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T4 Polynucleotide Kinase

Cat # ME311
Pack Size: 250U
Storage: -20°C

Introduction

T4 Polynucleotide Kinase is a high-quality, purified enzyme that catalyzes the transfer of the gamma-phosphate from ATP to the 5'-hydroxyl terminus of single- or double-stranded DNA, RNA, or synthetic oligonucleotides. The enzyme is derived from the T4 bacteriophage and is commonly used for a variety of applications, including 5' end-labeling of DNA and RNA, phosphorylation of oligonucleotide primers for PCR, and preparation of radiolabeled probes for hybridization experiments.

Storage buffer

The enzyme is supplied in a storage buffer containing Tris-HCl, MgCl2, and DTT.

Unit definition

One unit of T4 Polynucleotide Kinase catalyzes the transfer of one nanomole of phosphate from ATP to the 5'-hydroxyl terminus of a single-stranded DNA or RNA molecule per minute at 37°C in a 50 µl reaction volume.

Application

- Labeling of DNA and RNA: T4 Polynucleotide Kinase is commonly used for the labeling of DNA and RNA molecules with radioactive isotopes or fluorescent dyes.
- Phosphorylation of DNA and RNA: T4 Polynucleotide Kinase can be used to phosphorylate the 5'-termini of DNA and RNA molecules for subsequent ligation or other downstream applications.
- Cloning: T4 Polynucleotide Kinase can be used to phosphorylate the ends of DNA fragments for subsequent cloning into vectors.
- Mutagenesis: T4 Polynucleotide Kinase can be used for the site-directed mutagenesis of DNA molecules.isotopes.

Protocol

1. Thaw T4 Polynucleotide Kinase, 10x Reaction Buffer, and ATP on ice.

   Set up the reaction as follows:
   Component Volume per reaction (µl)
   DNA/RNA molecule variable (see step 3)
   T4 Polynucleotide Kinase 1
   10x Reaction Buffer 5
   ATP (10 mM) 1
   Water variable (see step 3)

2. Determine the amount of DNA or RNA molecule to be used based on the specific application and labeling or phosphorylation efficiency. Typically, 50-500 ng of DNA or RNA is used per reaction. Adjust the volume of water accordingly to achieve a final reaction volume of 50 µl.
3. Mix the reaction components thoroughly by pipetting up and down several times.
4. Incubate the reaction at 37°C for 30-60 minutes. Incubation times may vary depending on the specific application and labeling or phosphorylation efficiency.
5. Inactivate the enzyme by heat treatment at 65°C for 10 minutes or by phenol-chloroform extraction and ethanol precipitation of the labeled or phosphorylated DNA or RNA.
6. Analyze the labeled or phosphorylated DNA or RNA by the appropriate method (e.g. gel electrophoresis, autoradiography, fluorescence detection).
7. Note: The optimal reaction conditions may vary depending on the specific application and the type of DNA or RNA molecule being labeled or phosphorylated. It may be necessary to optimize the reaction time, temperature, and enzyme and substrate concentrations for each specific application.