



Product Information	
<b>T4 Polynucleotide Kinase</b>	
Part Number:	Y904L
Lot Number:	

*Certificate of Analysis*

2010-Y904-Rev.E

### **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  $\mu$ M ATP  
1.0 mM dithiothreitol  
0.1 mM EDTA  
50% glycerol  
pH 7.4 @ 25°C

### **Supplied With**

B904 10X T4 Polynucleotide Kinase Buffer

### **10X Polynucleotide Kinase Buffer (B904)**

700 mM Tris-HCl  
100 mM MgCl<sub>2</sub>  
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 [<sup>32</sup>P] (ATP donor) in 30 minutes at 37°C in 1X T4 Polynucleotide Kinase Reaction Buffer.

### Product Specification\*

Unit Size:	10,000 Units
Unit Concentration	10,000 U/mL
Protein Concentration	0.075 mg/ml
Purity (SDS-PAGE)	>99%
Specific Activity (est.)	133,333 U/mg
SS Exonuclease	2000 U <5.0% released
DS Exonuclease	2000 U <0.5% released
Endonuclease	2000 U <10% converted
<i>E.coli</i> 16S rDNA Contamination	2000 U <10 copies
Storage	-20°C

\* For a detailed summary of assay conditions and data, refer to the Quality Controls Analysis section below.

### **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 ([PNK]<sub>f</sub> = 0.075-0.0006 $\mu$ g/ $\mu$ L) and added to 50  $\mu$ L reactions containing 10  $\mu$ M Oligo dT single-stranded DNA, 1X PNK Reaction Buffer, and 66  $\mu$ M [ $\gamma$ -<sup>32</sup>P] ATP (6.6 $\mu$ Ci/mL). Reactions were incubated 10 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<sub>280</sub>) Measurement**

A 2.0  $\mu$ L sample of enzyme was analyzed at OD<sub>280</sub> 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 34,619 Daltons. Acceptance for this assay is +/- 5% of reference sample.

#### **SDS-Page (Physical Purity Assessment)**

2.0  $\mu$ L 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  $\mu$ L 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.

## **Nuclease Contamination Tests:**

### **Single-Stranded Endonuclease Activity**

A 50  $\mu$ L reaction containing 11,000 cpm of radiolabeled single-stranded DNA and 10  $\mu$ L of enzyme solution incubated for 4 hours at 37°C resulted in 5.0% release of TCA-soluble counts.

### **Double-Stranded Endonuclease Activity**

A 50  $\mu$ L reaction containing 5,000 cpm of a radiolabeled double-stranded DNA substrate and 10  $\mu$ L of enzyme solution incubated for 4 hours at 37°C resulted in less than 0.5% release of TCA-soluble counts.

### **Endonuclease Activity**

A 50  $\mu$ L reaction containing 0.5  $\mu$ g of pBR322 DNA and 10  $\mu$ L 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 5  $\mu$ L 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.

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.



#### **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.