Product Information	
***E. coli* Pyrophosphatase**	
Part Number	Y9380L
Concentration	100 U/mL
Unit Size	50 U
Storage Temperature	-25°C to -15°C
Lot Number	
Reference Number	

Product Description: *E. coli* pyrophosphatase catalyzes the Mg^{2+}-dependent reaction of $P_2O_7^{-4} + H_2O \rightarrow 2HPO_4^{-2}$ (1,2).

Product Specifications						
Y9380						
Assay	**SDS Purity**	**Specific Activity**	**SS Exonuclease**	**DS Exonuclease**	**DS Endonuclease**	***E. coli* DNA Contamination**
Units Tested	**n/a**	**n/a**	**35**	**35**	**35**	**35**
Specification	>95%	3,500 U/mg	<1.0% Released	<1.0% Released	No Conversion	<10 copies

Source of Protein: A recombinant *E. coli* strain carrying the *E. coli* Pyrophosphatase gene.

Unit Definition: 1 unit is the amount of enzyme that will liberate 1 µmol of phosphate per minute from inorganic pyrophosphate at 37°C and pH 8.5.

Molecular weight: 19.7 kDa

Quality Control Analysis:

Unit Activity The assay is based on that described by Taussky and Shorr (3). 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 37°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 (OD_{280}) is determined by OD_{280} 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:
20 mM Tris-HCl, 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol (pH 8.0 at 25°C)

Usage Instructions:
The *E. coli* Pyrophosphatase is widely used for *in vitro* transcription (IVT) or RNA synthesis reactions to reduce inhibitory effects of PPi. As a starting point for *in vitro* RNA synthesis reaction, add 0.1-1 units per mL of *E. coli* Pyrophosphatase to identify the optimal concentration.

Notes:
E. coli Pyrophosphatase catalysis is Mg^{2+}-dependent, therefore, it is important to have Mg^{2+} in the reaction buffer.

References:
1. Lahti, R. et al. (1988) J. Bacteriol., 170(12), 5901-7.
2. Baykov, A.A. et al. (1996) Biochemistry, 35(15), 4655-61.
3. Taussky, H.H. and Shorr, E. (1953) J. Biol. Chem., 202(2), 675-85.

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