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## *Product Information Sheet* *Y9040L Rev F*

| Product Information | |
|---|---|
| **T4 Polynucleotide Kinase** | |
| **Part Number** | Y9040L |
| **Concentration** | 10,000 U/mL |
| **Unit Size** | 10,000 U |

| Product Specification | |
|---|---|
| **Storage Temperature** | -25°C to -15°C |
| **TEST:** | **SPECIFICATION:** |
| **Purity (SDS-PAGE)** | >99% |
| **Specific Activity** | 133,333 U/mg |
| **SS Exonuclease** | 2000 U <5.0% released |
| **DS Exonuclease** | 2000 U <1.0% released |
| **DS Endonuclease** | 2000 U = No conversion |
| ***E.coli* DNA Contamination** | 2000 U <10 copies |

## 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, 3, 4, 5, 6).

### Source of Protein

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

### Supplied in

10 mM Tris-HCl
50 mM KCl
0.1 µM ATP
1.0 mM DTT
0.1 mM EDTA
50% glycerol
pH 7.4 @ 25°C

### Supplied with

B9040 (10X T4 Polynucleotide Kinase Buffer)

**10X T4 Polynucleotide Kinase Buffer (B9040):**
700 mM Tris-HCl
100 mM $MgCl_2$
50 mM DTT
pH 7.6 @ 25°C

### Unit Definition

One unit is defined as the amount of enzyme catalyzing the incorporation of 1 nmol of [$^{32}$P] (ATP donor) in 30 minutes at 37°C in 1X T4 Polynucleotide Kinase Reaction Buffer.

## Quality Control Analysis:

### Unit Characterization Assay

Unit activity was 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 ($OD_{280}$) Measurement

A 2.0 µL sample of enzyme was analyzed at $OD_{280}$ using a Nanodrop ND-1000 spectrophotometer standardized using a 2.0 mg/ml BSA sample (Pierce Cat #23209) and blanked with product storage solution. The observed average measurement of 3 replicate samples was converted to mg/mL using an extinction coefficient of 63,440 and molecular weight of 34,620 Daltons..

### SDS-Page (Physical Purity Assessment)

2.0 µL of enzyme solution was loaded on a denaturing 4-20% Tris-Glycine SDS-PAGE gel flanked by a broad-range MW marker and 2.0 µL of a 1:100 dilution of the sample. Following electrophoresis, the gel was stained and the samples compared to determine physical purity. The acceptance criteria for this test requires that the aggregate mass of contaminant bands in the concentrated sample do not exceed the mass of the protein of interest band in the dilute sample, confirming greater than 99% purity of the concentrated sample.

## Contamination Tests:

### Single-Stranded Endonuclease Activity

A 50 µL reaction containing 10,000 cpm of radiolabeled single-stranded DNA and 10 µL of enzyme solution incubated for 4 hours at 37°C resulted in 5.0% release of TCA-soluble counts.

*(additional information on reverse side)*

**Double-Stranded Endonuclease Activity**
A 50 µL reaction containing 5,000 cpm of a radiolabeled double-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C resulted in less than 1.0% release of TCA-soluble counts.

**Double-Stranded Endonuclease Activity**
A 50 µL reaction containing 0.5 µg of pBR322 DNA and 10 µL of enzyme solution incubated for 4 hours at 37°C resulted in no visually discernible conversion to nicked circular DNA as determined by agarose gel electrophoresis.

***E.coli* 16S rDNA Contamination Test**
Replicate 5uL samples of enzyme solution were 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. The acceptance criterion for the test is the threshold cycle count ($C_t$) produced by the average of 3 replicate no template control samples. Based on the correlation between the no template control $C_t$ values, and standard curve data, the detection limit of this assay is <10 copies genome/sample.

## 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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### 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.