

# T4 Polynucleotide Kinase

Code No. PNK-111

Lot No. \*\*\*\*\*

Storage Store at -20°C

Size 1,500units

Source : *Escherichia coli* JM109 which is carrying T4 pse T gene.

Reaction :  $(\gamma\text{-}^{32}\text{P})\text{-ATP} + 5'\text{-OH-polynucleotide} \rightarrow \text{ADP} + (5'\text{-}^{32}\text{P})\text{polynucleotide}$

Concentration : \*\*\* units/ $\mu\text{l}$

Unit Definition : One unit is the amount of enzyme activity that incorporates 1 nmole of  $(\gamma\text{-}^{32}\text{P})\text{-ATP}$  into micrococcal nuclease-treated calf thymus DNA in 30 minutes at 37°C.

Assay Condition : 50 mM Tris-HCl(pH7.6)  
100  $\mu\text{M}$   $(\gamma\text{-}^{32}\text{P})\text{-ATP}$   
10 mM  $\text{MgCl}_2$   
10 mM 2-mercaptoethanol  
0.2 mg/ml micrococcal nuclease-treated calf thymus DNA

Storage Buffer : 50 mM Tris-HCl(pH7.5)  
1 mM DTT  
0.1 mM EDTA  
50 mM KCl  
0.1  $\mu\text{M}$  ATP  
50 % Glycerol

10 $\times$  protruding end kinase buffer : 0.5 M Tris-HCl(pH8.0)  
0.1 M  $\text{MgCl}_2$   
50 mM DTT

10 $\times$  blunt end or recessed end kinase buffer : 0.5 M Tris-HCl(pH9.5)  
0.1 M  $\text{MgCl}_2$   
50 mM DTT

Denaturation Buffer : 20 mM Tris-HCl(pH9.5)  
1 mM Spermidine  
0.1 mM EDTA

## Contaminant Assay

1. Nonspecific Endonuclease : When 10 units of enzyme were incubated with 1 $\mu\text{g}$  of *Hind*III digest of  $\lambda$ -DNA for 20 hours at 37°C in 50  $\mu\text{l}$  reaction volume, no degradation of the DNA fragments is observed after agarose gel electrophoresis.

2. Nonspecific Exonuclease : 30 units of enzyme, when incubated with 1  $\mu\text{g}$  of *E. coli*  $^3\text{H}$ -DNA for

4 hours at 37°C in 50 µl reaction volume, will release less than 0.01 % acid soluble counts.

### 3. Nicking Activity

: After incubation of 1 µg of ΦX174DNA(RFI) with 25 units of this enzyme for 4 hours at 37°C in 50 µl reaction volume , the supercoiled structure is observed after agarose gel electrophoresis .