T3 RNA Polymerase (RNase-free)

Product description

The phage T3 RNA Polymerase, with a molecular weight of about 99 kDa, is a DNA-dependent 5'-3' RNA polymerase, and highly specific to the T3 phage promoter (5'-AATTAACCCTCACTAAAGGGAGA-3'). This enzyme can be used to synthesize RNA strands complementary to the template DNA downstream of the T3 promoter by catalyzing the incorporation of NTP.

T3 RNA polymerase can recognize NTP substrates containing modified nucleotides such as biotin, fluorescein, digoxin labeling, commonly used in RNA hybridization probe preparation, genomic DNA sequence analysis, RNase protection assay, antisense RNA and RNAi synthesis, in vitro translation, RNA structure and function studies.

Composition and storage conditions

Components \ Size	2000 U	5000 U	10000 U
T3 RNA Polymerase (50 U/μL)	0.04 mL	0.1 mL	0.2 mL
10X T3 RNA Polymerase Reaction Buffer	0.2 mL	0.5 mL	1 mL
Store the components at -20 °C.			

Experimental operation

1. DNA template preparation

PCR products containing T3 polymerase promoter sequence, linearized plasmid DNA, cDNA, or oligonucleotides can all be used as templates for in vitro transcription. Fig.1 illustrates the in vitro transcription process of T3 RNA polymerase.

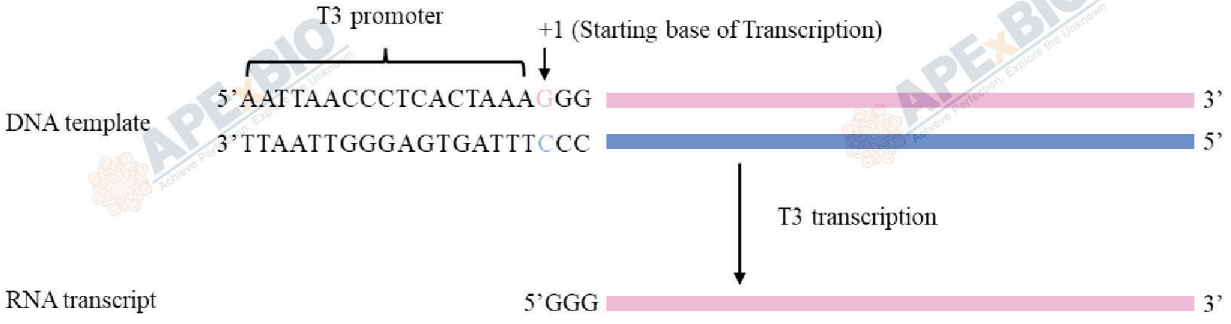

Fig.1 T3 RNA polymerase binds to the T3 promoter and transcribes RNA.

2. RNA synthesis

1) Configure the reaction system at room temperature according to the following table:

Total Reaction Volume	20 μL	
Template DNA	X μL	0.2~1 μg
10X T3 RNA Polymerase Reaction Buffer	2 μL	
NTP Mixture (10 mM each)	4 μL	final 2 mM each
Ribonuclease Inhibitor	X μL	20 U
T3 RNA Polymerase	1 μL	
Nuclease-free Water	X μL	

2) Mix the reaction system gently (pipette to mix by blowing or vortexing at lowest speed), then incubate at 37 °C for 1-2 h.

3) Add 2 μL of 0.5 M EDTA (pH 8.0) into the reaction system or cool at -20 °C to stop the reaction.

4) Transcription product can be detected by gel electrophoresis or other appropriate methods.

Notes

1. The entire reaction system must be carried out without RNase.
2. The reaction system needs to be configured at room temperature because DNA precipitates in the presence of spermidine at 4 °C.
3. The template DNA can be linear plasmid DNA containing the T3 promoter, PCR products, or genomic DNA containing the T3 promoter.
4. Incomplete linearization of the template DNA can result in the transcription of RNA longer than the expected, and a decrease in the proportion of transcripts of the expected length.
5. The reaction system can be scaled up or down proportionally according to the above table.

Product description

1. Enzyme activation unit (U) definition: The amount of enzyme required to catalyze the incorporation of 1 nmol ATP into polynucleotides at 37 °C for 1 h is defined as 1 U.
2. Stored solution composition: 100 mM NaCl, 50 mM Tris-HCl (pH 7.9), 1 mM EDTA, 20 mM β-mercaptoethanol, 0.1% Triton® X-100, 50% (w/v) Glycerol.
3. 10X T3 RNA Polymerase Reaction buffer: 400 mM Tris-HCl (pH 7.9 @ 25°C), 60 mM $MgCl_2$, 10 mM DTT, 20 mM spermidine。
4. Quality assurance:
   - Purity: SDS-PAGE detection purity > 95%.
   - Non-specific nuclease: 250 U of this enzyme was incubated with Lambda DNA for 16 h at 37 °C without changing the electrophoresis band.
   - Non-endonuclease: 150 U of this enzyme was incubated with 1 μg of supercoiled PhiX174 DNA for 4 h at 37 °C, the DNA de-supercoiled < 10% by agarose gel electrophoresis.
   - Non-exonuclease: 150 U of this enzyme was incubated with HindIII-DNA for 4 h at 37 °C without changing the electrophoresis band.

- Non-RNase: 50 U of this enzyme and 200 ng of 300 base RNA substrate were reacted for 4 h at 37 °C without changing the electrophoresis band of RNA.

5. Inactivation conditions: T3 RNA polymerase can be inactivated by heating at 70 °C for 10 minutes. Adding an appropriate amount of EDTA can also inactivate T3 RNA polymerase.
6. This product is for scientific use only.