999) Arch. Biochem. Biophys., 363(1):135-47.
3. Meyer, W. et al. (1995) Arch. Biochem. Biophys., 319(1):149-56.
4. Taussky, H.H. and Shorr, E. (1953) J. Biol. Chem., Jun;202(2):675-85.
5. Moldes, C. et al. (2004) Appl. Environ. Microbiol., 70(8): 4642-4647.
6. Dignam, J.D. and Deutscher, M.P. (1979) Biochemistry, 18(14):3165-70.
7. Gibson, N.J. et al. (1997) Anal. Biochem., 254(1):18-22.

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Use of this enzyme in certain applications may be covered by patents and may require a license. Purchase of this product does not include a license to perform any patented application; therefore, it is the sole responsibility of the users of the product to determine whether they may be required to engage in a license agreement depending upon the particular application in which the product is used.

Limitations of Use

This product was developed, manufactured, and sold for *in vitro* use only. The product is not suitable for administration to humans or animals. SDS sheets relevant to this product are available upon request.