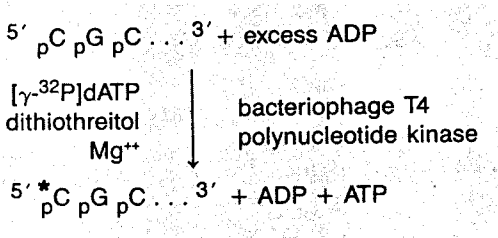
Bacteriophage T4 Polynucleotide Kinase

Activity: Kinase (exchange reaction)

Substrate: Single-stranded DNA with a 5′-phosphate terminus is most efficiently labeled (96%). Recessed 5′-phosphate termini are labeled to 70% with sufficient enzyme. 5′-phosphate groups at nicks are labeled 30-fold less efficiently than single-stranded 5′-phosphate termini.

Reaction:



Bacteriophage T4 Polynucleotide Kinase

Activity: Kinase (forward reaction)

Substrate: Single- or double-stranded DNA with 5′-hydroxyl terminus; RNA with a 5′-hydroxyl terminus. The enzyme phosphorylates protruding 5′ single-stranded termini more rapidly than blunt ends or recessed 5′ termini; however, with sufficient enzyme and ATP, such termini can be completely phosphorylated. The reaction at nicks or gaps in double-stranded DNA is less efficient than for single-stranded termini; however, with sufficient concentrations of ATP and enzyme, gaps can be completely phosphorylated and nicks can be phosphorylated to 70%.

Reaction:

$$DNA_{OH}{}^{5'} \text{ or } RNA_{OH}{}^{5'} \xrightarrow[\substack{[\gamma\text{-}^{32}P]ATP\\ \text{dithiothreitol}\\ Mg^{++}}]{\substack{\text{bacteriophage T4}\\ \text{polynucleotide kinase}}} 5'[^{32}P]DNA \text{ or } 5'[^{32}P]RNA + ADP$$

For example: