Bacteriophage T4 Polynucleotide Kinase

(Bacteriophage T4-infected *E. coli*)

Bacteriophage T4 polynucleotide kinase catalyzes the transfer of the γ-phosphate of ATP to a 5′ terminus of DNA or RNA (Richardson 1971). Two types of reactions are commonly used. In the *forward* reaction, the γ-phosphate is transferred to the 5′ terminus of dephosphorylated DNA (Richardson 1971). In the *exchange* reaction, an excess of ADP causes bacteriophage T4 polynucleotide kinase to transfer the terminal 5′ phosphate from phosphorylated DNA to ADP; the DNA is then rephosphorylated by transfer of a radiolabeled γ-phosphate from [γ-^{32}P]ATP (Berkner and Folk 1977). In addition to the phosphorylation activity of bacteriophage T4 polynucleotide kinase, the enzyme also contains a 3′ phosphatase activity (Richardson 1981). The properties of this enzyme are summarized in Table 5.9 and Richardson (1981).

USES

1. Radiolabeling 5′ termini in DNA for sequencing by the Maxam-Gilbert technique (Maxam and Gilbert 1977), for nuclease-S1 analysis, and for other uses requiring terminally labeled DNA.
2. Phosphorylating synthetic linkers and other fragments of DNA that lack terminal 5′ phosphates in preparation for ligation.

Notes

i. Bacteriophage T4 polynucleotide kinase is difficult to purify from infected cells, and impure preparations are not uncommon. Wherever possible, use bacteriophage T4 polynucleotide kinase that has been purified from cells expressing high levels of a cloned copy of the bacteriophage T4 gene.

ii. When setting up reactions involving the termini of nucleic acid molecules, the concentration of the reacting species can be calculated using the following table as a guide.

Size of double-stranded DNA (in base pairs)	Amount of DNA required to contribute 1 pmole of 5′ termini (in μg)
50	1.7×10^{-2}
100	3.3×10^{-2}
250	8.4×10^{-2}
500	1.7×10^{-1}
1000	3.3×10^{-1}
2500	8.4×10^{-1}
5000	1.7

iii. Spermidine stimulates incorporation of [γ-^{32}P]ATP and inhibits a nuclease present in some preparations of bacteriophage T4 polynucleotide kinase.

iv. ATP should be present at a concentration of at least 1 μM in the forward

reaction and at least 2 μM in the exchange reaction, but optimal activity occurs at higher concentrations (see Table 5.9).

v. The dephosphorylated DNA should be rigorously purified by gel electrophoresis, density gradient centrifugation, or chromatography on columns of Sepharose CL-4B in order to remove low-molecular-weight nucleic acids. Although such contaminants may make up only a small fraction of the weight of the nucleic acids in the preparation, they provide a much larger proportion of the 5′ termini. Unless steps are taken to remove them, contaminating low-molecular-weight DNAs and RNAs can be the predominant species of nucleic acids that are labeled in bacteriophage T4 polynucleotide kinase reactions.

vi. Ammonium ions are strong inhibitors of bacteriophage T4 polynucleotide kinase. Therefore, DNA should not be dissolved in, or precipitated from, buffers containing ammonium salts prior to treatment with the kinase.

vii. Low concentrations of phosphate also inhibit bacteriophage T4 polynucleotide kinase; imidazole buffer (pH 6.4) is therefore the buffer of choice for the exchange reaction, and Tris buffer is the buffer of choice for the forward reaction.