Product Information	
T4 Polynucleotide Kinase	
Part Number	Y9040L
Concentration	10,000 U/mL
Unit Size	10,000 U
Storage Temperature	-25ºC to -15ºC
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 terminus of double-and single-stranded DNA, RNA and nucleoside 3'-monophosphate molecules (1). T4 PNK also exhibits 3'-phosphatase and 2', 3' cyclicphosphodiesterase activities (2-6).

Product Specifications						
Y9040						
Assay	**SDS Purity**	**Specific Activity**	**SS Exonuclease**	**DS Exonuclease**	**DS Endonuclease**	***E. coli* 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 donor) in 30 minutes at 37°C in 1X T4 Polynucleotide Kinase Reaction Buffer.

Molecular weight: 34,620 Daltons

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-^{32}P] ATP Reactions were incubated 30 minutes at 37°C, plunged on ice, and analyzed using the method of Sambrook and Russell (Molecular Cloning, v3, 2001, pp. A8.25-A8.26).

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.

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.

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 **r**eplicate 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: 10mM Tris-HCL, 50 mM KCl, 0.1 µM ATP, 1mM DTT, 0.1mM EDTA, 50% glycerol (pH 7.4 at 25ºC).
Supplied with:
10X T4 Polynucleotide Kinase Buffer (B9040): 700mM Tris-HCl, 100mM $MgCl_2$, 50mM DTT (pH 7.6 at 25ºC).

For phosphorylation of 5' termini:

Usage Instructions:

1. Set up the following reaction mixture in a total volume of 50 µL on ice:
   - Up to 50pmol of 5' termini
   - 5µl Buffer 10x T4 Polynucleotide Kinase Buffer
   - Add 5 µL dATP (10 mM) to a final concentration 1 mM dATP
   - Add 1 µL of T4 PNK (10 units)
   - Add Nuclease-free water up to 50 µL
2. Incubate for 30 minutes at 37°C.
3. Inactivate the enzyme by heating for 20 minutes at 65°C.

References:

1. Richardson, C.C. (1981) P.D. Boyer (Eds.), The Enzymes, 14, pp. 229-314. San Diego: Academic press.
2. Morse, D. P. et al. (1997) Biochemistry 36, 8429-8434.
3. Cameron, V. et al. (1977) Biochemistry 16, 5120-5126.
4. Wand, L. K. et al. (2002) Nucl. Acids Res. 30, 1073-1080.
5. Galburt, E., et al. (2002) Structure 10, 1249-1260.
6. Wang, L. K., et al. (2002) EMBO J. 21, 3873-3880

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