Version: 03
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DATASHEET

T7 RNA Polymerase

Cat. No.: E00066-5; E00066-10
Size: 5 kU/ 10 kU

Product Introduction

Bacteriophage T7 RNA Polymerase is a DNA-dependent RNA polymerase with strict specificity for the T7 phage promoter. The enzyme is widely used for the synthesis of specific transcripts from DNA in the 5'→ 3' direction, as well as being a suitable model for studying the mechanisms of transcription. The RNA produced by T7 RNA Polymerase is suitable for many downstream applications.

GenScript is offering T7 RNA Polymerase produced by expression in an *E. coli* strain carrying a plasmid encoding the T7 RNA Polymerase.

Source: Recombinant T7 RNA Polymerase expressed by E.*coli*
Species: phage T7
Molecular Weight: ~100 kDa
Unit Definition: One unit is defined as the amount of enzyme that will incorporate 1 nmol ATP into acid-insoluble material in a total reaction volume of 50 µl in 1 hour at 37°C.
Optimal active temperature: 37 °C.
Storage Buffer: This enzyme is supplied in 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, 10 mM DTT, 50% Glycerol, 0.1% Triton X-100, pH 8.0.
Storage & Stability: This product remains stable for up to 12 months at -20 °C. Avoid repeated freeze-thaw cycles.
Application:

- Synthesis of the single-strand RNA
- Synthesis of highly labeled RNA probes
- Synthesis of precursors of siRNA
- Synthesis of precursors for RNA splicing reactions
- Synthesis of capped mRNA when a cap analog is used as a primer

Quality Control Specifications

Assay	Specifications
Appearance	Clear, colorless liquid
Purity	≥ 95% as analyzed by SDS-PAGE
Enzyme Activity	≥ 50 U/µl
Endotoxin Level	≤ 0.1 EU/µg of protein as analyzed by gel clotting method
Residual Endonuclease	Non-detectable
Residual Exonuclease	Non-detectable
Residual RNase	Non-detectable

Reagents Supplied:

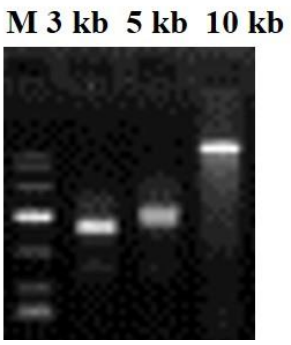
Components	Amount		Storage
T7 RNA Polymerase	5 kU	10 kU	-20 °C
10 × Transcription Buffer	1 ml*1 vial	1 ml*2 vials	-20 °C

Protocols for *in vitro* transcription

Assemble the reaction in a nuclease-free microfuge tube or PCR strip tube on ice with the following order:

Components	Volume
T7 RNA Polymerase (E00066)	100 U
10 × Transcription Buffer	2 µl
NTP Mix, 10 mM each, nuclease free (C01731)	4 µl
Linearized template DNA	0.2-1 µg
RNase Inhibitor (E00070)	20 U
DTT (optional)	5 mM final
RNase free water	to 20 µl
Incubate at 37 °C for 1 hour. High yields of long transcripts (> 10 kb) are achieved by increasing the amount of T7 RNA Polymerase.	
We recommend removing template DNA with DNase I. Add 2 U of DNase I (E00053) into the reaction solution, mix thoroughly, and incubate at 37 °C for 30 min.	

Data Images



In vitro transcription of RNA with the recommended reaction conditions, the transcribed RNA can be up to 10 kb.

References

1. J Kochetkov, S. N., E. E. Rusakova, and V. L. Tunitskaya. "Recent studies of T7 RNA polymerase mechanism." FEBS letters 440.3 (1998): 264-267.
2. Sousa, Rui, Debabrata Patra, and Eileen M. Lafer. "Model for the mechanism of bacteriophage T7 RNAP transcription initiation and termination." Journal of molecular biology 224.2 (1992): 319-334.

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