

Product Information	
T4 Polynucleotide Kinase	
Part Number	Y9040L
Concentration	10,000 U/mL
Unit Size	10,000 U
Storage Temperature	-25°C to -15°C
Lot Number	
Reference Number	

Product Description: T4 Polynucleotide Kinase (PNK) catalyzes the transfer and exchange of the terminal gamma position phosphate of ATP to the 5'-hydroxyl termini of double- and single-stranded DNA, RNA, and nucleoside 3'-monophosphate molecules (1). T4 PNK also exhibits 3'-phosphatase and 2', 3' cyclic phosphodiesterase activities (2-6).

Product Specifications						
Y9040						
Assay	SDS Purity	Specific Activity	SS Exonuclease	DS Exonuclease	DS Endonuclease	<i>E. coli</i> DNA Contamination
Units Tested	n/a	n/a	2000	2000	2000	2000
Specification	>99%	133,333 U/mg	<5.0% Released	<1.0% Released	No Conversion	<10 copies

Source of Protein: Purified from a strain of *E. coli* that expresses the recombinant T4 Polynucleotide Kinase gene.

Unit Definition: 1 unit is defined as the amount of enzyme catalyzing the incorporation of 1 nmol of ³²P-ATP in 30 minutes at 37°C in 1X T4 Polynucleotide Kinase Reaction Buffer.

Molecular weight: 34.6 kDa

Quality Control Analysis:

Unit Activity is measured using a 2-fold serial dilution method. Dilutions of enzyme were made in 1X reaction buffer and added to 50 µL reactions containing 10 µM Oligo dT single-stranded DNA, 1X PNK Reaction Buffer, and 66 µM ATP and [γ -³²P] ATP. Reactions were incubated 30 minutes at 37°C, plunged on ice, and analyzed using the method of Sambrook and Russell (7).

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.

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. MSDS sheets relevant to this product are available upon request.

***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 KCl, 0.1 µM ATP, 1 mM DTT, 0.1 mM EDTA, 50% glycerol (pH 7.4 at 25°C)

Supplied with:

10X T4 Polynucleotide Kinase Buffer (B9040): 700 mM Tris-HCl, 100 mM MgCl₂, 50 mM DTT (pH 7.6 at 25°C)

Usage Instructions: Phosphorylation of 5'-terminus

1. Set up the following reaction mixture in a total volume of 50 µL on ice:

Components	Final Concentration	Volume
Nuclease free water	N/A	X µL
10X T4 Polynucleotide Kinase Buffer (B9040)	1X	5 µL
10 mM ATP	1 mM	5 µL
DNA 5'-termini	up to 50 pmol	X µL
T4 Polynucleotide Kinase (Y9040) (10 U/µL)	10 units	1 µL
	Total Volume =	50 µL

2. Incubate for 30 minutes at 37°C.

3. Inactivate the enzyme by heating for 20 minutes at 65°C.

References:

- Richardson, C.C. (1981) P.D. Boyer (Eds.), The Enzymes, 14, pp. 229-314. San Diego: Academic press.
- Morse, D. P. et al. (1997) Biochemistry, 36, 8429-8434.
- Cameron, V. et al. (1977) Biochemistry, 16, 5120-5126.
- Wand, L. K. et al. (2002) Nucl. Acids Res., 30, 1073-1080.
- Galburt, E., et al. (2002) Structure., 10, 1249-1260.
- Wang, L. K., et al. (2002) EMBO J., 21, 3873-3880
- Sambrook, J. et al. (1989) Cold Spring Harbor Laboratory Press, Molecular Cloning: A Laboratory Manual., (2nd ed.), 5.40-5.43.

Disclaimer:

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.

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