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# **Product Information**

# Polynucleotide Kinase from T4-infected Escherichia coli

Catalog Number **P4390** Storage Temperature –20 °C

CAS RN 37211-65-7 EC 2.7.1.78

## **Product Description**

Polynucleotide Kinase catalyzes a "forward reaction" transfer of the  $\gamma$ -phosphate of ATP to the 5'-hydroxyl terminus of single and double-stranded nucleic acids (DNA and RNA) and 3'-nucleoside monophosphates. In exchange reactions containing ADP, the enzyme will catalyze the exchange between 5'-terminal phosphate groups and ATP. 3'-phosphatase activity enables the enzyme to remove 3'-phosphoryl groups from phosphorylpolynucleotides. <sup>1-3</sup>

### **Forward Reaction**

Transfer of labeled  $\gamma$ -phosphate from [ $\gamma$ -<sup>32</sup>P]-ATP to the free 5'-hydroxyl group of the substrate.

5'-HO-DNA + 
$$[\gamma^{-32}P]$$
-ATP  $\rightarrow$  5'- $^{32}PO$ -DNA + ADP

Substrates that do not have a free 5'-hydroxyl group require prior dephosphorylation by alkaline phosphatase.

# Exchange reaction

First, the terminal 5'-phosphate group is transferred from the substrate to ADP present in the reaction mixture. Then, the labeled  $\gamma$ -phosphate from [ $\gamma$ - $^{32}$ P]-ATP is transferred to the free hydroxyl group of the substrate.

5'-PO-DNA + ADP → 5'-HO-DNA + ATP

5'-HO-DNA + 
$$[\gamma^{-32}P]$$
-ATP  $\rightarrow$  5'- $^{32}PO$ -DNA + ADP

Applications for Polynucleotide Kinase include:

- Sequencing or nucleic acid tagging (DNA and RNA) by 5'-end labeling
- 5' phosphorylation of oligonucleotides
- Removal of 3'-phosphate groups from phosphorylpolynucleotides

The product is supplied in a solution of 50% (v/v) glycerol, 20 mM Tris-HCl, pH 7.5, with 25 mM KCl, 2 mM DTT, 0.1 mM EDTA, and 0.1  $\mu$ M ATP.

Activity: ~10 units/μl

Unit Definition: One unit catalyzes the transfer of one nanomole of  $^{32}P$  to the 5′-end of micrococcal nuclease-treated DNA in 30 minutes at 37 °C. Transfer is detected as incorporation into acid-insoluble material. Activity is determined in a reaction mixture containing 40 mM Tris-HCl, pH 7.5, with 10 mM MgCl<sub>2</sub>, 5 mM dithiothreitol, 0.5 mM 5′-OH polynucleotide ends, and 0.1 mM [ $\gamma$ - $^{32}P$ ]-ATP.

No endonuclease or exonuclease activity detected.

### **Precautions and Disclaimer**

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

# Storage/Stability

The product ships on wet ice and storage at  $-20~^{\circ}$ C is recommended. Do not store in a frost-free freezer. The frequent freeze-thaw cycles may decrease enzyme stability.

Centrifuge to improve recovery of product from its storage container.

#### References

- Berkner, K.L., and Folk, W.R., J. Biol. Chem., 252, 3176 (1977).
- 2. Sirotkin, K., et al., J. Mol. Biol., **123**, 221-233 (1978).
- 3. Cameron, V., and Uhlenbeck, O.C., Biochemistry, **16**, 5120-5126 (1977).

NC,KH,JB,MAM, NRC 11/08-1