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.

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 37-52°C. The optimal temperature is 37°C. It still retains more than 50% of its activity 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
- These enzymes are purified from recombinant *E. coli BL21* strain without nuclease contamination

Kit Contents

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

	25 KU
T7 Thermostable RNA Polymerase (50 U/μl)	500 μl
5 × Transcription Buffer	1 ml × 5

- **1 × Transcription Buffer:** 40 mM Tris (pH 7.8), 20 mM NaCl, 18 mM MgCl2, 2 mM Spermidine HCl, 10 mM DTT
- **Storage Buffer:** 10 mM Tris-HCl, 0.1 M NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 0.01% (w/v) Triton X-100, pH 7.5

Protocol

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

Component	Volume
5 × Transcription Buffer	4 μl
NTP Mixture (25 mM each, *Cat. No.:NTP-25*)	3.2 μl
Linearized Template DNA	0.2-1 μg
RNasin (RNase inhibitor, 40 U/μl, *Cat. No.: FRN*)	0.5 μl
T7 Thermostable RNA Polymerase (50 U/μl)	1-2 μl
RNase Free H_2O	Up to 20 μl

2. After the reaction system is prepared, carry out the reaction at 50°C for 1-3 h.
3. Optional: Using DNase I (RNase Free) to remove DNA template.

   After in vitro transcription, add 5 μl 10 × DN Buffer, 2 μl DNase I (RNase Free) (10 U/μl, *Cat. No.: DN1*), and 23 μl RNase Free H2O. Incubate at 37°C for 15 min.
4. Purify the transcribed product (e.g. LiCl Precipitation).

Storage

Minimum shelf life is 2 years at -20°C.

Note

- It is recommended to use the linearized plasmid containing T7 promoter and PCR products as templates.
- The purity of the template can significantly affect the *in vitro* transcription reaction. The residual RNase A in the process of plasmid DNA extraction can significantly affect the quality of transcriptional RNA. The plasmid DNA extracted by phenol chloroform is the best template.
- The enzyme is not tolerant to high concentration of NaCl or KCl. Its activity will be significantly inhibited when the concentration is higher than 150 mM.
- In 20 μl reaction system, the addition of 0.02 U Thermostable Inorganic Pyrophosphatase (*Cat. No.: TIPP*) can significantly increase the transcription yield.

Only for research and not intended for treatment of humans or animals