T7 Thermostable RNA Polymerase

User's Instruction

Description

With T7 phage promoter and template DNA sequence, T7 Thermostable RNA polymerase can synthesize RNA *in vitro*. Both linear blunt end and 5' sticky end of double-stranded DNA can be used as the template, so the linear plasmids and PCR products are ideal for the reaction. Whether the synthesized RNA strand is sense or antisense depends on the relative position of template DNA sequence and T7 promoter. When the template DNA sequence is downstream of T7 promoter, T7 Thermostable RNA polymerase will transcribe sense RNA strand. Otherwise it will transcribe antisense RNA strand.

Kit Contents

	5 KU
T7 Thermostable RNA Polymerase (50 U/μl)	100 μl
10 × Transcription Buffer	1 ml

Unit Definition

One unit is defined as the amount of enzyme required to add 1 nmol of [^{3}H] GMP into acid-insoluble substances within 1 hour at 50°C under the standard reaction system (pH 8.0).

Features

Compared with wild-type phage T7 RNA polymerase, T7 Thermostable RNA polymerase can transcribe *in vitro* at higher temperature. It can efficiently transcribe *in vitro* at 50°C, while the wild type has no activity at this temperature. Based on this heat-resistant *in vitro* transcription reaction characteristics, it has the following advantages:

- Improving the transcription efficiency of RNA with high GC content
- Improving the synthesis ability of long segment RNA
- Improving the capping efficiency of co-transcription when using cap analogues
- Reducing the formation of dsRNA by-products and the immunogenicity of synthetic RNA

- ❖ Improving the detection performance of NASBA and CRISPR nucleic acid amplification

Protocol

1. Set up the reaction system as the following table:

Component	Volume
10 × Transcription Buffer	2 μl
NTP Mixture	2 mM each
Template DNA	20 ng-1 μg
T7 Thermostable RNA Polymerase (50 U/μl)	1 μl
RNase Free H_2O	Up to 20 μl

2. After the reaction system is prepared, carry out the reaction at 50°C for 1 h.

Storage

The minimum shelf life is two years at -20°C. Avoid repeated freezing and thawing.

Note

- RNase Inhibitor can be added to the reaction system to 1U/μl, preventing RNase contamination.
- In order to effectively transcribe a specific region, it is recommended to pre-cut the template DNA into blunt or 5' protruding ends at the downstream of the region.
- The combination of spermidine and nucleic acid in the buffer may form insoluble substances, so it is recommended to add template DNA at last.
- The template DNA should be RNase A-free and of high purity, with a recommended OD260/280 of 1.8 to 2.0.