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T7 RNA Polymerase

Cat # ME305
Pack Size: 1000U
Storage: -20°C

Introduction

T7 RNA Polymerase is a recombinant enzyme derived from the T7 bacteriophage. It is a DNA-dependent RNA polymerase that synthesizes RNA in a 5'-3' direction. The enzyme can transcribe DNA templates that contain a T7 promoter sequence. It is supplied in a buffer solution containing $MgCl_2$.

Storage buffer

The enzyme is supplied in a storage buffer containing 50 mM Tris-HCl (pH 7.9), 1 mM EDTA, 50% glycerol.

Unit definition

One unit is defined as the amount of enzyme required to incorporate 1 nmol of ATP into acid-precipitable material in 1 hour at 37°C using a linearized plasmid containing the T7 promoter as template.

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

Materials Required

T7 RNA Polymerase
DNA template containing a T7 promoter sequence
Reaction buffer (supplied with enzyme)
NTPs (A, C, G, U)
Water

1. Thaw T7 RNA Polymerase, reaction buffer, and NTPs on ice.
2. Set up the reaction as follows:

Component	Volume per reaction (µl)
DNA template	variable (see step 3)
T7 RNA Polymerase	1
Reaction buffer (10x)	2
NTPs (10 mM each)	1
Water	variable (see step 3)

3. Determine the amount of DNA template to be used based on the specific application and the desired RNA yield. Typically, 0.1-1 µg of DNA template is used per reaction. Adjust the volume of water accordingly to achieve a final reaction volume of 20 µl.
4. Mix the reaction components thoroughly by pipetting up and down several times.
5. Incubate the reaction at 37°C for 1-3 hours, depending on the desired RNA yield.
6. Inactivate the enzyme by heat treatment at 65°C for 10 minutes or by adding EDTA to a final concentration of 10 mM.
7. Analyze the transcribed RNA by the appropriate method (e.g. gel electrophoresis, autoradiography, fluorescence detection).