

## T7 RNA Polymerase

REF: EG20124-S/M

### Storage Condition

-20°C

### Components

Component	EG20124S	EG20124M
T7 RNA Polymerase (50 U/μl)	100 μl	500 μl
10× T7 RNA Pol Buffer	1.25 ml	1.25 ml

### Description

T7 RNA Polymerase is derived from recombinant expression in *E. coli*. It is a DNA-dependent RNA polymerase that exhibits high specificity for the promoter sequence of bacteriophage T7. T7 RNA Polymerase utilizes double-stranded DNA templates containing the T7 promoter sequence and NTPs as substrates to synthesize single-stranded RNA complementary to the downstream of the promoter.

### Definition of Activity Unit

One unit is defined as the amount of enzyme that incorporate 1 nmol of ATP into acid-insoluble material in 1 hour at 37°C .

### Applications

1. Synthesis of single-stranded RNA, including mRNA, siRNA, gRNA, and other types of RNA precursors.
2. Synthesis of labeled or unlabeled highly specific RNA probes.
3. Synthesis of capped mRNA using cap analogs.

### Quality Control Assays

#### Protein Purity

The enzyme is ≥95% pure as determined by SDS-PAGE analysis using Coomassie Blue staining.

#### Endonuclease Activity

A 20 μl reaction containing 1 μg of supercoiled plasmid and 50 U of T7 RNA Polymerase incubated for 4 hours at 37°C results in <10% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

#### Non-Specific Nuclease Activity

A 20 μl reaction containing 15 ng of dsDNA fragments and 50 U of T7 RNA Polymerase incubated for 16 hours at 37°C results in no detectable degradation of the dsDNA fragments as determined by agarose gel electrophoresis.

#### RNase Activity

A 10 μl reaction containing 500 ng of total RNA and 50 U of T7 RNA Polymerase incubated for 1 hour at 37°C results in >90% of the substrate RNA remains intact as determined by agarose gel electrophoresis.

### Protocol

1. Prepare the following reaction mixture on ice:

Reagent	Amount	Final Concentration
10×T7 RNA Pol Buffer	2 μl	1×
CTP/ GTP/ ATP/ UTP (100 mM each)	0.1~0.4 μl each	0.5~2 mM each
RNase Inhibitor (40 U/μl)	0.5~1 μl	1~2 U/μl
Template DNA	0.1~1 μg	-
T7 RNA Polymerase (50 U/μl)	1~2 μl	-
Nuclease-Free Water	up to 20 μl	-

**Note:** It is recommended to add Nuclease-Free Water first, followed by CTP/GTP/ATP/UTP.

2. Mix gently and spin down, then incubate at 37°C for 1 h. If the length of transcript is less than 300 nucleotides, the reaction time can be extended to 2~16 hours.

3. After the in vitro transcription (IVT) reaction, add 1 μl of dsDNase (REF: EG20206) to the product and incubate at 37°C for 15 minutes to remove the DNA template.

### Notice

1. The purity of the template DNA is crucial for IVT reactions. Residual RNase A introduced during plasmid DNA extraction can significantly affect the quality of transcribed RNA. It is recommended to use high-purity RNase-free template with an  $A_{260}/A_{280}$  ratio of 1.8~2.0.

2. The template DNA can be obtained from linearized circular plasmids or PCR. The upstream region of the template DNA should contain a T7 promoter sequence, while the downstream region should have a blunt end or a 5'-overhang.

3. For your safety and health, please wear a lab coat, disposable gloves, and a mask while conducting the experiment.